THE NEWER KNOWLEDGE OF NUTRITION

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THE NEWER KNOWLEDGE OF NUTRITION

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TO

RUSSELL H. CHITTENDEN

Founder of the First Laboratory of Physiological Chemistry in America, Distinguished Investigator, and Inspiring Teacher.

PREFACE TO THE FIFTH EDITION

THE fourth edition of THE NEWER KNOWLEDGE OF NUTRITION appeared in 1929 and has been out of print since 1935. The book has been completely rewritten, but the plan of the present edition is essentially the same as that of the previous editions. It represents an attempt to interpret the meaning of the extensive experimental work relating to nutrition and described in numerous journals during recent years. Our purpose has been to present the subject matter in a form intelligible and useful to the large number of students, teachers, physicians, dentists, and others who are interested in being informed on the present knowledge of nutrition.

In order to place the modern viewpoints in their proper setting the first two chapters are essentially historical and trace the development of modern concepts of nutrition, only the publications which constitute distinct advances being mentioned. In other chapters, dealing with the several known nutrients, only such references are made to the older literature as are necessary for making clear their importance as foundations for the more recent researches. But an attempt has been made to include all of the essential information about each nutrient, thus making the volume useful as a textbook. In presenting the data from many sources the title, THE NEWER KNOWLEDGE OF NUTRITION, has been kept in mind. The facts and opinions in what appear to be the more significant original publications have been recorded as nearly as possible in the form of a presentation of the conclusions of the investigators and the type of experimental procedure employed, only a minimum of data being presented which illustrate the basis for conclusions. Where conflicting views are supported by experimental evidence these are cited, and where critical remarks seem justified these are included. A careful re-examination was made by the authors of all the literature which appeared to be significant.

An attempt has been made to prepare a concise survey of the field of nutrition which is more extensive and more inclusive than any other now available and which is kept close to the scientific literature by means of citations from carefully selected references for the benefit of those who may care to extend their reading in the original literature. It is hoped that the book will meet the needs of all who are, like the authors, seeking to determine what is sound and what is speculation

or misinformation in respect to the nutritive needs of several species, including man; the dietary properties of foodstuffs; the characterization of malnutrition due to specific or multiple deficiency states; the occurrence of various types of malnutrition in man and animals in different parts of the world; and the means of dealing effectively with these nutritional problems.

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Baltimore, Md.
August, 1938.

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CHAPTER

I

The Science of Nutrition, Early History and Development of Concepts

PRIMITIVE MAN appears nowhere to have given much thought to the nature of the processes of nutrition. However, he exhibited much thought and foresight in providing a food supply, for throughout human history food has been one of man's most pressing problems. This is reflected by the biblical maxim, "In the sweat of thy face shalt thou eat bread." The ancient hunter ate the heart of his adversary in the belief that this gave him strength and courage. This appears to have been the earliest distinction of special nutritive quality in food. Primitive peoples experimented with everything available in the way of food. Long before the dawn of history, the chewer of the coca leaf in South America, the devotees of kat in the near East, and the fabled lotus eaters of the Orient, had discovered that these vegetable products would allay pain and act as stimulants or produce dreamy contentment. The biblical story of Noah's misadventure with wine of the grape illustrates the early experience with alcohol and showed that this beverage could cheer and give respite from care, although it brought the blush of shame to the cheeks of his modest family.

With the development of a relatively stable society the more favored individuals acquired a keen appreciation of the appetizing qualities of certain foods and beverages. The earliest extensive account of the views of the Greeks and Romans concerning the merits of all the more attractive foods available in the Mediterranean region is that of Athenaeus, written about 200 A. D., which is a record of the conversation about foods and drinks by a company of gentlemen at a banquet. Also it includes their views about the merits of waters from various sources.

From time immemorial mothers must have given much thought to foods which were suitable for young children. It is stated by Chief

Standing Bear ('33) of the Western Sioux Indians, that grandmothers believed a cake, made of a meal composed of dried meat and dried chokeberries, and held together by the fat skimmed from the boiled bones of the buffalo, was especially good for young children just beginning to eat solid food. The Sioux were in great measure a carnivorous people.

Diodorus (1st C. B.C.) tells of the understanding by the Ancient Egyptians of the fact that many diseases were caused by excess of eating, and that they employed emetics and aperients to relieve the body (Barach, '28). Much as they knew about several arts such as metallurgy, dyeing, soap, glass making, alloys, amalgams, and medicines, they knew scarcely anything of the nature of foods or the processes of nutrition.

The concept of four elements, fire, air, earth, and water, introduced by Empedocles (504-432 B.C.), was inadequate for progress in an understanding of the nature of nutrition. Hippocrates (460-359 B.C.) insisted that those who attempted to speak on medicine, using only the concepts of hot or cold, and moist or dry, were clearly mistaken. He pointed out that a man who eats raw wheat from the threshing floor, raw meat, and water, suffers severely in his bowels and does not live long, whereas if he eats bread, boiled meat and takes wine, he is well nourished. He observed that some could not eat cheese without distress, whereas others with whom it agreed gained from it wonderful strength. One of his aphorisms is translated: "Growing bodies have the most innate heat; they therefore require the most food, for otherwise their bodies are wasted. In old people the heat is feeble and they require little fuel, as it were, to the flame, for it would be extinguished by much." Modern calorimetry is foreshadowed in this aphorism. Hippocrates also noted that very fat people are likely to die earlier than those who are slender. In his system of medicine, dieting, exercise, exposure to sunshine, and psychic diversions were prominent.

Erasistratus (310-250 B.C.) apparently performed the first experiment in nutrition (Garrison, '29). Believing that the pneuma of the air was transformed into spirit in the body, he placed fowls in a jar and weighed them and their excreta before and after feeding. This was the beginning of respiration studies. Galen (130-200 A.D.) studied the digestion of hogs and stated that the stomach was the place in which food was resolved into particles small enough to be absorbed (Walsh, '27). Barach (l.c.) states that about 229 A.D. a Chinese observer noted that the urine of a diabetic was so sweet that dogs drank it. By the sixth century the Hindus had records that "honey urine" was caused by excessive eating of rice, flour, and sugar.

Leonardo da Vinci (1452-1519) discerned that the body of an animal constantly dies and is constantly born again (Herzfeld, '26).

That nourishment can only enter into places where past nourishment has expired; that if one restores as much as is consumed day by day just so much life is reborn as is consumed; as the flame of a candle is fed by the nourishment given by the liquor of the candle. He also said, "Where there is life there is heat, and where there is vital heat there is movement of the humors." Da Vinci propounded certain rules for the investigator, among which are: "You must first have a theory, afterwards practical work. There is no certainty where mathematics is not involved; an experiment is a repetition of a natural process designed to discover the laws of relations presented by science; no action of nature is without a cause. If you understand the cause you need no experiment; an experiment is never fallacious, only our interpretation of it may be wrong."

Sanctorious (1561-1636), Professor at Padua, made an avocation of weighing himself to determine the amount of "insensible perspiration" during periods between the taking of food and when no sensible evacuations had occurred. He estimated that there was half a pound loss of moisture daily in the breath. His test for expired moisture was made by breathing on a cold glass.

An understanding of nutrition was not possible until many discoveries were made in the field of chemistry, since nutrition is a chemical process. A list of the most important chemical discoveries of significance in paving the way to effective researches in nutrition is given to provide a view of the landmarks along the road of progress which have led to the present imposing monument of nutritional science. Each discovery was a beacon which illuminated new ground and enabled the eyes of succeeding inquirers to discern new facts and relationships which hitherto had escaped detection.

In 1669 John Mayow heated antimony with a burning glass and showed that it increased in weight, and correctly interpreted the observation to signify the formation of an oxide. He also showed that part of the air was consumed in respiration. He placed a small animal and a lighted candle in a closed jar, and noted that the candle went out first and the animal died later. If there was no lighted candle the animal lived longer.

1736. H. S. Duhamel De Monceau discovered sodium in plant ash.

1743. A. S. Marggraf, the discoverer of sucrose, demonstrated the presence of phosphorus in plants.

1757. J. Black discovered carbon dioxide. This had been previously known to Van Helmont, who had compared the properties of the gas evolved in alcoholic fermentation and in the burning of charcoal, with that evolved when marble dissolved in acid, and the gas emanating from the Grotto del Cane, near Naples.

1761. A. S. Marggraf named potassium "Gewachslaugensalz" (Growth lye salt).

1774. J. Priestley discovered oxygen. His "fire air" disappeared during respiration and was replaced by "fixed air."

1770-1794. A. Lavoisier made clear, by experiments, the nature of respiration and of oxidation. He first used the thermometer and the balance in the study of metabolism. He believed that organic substances were composed of carbon, hydrogen, and oxygen.

1777. A. Crawford made experiments on animal heat and on combustion, and correctly interpreted his observations.

1779. A. J. Ingenhouss demonstrated the power of plants to utilize carbon dioxide, and their dependence upon chlorophyll.

1786. C. L. Berthollet showed that nitrogen was a constant constituent of animal tissues. Older chemists had found that ammonia was produced by dry distillation of both animal and plant tissues, but the role of nitrogen as a constituent of living matter gained recognition slowly.

1789. A. Fr. de Fourcroy distinguished three kinds of animal products on the basis of their nitrogen content and laid the foundation for the study of the proteins by noting the similarity of gluten of wheat and animal flesh, and by showing that plant juices afford a coagulum like animal protein when heated. He compared the coagulated proteins of plant juices with gelatin. He also discovered adipocere (corpse wax) and described cholesterol which he obtained from bile.

1742-1786. S. W. Scheele prepared and described tartaric, citric, oxalic, malic, lactic, uric, and gallic acids.

1789. J. H. Hassenfratz first demonstrated the presence of calcium, sulfur, and silicon in plants.

1789. C. A. Rückert combated the view that only organic substances in the soil were of value to plants. He held that the carbonic acid of soil dissolved inorganic nutrients, and emphasized the importance of soil analyses and the need of fertilizing soil with inorganic salts.

1798. L. N. Vanquelin discovered benzoic acid.

1804. Th. de Saussure, by numerous experiments, clarified somewhat the problem of the nutrition of plants.

1814. M. E. Chevreul discovered that fats are composed of fatty acids and glycerol. In 1832 he isolated creatine from muscle.

1828. Th. Wöhler synthesized urea from ammonium cyanate, thus demonstrating for the first time that an "organic" substance can be made "without the aid . . . of an animal."

1830. Liebig perfected methods for the quantitative determination of carbon, hydrogen, and nitrogen in organic compounds.

1839. Boussingault first undertook to make a balance of intake and

outgo of nutrients in food and excreta, using a cow as his subject. He published the first system of analysis of feeding-stuffs and fertilizers.

1849. Regnault and Reiset first devised and used a closed circuit system of apparatus for respiration experiments.

Early important discoveries were made concerning simple sugars and other carbohydrates, thus permitting the development of important concepts regarding the nutritive roles of these substances. In 1747 Marggraf discovered milk sugar, and in 1806 Prout discovered malt sugar. The latter also crystallized glucose from grape juice. Kirschoff, 1815, studied the acid hydrolysis of starch. Lagrange, 1817, discovered mannite, and observed that it was not fermentable. Several chemists established between 1830 and 1840 the empirical formula for glucose. Dubrunfaut, 1847, learned the true nature of malt sugar and fructose. The latter was formed through the acid hydrolysis of cane sugar. Hunefeld, 1836, discovered dulcit and Pelouze detected sorbose in 1852. Vogel discovered in 1815 the property of glucose solutions of reducing alkaline compounds of metallic salts. In 1870 von Beyer proposed the aldehyde nature of glucose. Tollens, Kiliani, and finally E. Fischer, greatly extended the knowledge of the structure of the different sugars after 1870. But not until 1875 was it discovered that glucose is the sugar of the blood. This important observation was made by Claude Bernard, only three years before his death.

Although casein of milk as it separates from sour milk, gelatin in cooled solutions in which meat had been boiled, egg white, and flesh of animals, were familiar examples of protein materials from time immemorial, no conception of their nature was gained until Beccaria, in the 18th century, described the gluten of wheat. Rouelle discussed the similarity of wheat gluten and the coagulated protein obtained from plant juices. Fourcroy, 1791, called attention to the similarity of plant and animal proteins. Bostock, 1808, first precipitated proteins with tannic acid and salts of heavy metals; and Prout, 1820, made the first elementary analysis of proteins. Bracconnot, 1822, first hydrolyzed proteins with acid. He discovered the amino acids glycine and leucine. Berzelius and Einhof, 1828, compared the analytical data from animal and vegetable proteins and found them to be alike. Mulder, 1838, coined the term protein. He believed that the sulfur and phosphorus content determined the differences in proteins, which, otherwise, he believed to consist of a nucleus of $C_{40}H_{62}N_{10}O_{12}$.

In 1872 Ritthausen wrote his book on the proteins of seeds, a notable contribution, describing as it did, a great amount of original work on the isolation of plant proteins. From this time onward the studies of Hoppe-Seyler, Weyl, Schmiedeberg, Drechsel, and others contributed many observations on the use of neutral salt solutions as solvents and, in stronger concentrations, as precipitants for proteins,

by means of which proteins could be purified without coagulating or denaturing them. E. Fischer, Kühne, Hofmeister, Kossel, Osborne, Chittenden, Van Slyke, Vickery, Dakin, Abderhalden, and a number of other workers, extended our knowledge of the properties of proteins, and their content of different amino acids which are formed on acid hydrolysis. Hoffmeister was the first to crystallize a protein. This he accomplished by half saturation of egg albumen solution with ammonium sulfate.

Early Studies in Animal Nutrition.—Physiologists have always welcomed the discoveries of chemists and physicists which they might turn to account in their own experimental studies. As fast as new knowledge became available it pointed the way to new methods of study. But even before any clear concept of the nature of food substances was possible a few men sought to learn something of digestion and the processes of nutrition. Thus, contemporaneously with Lavoisier, Spallanzani (1729-1799), inspired by his experiments on respiration, demonstrated the consumption of oxygen and the expiration of carbon dioxide by isolated glandular organs. He also studied digestion by enclosing bits of foods in cages, which he inserted into the stomachs of birds, afterward recovering these by regurgitation or by withdrawal with a string. He swallowed such cages himself, and later drew them from his stomach. He found that foods which were not dissolved by water were rendered soluble by the gastric juice, and postulated chemical changes in digestion.

Magendie (1783-1855) first studied the unlike nutritive effects of the three outstanding types of food substances which are still accepted today, i.e., proteins, fats, and carbohydrates. He experimented with dogs, restricting them to fatty, saccharine, and albuminous substances, such as sugar alone, or butter or gelatin, together with distilled water, and noted that they died in 30 to 36 days. He was the first to describe the eye condition known as xerophthalmia, now known to be caused by deprivation of vitamin A.

During the early decades of the nineteenth century the view of Hippocrates was generally accepted that there is but one kind of aliment, a universal nutrient substance present in various kinds of foods and which is abstracted from them during digestion. This view was reiterated by Richerand (1813) in his *Elements of Physiology*. The outstanding early American investigator in the physiological aspects of nutrition was William Beaumont, a surgeon in the U. S. Army, who experimented during many years on his famous patient, Alexis St. Martin. The latter, as the result of a gunshot wound, had a permanent external fistulous opening into his stomach. Beaumont published his classic *Experiments and Observations on the Gastric Juice, and The Physiology of Digestion* in 1833. He was satisfied with

the idea that there is but one kind of aliment which, in digestion, is abstracted in the form of gastrite of aliment, the gastric juice furnishing the gastrite.

William Prout (1785-1850) published in 1834 his *Chemistry, Meteorology, and The Function of Digestion* (Lusk, '33). In this book he set forth the modern view that several kinds of nutrient substances are essential. These he distinguished as the saccharine group, the oleaginous group, and the albuminous group. He was familiar with the then known facts concerning the diversity of saccharine substances, among which were starch and several kinds of sugars; with a variety of oily and other fatty substances; and with gelatin and egg white and the solid protein material of cooked flesh after gelatin was dissolved out. He drew attention to fermentation of sugar into spirit of wine, or alcohol, as an example of the biological transformation of one kind of food into a totally dissimilar substance.

Gmelin, 1836, was the first to use the term Stoffwechsel (metabolism), a general term which includes all the chemical transformations which food undergoes in the constructive and destructive processes concerned with nutrition.

Regnault, 1849, first determined the respiratory quotient, or volume of carbon dioxide expired divided by the volume of oxygen consumed. With Reiset he studied oxygen consumption by animals of different sizes, and found that small animals used far more oxygen per unit of body weight than did large animals. It was ten times greater in sparrows than in chickens. They rightly assigned the difference to the greater relative body surface presented for cooling the creature of small weight. This was the beginning of the study of the relation of surface area to metabolic rate. This relationship was first clearly enunciated by Bergmann in 1845.

In 1852 Bidder and Schmidt published their book *Die Verdauungssäfte und der Stoffwechsel.* They described experiments which proved that energy metabolism, as represented by the amount of heat lost to the environment, is, in the fasting animal, a constant having the same value for animals with similar body volume, surface, and temperature.

Claude Bernard (1813-1878) studied the sugar of the portal and systemic blood and discovered that the liver could store glucose as glycogen, which he first prepared and described, and could reconvert it into glucose.

Mulder (1802-1880) was the first investigator to emphasize the protein element in nutrition. He examined protein materials from plant and animal sources, and stated his belief that protein is the most important of all known substances in the organic kingdom. This view received immediate recognition. His writings in 1838-1839 led Bouss-

ingault, in 1844, to assess the values of rations for farm animals largely on the basis of their content of nitrogen. Boussingault appreciated, however, that the non-nitrogenous principles were of value, and also that the mineral constituents in foods played an indispensable role.

The earliest attempts to analyze feeding-stuffs appears to have been made by Einhof about 1800. He compared the amounts of substances in different animal feeds which were soluble in water, alcohol, dilute acids, and dilute alkalies. The results were used by Thaer in Germany in 1809 (Armsby, '17) to express the values of different farm products in terms of "hay values." Good meadow grass was universally recognized to be a complete food, and it was naturally taken as a standard with which other feeds could be compared. This work was the beginning of experiments by animal husbandrymen in stock feeding, of a type which has persisted to the present day, and consists in comparing the results of substituting one feed for another in a ration. After decades of trials of many kinds to determine the values of foods by chemical analysis, a procedure was evolved by Professor Henneberg, which was known as the Weende method, and which was presented at the second Convention of German Agricultural Chemists in 1864. By this method the protein, carbohydrate, fat, mineral matter, and moisture were estimated.

Liebig exerted great influence on the thoughts of physiologists and of practical feeders of animals. In his famous book on *Animal Chemistry,* published in 1846, he stressed the idea that only those nutrients which could be transformed into blood were of value to the organism. He stated that the investigation of substances adapted to this purpose should be restricted to the determination of the composition of foods and comparison made thereof with the make-up of blood.

Liebig believed that such physiological functions as muscular contraction and glandular activity appeared to proceed at the expense of the albuminous tissue structures. The function of nutrition, therefore, was to replace destroyed tissue protein. The nitrogenous group of nutrients he designated plastic foods. Fats, starch, sugars, beer, wine, brandy, he called respiratory foods, which served as a source of heat. Liebig's view that nutrition was identical with reconstruction of protein in the tissues which had been destroyed by work persisted for many years, due largely to the force of his great reputation.

At the beginning of the last third of the nineteenth century, the Munich School of physiologists, under the leadership of Carl Voit, was the center of learning and progress in the field of nutrition. Voit showed that geese could transform carbohydrate into fat. Workers influenced by his school demonstrated that the muscles could do work at the expense of carbohydrate or fat, and that a part of the protein in the food could be converted into carbohydrate. It is not possible

here to follow the progress of such experiments further than to point out that Voit's school demonstrated the dual function of carbohydrate and protein and the interchangeability, to a certain extent, of these substances.

Voit established the fact that even when the daily protein intake is high, protein is not stored, but is, except in the growing animal, degraded during a period of about 24 hours into urea, and other metabolic end-products of protein metabolism, and these products are excreted in the urine. Fats may be retained in large amounts and deposited in the adipose tissues. Carbohydrates may likewise be stored as glycogen in liver and muscle, after being digested and transformed into glucose. When a need arises glycogen stores may be reconverted into glucose, which is contributed to the blood. If carbohydrate is ingested in excess over the requirements for energy (which may also be derived from fats or proteins) it may be converted into fat and deposited as an energy reserve.

With the perfection, through the years, of chemical methods for the analysis of foodstuffs, it appeared to students of nutrition that natural foods consist, aside from cellulose, lignin, pentosans, waxes, etc., of protein, carbohydrates (starch, sugars), fats, and ash constituents. Since it was found that isolated proteins contained, on an average, about 16 per cent of nitrogen, and since direct estimation of proteins was not possible, it became the custom of chemists to estimate the protein content of foods by multiplying the content of nitrogen by the factor 6.25. Generally the carbohydrate fraction was determined by differentiation between cellulose and "nitrogen-free extract." The latter contained the pentose sugars, which are present in notable amounts in many vegetable foods, and are prominent constituents of straw, as pentosans. Although it was well recognized that the methods of food analysis were only approximate, the results of separate determinations of the above-named components, summed nearly enough to 100 per cent to convince investigators that the chemist was actually estimating all of the important constituents of foods. Hence the conclusion that proteins, fats, carbohydrates, and certain mineral elements, were the only nutrients which man and animals require.

With the accumulation of experimental data from animal feeding studies, it became evident that the quality of a ration could not be accurately predicted on the basis of its chemical composition. Therefore, animal husbandrymen began to speak of "the specific effects of nutrients" to account for the better results obtained from certain rations when one food was included, as compared with the same mixture with one constituent substituted by another. It was not until about 1900 that chemical studies showed that proteins from different sources yielded on digestion very different proportions of the then

known amino acids. Other amino acids were discovered from time to time, and by 1900 about 16 were known. It was easy to account for observed differences in the quality of foods by assuming that the cause lay in the quality of their proteins. This was a correct judgment, but as later studies showed, was by no means the only factor involved.

Atwater (1895), a student of Voit, returned to America about 1873 (Chittenden, '30) with a glorious program of research. He believed that if he knew the chemical composition and the fuel values of all important foods and feeding-stuffs, in terms of their content of protein, carbohydrate, and fats, and the digestibility factors for these, together with the energy requirements of human beings and animals, it would be possible to place nutrition of man and animals on a sound economic basis. He set to work, with the support of the U. S. Department of Agriculture, to analyze all American foods, taking into account values as purchased, and waste in preparation. In 1895 he published a compilation of food analyses, emphasizing their energy-yielding functions, and discussed both the chemistry and economy of foods. To illustrate wise and unwise food purchases he presented a table showing the amounts of nutrients which could be purchased in the Eastern United States in 1895 for 25 cents. The following items are selected to illustrate his advice.

25 cents will pay for:

	Total Pounds	Protein Pounds	Fat Pounds	Carbohydrates Pounds	Calories of Potential Energy
Milk @ 8 cts. per qt. . . .	.81	.23	.25	.29	2,020
Cheese @ 18 cts. per lb. . .	1.32	.96	.40	.49	2,850
Potatoes @ $1.00 per bu. . .	2.69	.27	.01	2.29	4,785
Sugar @ 5 cts. per lb. . . .	4.90	0	0	4.89	9,095
Dried beans @ 5 cts. per lb. .	4.37	1.15	.10	2.96	8,065
Corn meal @ 3 cts. per lb. . .	7.08	.77	.32	5.88	13,720
Wheat flour @ 3.5 cts. per lb.	6.25	.79	.07	4.68	10,285
Oatmeal @ 5 cts. per lb. . .	4.62	.76	.36	3.41	9,275
Eggs @ 35 cts. per doz. . .	.23	.12	.10	0	645

Atwater regarded fruits and water-rich fresh vegetables, eggs, etc., as extravagent food purchases. He saw no reason why one should not remain in health while taking a diet selected from the cheapest dried food products.

Atwater visualized the coming of a time when farmers should be able to consult tables showing the cost of protein and energy in various farm crops, and taking into account digestibility of their food ele-

ments, to select the cheapest sources of these nutrients for compounding their rations for feeding animals. Fortunately for his peace of mind he never saw the effects of restricting animals or men to diets which might have been compounded on his advice. It is also very fortunate that housewives did not, so far as we are aware, attempt to follow his advice in the feeding of their families.

Voit set the average protein requirement of a man at 118 gm. per day. The value was placed at 145 grams for men doing hard work. Atwater thought 125 grams the appropriate figure for men doing an ordinary amount of work. For several decades these standards were followed in estimating the nutritive requirements of soldiers, prisoners, and laboring men where group feeding was practised. The stimulus given by Voit and by Atwater to nutrition studies resulted in many dietary studies in different parts of the world. Dietary studies were made on laborers, sedentary workers, students, Alabama negroes, Mexican families, football teams, and others. McCay studied the dietaries of various peoples in India, where the protein consumption varied from 67 grams daily for Bengali students, to Bhutias taking 150 to 160 grams daily. He stated (McCay, '12), "We may conclude this study of the effects of the level of protein metabolism on the physique and general efficiency of different tropical tribes and races by stating that the facts afford ample proof of the all-important influence exerted by food, and particularly protein, in determining the degree of muscular development, the general physical endowment, the powers of endurance, resistance to disease, and most important of all, the place a tribe or race has won for itself in manliness, courage, and soldierly instincts . . . the higher the level of protein interchange, the more robust and energetic, and the more manly the race."

There were, during half the century following 1875, many differences of opinion expressed concerning man's requirement for protein. While C. Voit and Atwater laid emphasis on the point that it was hazardous to transgress below the limits they set for protein consumption, Hirschfeld remained in nitrogen equilibrium for a fortnight on 40 grams of protein intake daily, and E. Voit found vegetarians to be in nitrogen balance on 50 grams daily of protein, augmented by fats and carbohydrates to meet their energy requirements. Several other investigators confirmed the fact that protein equilibrium was attainable at these low planes of intake. C. Voit, himself, enunciated the principle that the smallest amount of protein, in addition to energy sources as fats and carbohydrates, capable of maintaining the body in health and vigor is best, but he did not amplify his view as to this requirement.

In 1901 Professor Russell Chittenden, at Yale University, turned his attention to a study of the protein requirements of man. Horace Fletcher and Dr. Van Someren, a vegetarian, had found each other

excellent company because of their common interest in the alleged virtues of intensive mastication. They had convinced themselves that careful attention to the mastication of each morsel of food, as long as it could be kept under control in the mouth, made the appetite more discriminating, diminished the total requirement of food by one-half, and reduced the protein requirement by two-thirds! They submitted themselves to the observation of Sir Michael Foster, and had the gratification of demonstrating these convictions by experimental methods. In 1902-03 Fletcher spent several months with Dr. Chittenden, and demonstrated that he was able to maintain his weight at 75 kilos and perform the work of a trained athlete in the gymnasium on about 43 grams of protein daily.

Chittenden instituted prolonged experiments on 5 brain workers (professors), 13 soldiers, and 8 university athletes, who worked hard both mentally and physically. During 225 days a careful record was kept of food consumed, and the excreta were analyzed chemically. Dr. Chittenden, himself, who weighed 57 kilos, maintained himself in nitrogen equilibrium and greatly improved his health, on 36 grams of protein daily, with a calorie intake of 2000. Dr. Mendel, who weighed 70 kilos, was in equilibrium on 41 grams of protein. The soldiers and athletes were in equilibrium on 50 and 55 grams of protein respectively. All attested the maintenance of perfect health on these low protein levels. Chittenden crystallized his view that the low protein mixed diet is most conducive to health for the following reasons:

Because protein cannot be stored up in the tissues.

Because energy is dissipated in excreting the surplus protein.

Because excess of protein tends to disseminate toxins throughout the body, arising through putrefaction in the colon.

Because there is no necessity to engage in muscular work to aid in working off excess of protein, hence energy is saved.

Because a combination of animal and vegetable proteins is essential to satisfy the requirement of the body.

The low protein regimen advocated by Chittenden was characterized as *physiological economy in nutrition*. His books (Chittenden, '04, '07) *Physiological Economy in Nutrition* and *Nutrition of Man* were published in 1904 and 1907 respectively. In 1909, Sir James Crichton-Browne ('09) published his *Parcimony in Nutrition,* in which he controverted Chittenden's views. He asserted that "the success of the races, their vitality and energy, might almost be measured by the degree in which animal flesh has entered into their diet. All the successful races have habitually consumed protein far in excess of the Chittenden standard and far in excess of what was required for

tissue repair, and when we find a definite relation between protein consumption and racial success there is good ground for believing that behind it there is a biological law."

The controversy over the role of the protein element in nutrition was the most outstanding feature of discussions relating to diet during the first decade of the twentieth century. The observations underlying this discussion were not of a nature to lead to progress in the understanding of the fundamentals of nutrition. Meanwhile animal experimenters were puzzling their heads over the cause or causes which determined the extraordinary differences in the nutritive values of rations which on chemical analysis appeared to be of similar composition. Feeding experiments conducted with mixtures of natural feedingstuffs, and the by-products of the milling industry and the slaughter house, were not of a nature which could lead to an understanding of the problem. A new method of approach to the study of the nutritive requirements of the body and the properties of individual foodstuffs was necessary. Unknown to those who were vocal in that decade in expressing their views about diet there lay buried in the literature of physiology and biochemistry, several fundamentally important contributions which pointed the way to an experimental procedure which was destined to eventually clarify the problems in this department of knowledge and to create a science of nutrition as a department of the science of chemistry. In the following chapter the early development of the modern method of study of nutrition problems which resulted in a clear statement of the nutritive requirements of the body, and the concept of dietary essentials, will be briefly reviewed.

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CHAPTER

II

Nutritive Requirements of the Body, the Modern Concept of Dietary Essentials

IT IS to the merit of Magendie (1816) that during the first quarter of the nineteenth century he introduced the experimental procedure of feeding to animals diets which in composition were simplified to a degree that made it possible to name their chemical constituents. His knowledge of what constituted purity in food substances such as proteins, fats, and carbohydrates was necessarily very limited since chemical science was in its infancy. Certain notable investigators never lost sight of his demonstration that animals could not remain in health when fed certain simple mixtures containing the "staminal principles," the saccharine, oleaginous, and albuminous. Pereira (1843), in his *Treatise on Food and Diet,* discussed the views of Prout, saying that after mature consideration he was satisfied of the impossibility of reducing all nutritive principles to these four heads. Common salt, for example, could not be considered only as a luxury, but as a substance as essential to life as nitrogenous or non-nitrogenous food and water. He pointed out that lemon juice, which is now recognized as one of our most valuable antiscorbutic foods, does not owe its nutritive importance to water, sugar, albumen, or oil.

Voit (1881, p. 19) clearly shared this view when he wrote "Unquestionably it would be best for the purpose (experimental study) if one could feed only pure chemical compounds (the pure foodstuffs), for example, pure protein, fat, sugar, starch, and ash constituents, or mixtures of the same. However, inasmuch as men and also animals only rarely tolerate continuously such tasteless mixtures, it is necessary in most cases to choose foods as they are provided by nature. Nevertheless, it would probably be possible and very desirable to repeat the tests with the natural food products by using the pure substances, although the results yielded thereby might not be essentially different."

Voit's words do not intimate a suspicion that the predicted unfavorable outcome of such experiments might be attributable to any cause other than loss or perversion of appetite. The first to put such experiments to test was Lunin (1881) whose experiments were performed in the same year. He employed a diet of casein, milk fat, and cane sugar, to which, in different experiments, he added various inorganic salts including an imitation of the ash constituents of milk. Mice survived but a short time on the ash-free diet and about double the time with certain mineral additions. He found it impossible to keep mice in health on his diets. His illustrious teacher, G. v. Bunge, raised the question whether milk did not contain nutrients of unknown nature which were indispensable for health.

Pekelharing ('05), in Holland, restricted mice to a diet consisting of bread baked with casein, albumin, rice flour, lard, and a mixture of all the salts which he thought ought to be in their food. When they were given this ration with water the mice eagerly nibbled the food for a few days, but they soon began to lose their appetite, grew thin, and in 4 weeks all were dead. He observed that when they had milk to drink, in addition to this bread, they remained in good health although the quantity of albumin, lactose, and fat which they assimilated from the milk was "quite negligible in comparison with what the bread on which they fed contains." He stated that the element in the milk which kept the animals alive also occurred in the whey from which casein and fat had been removed. He wrote "My intention is to point out that there is a still unknown substance in milk, which, even in very small quantities, is of paramount importance to nourishment. If this substance is absent, the organism loses the power properly to assimilate the well-known principal parts of food, the appetite is lost and with apparent abundance the animals die of want. Undoubtedly this substance not only occurs in milk but in all sorts of foodstuffs, both of vegetable and animal origin."

Hopkins ('06) wrote "But further, no animal can live upon a mixture of pure protein, fat and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than the proteins, carbohydrates and fats. Physiological evolution, I believe, has made some of these well nigh as essential as are the basal constituents of the diet; lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something familiar, and a substance which happens to have been tried. The field is almost unexplored, only it is certain that there are many minor factors in all diets, of which the body takes account. In diseases such as rickets, and particularly in scurvy, we have

had for long years knowledge of a dietetic factor, but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention, but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors."

Hopkins reported that when rats were fed a food mixture containing casein, starch, cane sugar, lard, and inorganic salts, they remained alive and grew a little when the components were supplied in a crude condition. When they were carefully purified, growth invariably ceased in a very short time and the animals declined and died. He showed that their failure was not due to insufficient food ingestion. When a small daily allowance of whole milk was given, which did not provide more than 4 per cent of the food eaten, the rats were able to grow to a certain extent. These experiments marked the beginning of an appreciation of what Hopkins called the "accessory factors of the diet."

Takaki (1887) reported a remarkable investigation on the prevention of beriberi by diet. About the year 1880 this disease had become a scourge among Japanese sailors. Takaki became impressed with the superior health of sailors in the British Navy, as compared with that of men in the Japanese Navy, and set about securing an improvement of the diet of the latter, which he judged to be of inferior quality. After many vicissitudes caused by resistence of officials, he succeeded in increasing the content of meat and vegetables, and of introducing condensed milk into the rations of the sailors. Through his influence a training ship was commissioned to make a nine months cruise, the men receiving the standard ration. While during this cruise there were no cases of beriberi among the officers who had a superior diet, there occurred 169 cases of the disease among 276 sailors. Another ship was then commissioned to make the same cruise, using the improved dietary. There were but 14 cases of beriberi among 276 men on this cruise. The results were so striking that a reform in the rationing of sailors was instituted which resulted in practical eradication of the disease from the Navy.

Hopkins was apparently not aware, in 1906, of the studies of Takaki, nor those of Eijkman (1897) in Batavia, who had produced experimentally in fowls a disease similar to beriberi, which had for centuries constituted one of the major causes of ill health among rice-eating peoples. Eijkman found that chickens restricted to polished rice developed polyneuritis, the cardinal symptom of beriberi, whereas they did not do so when fed unpolished rice. He further demonstrated

that a water or an alcoholic extract of rice polishings would produce dramatic cures of polyneuritic birds.

Eijkman did not at first correctly interpret his experimental observations. He concluded that rice, over-rich in carbohydrate, produced in the intestine a substance which acted as a poison to nerve cells, and that the outer layers of the seed contained a pharmacological antidote. Grijns ('01) expressed the view that beriberi, in man and in birds, is the result of a deficiency in an essential nutrient. Eijkman's interpretation of his findings furnishes an illustration of the difficulty which pathologists had in accepting the view that a deficiency of anything could cause disease. This was recognized in 1919, in the *Report on the Present State of Knowledge Concerning Accessory Food Factors* (*Vitamines*), prepared for the British Medical Research Committee ('19):

"Disease is so generally associated with positive agents—the parasite, the toxin, the *materies morbi*—that the thought of the pathologist turns naturally to such positive associations and seems to believe with difficulty in causation prefixed by a *minus* sign. Even in connection with deficiencies arising within the body there is or was a similar tendency. When the importance of the internal secretions was first recognized there seemed to be much hesitation in believing that symptoms might be frankly due to their failure. When each fresh internal secretion was described there was always an effort to show that its function was to 'neutralize' some, always hypothetical, toxic substance. Symptoms, on this view, were due to the unmasking of a deleterious agent rather than to simple deficiency in a normal and necessary agent. To distinguish between these two possibilities was of course a scientific duty; but, at any rate in the earlier literature of internal secretions, a bias against the simpler view interfered with the fair interpretation of experimental results. So (A similar situation existed) in connection with the newer conception of disease as due to dietetic deficiencies."

Stepp ('09-'12) carried out significant experiments to determine whether lipids are essential for normal nutrition. He found that mice, when fed wheat bread and milk, remained in health during several months, but the same foods, after thorough extraction with alcohol and ether, did not support life longer than one month. Restoration of the extract made the food complete once more. He tried adding cholesterol, lecithin, kephalin, or cerebrone, but found that these were not able to protect mice against early nutritive failure. Stepp wrote "It is not impossible that unknown substances indispensable for life go into solution with the lipids, and that the latter thereby become what may be termed carriers for these substances."

Impressed by the investigations of beriberi—notably those of Fletcher, Fraser, and Stanton, Schaumann, Grijns, and others—Funk

began at the Lister Institute in London, in 1911, an attempt to isolate the antiberiberi substance from rice polish. Similar attempts, but less comprehensive, were made by others. He succeeded in making a far more potent concentrate of the curative substance than had been hitherto obtained, but did not succeed in isolating it. In 1912 Funk ('12-13) published a paper entitled "The Etiology of the Deficiency Disease," in which he introduced the term vitamine. This paper attracted a great deal of attention and stimulated much thought by others. After reviewing the literature relating to scurvy, beriberi, and pellagra, he expressed the opinion which was to be later verified, that these were all to be considered deficiency diseases. He suggested further that rickets and sprue were in the same category.

Vitamin A.—Beginning in the year 1907 McCollum and his associates, (McCollum and Davis, '13) at the University of Wisconsin, initiated studies with diets of purified foodstuffs. These studies were stimulated by the observations of Lunin and of Hopkins. McCollum and coworkers had not learned of the writings of Pekelharing, already referred to, since they appeared in Dutch and were not generally known. In 1909 Osborne and Mendel ('11, '13-14) of Yale University, undertook similar investigations. After many vicissitudes and wrong interpretations, due to the fact that certain ingredients of their mixtures, notably milk sugar, were not sufficiently purified, and carried significant amounts of "accessory food substances," these two groups of workers were able to demonstrate that certain fats, but not all, carried an indispensable nutrient. Thus McCollum and Davis found that a certain diet, composed of purified foodstuffs, proved a failure in the nutrition of young rats when it contained lard as the fat constituent, but was far more satisfactory when butter fat or ether extract of egg yolk was substituted for the lard. They, thereupon, announced the discovery of a hitherto unknown fat-soluble nutrient, which had been foreshadowed by the studies of Stepp. Later this factor was named vitamin A.

In 1921 Steenbock and his coworkers showed that carotene, the yellow pigment of carrots and of many other yellow and green vegetable foods, when fed to rats had a vitamin A-like effect. This conclusion was controverted by several investigators, but in 1928 von Euler and his coworkers reopened the question. They pointed out that at the time of Steenbock's investigation vitamin D was unknown and that in its absence young rats could not develop normally; hence a clear demonstration of the effects of vitamin A on health and growth could not well be made. They administered vitamin D together with crystalline carotene, and confirmed the findings of Steenbock that the yellow pigment could serve as a source of vitamin A. It was clear, however, that vitamin A itself was not a yellow pigment

since almost colorless fish liver oil contained it in abundance. Vitamin A must, therefore, be formed in the body from carotene, a surmise which was later fully justified by experiments. Vitamin A is now known to occur preformed in various liver oils and oils from different mammals. It does not occur in vegetable substances, so far as is known, but is found there in the form of its precursors, carotene, or cryptoxanthin.

Osborne and Mendel, after failing to secure growth or survival of young rats on a diet of purified casein, starch, lard, and a salt mixture, had the happy thought to prepare an evaporated whey, from which both casein and lactalbumin had been removed, as an adjuvant to their purified food mixture. When 27 per cent of the diet consisted of this "protein-free milk," they succeeded in growing and maintaining rats for a year or more in apparent health. They regarded their "protein-free milk" as essentially a source of a well-balanced salt mixture, but later, in the light of accumulating knowledge, they came to realise that it supplied other essential nutrients. From this product they proceeded to make up an "artificial protein-free milk" from "carefully purified lactose" and inorganic salts, and discovered that this mixture was very inadequate as compared with the "natural" product. At one time they reported (1912) that all true fats were dispensable in the diet, and that their experiments pointed in the same direction with regard to the lipids in general. But the following year (1913) they were led to announce as the result of early failure of rats on their "artificial protein-free milk" made of more highly purified materials, that the addition of butter (containing about 15 per cent of water, curd, salts, and whey constituents) caused an extraordinary improvement in the growth and general condition of their animals. On November 4, 1913, they reported similar recoveries of their animals when butter fat was employed instead of butter, and differentiated clearly between the growth-promoting properties of butter fat and of cod liver oil, in contrast to lard and almond oil, as had McCollum and Davis between butter fat and egg yolk fats on the one hand, and lard and olive oil on the other. McCollum and Davis had saponified butter fat and shaken the resulting soaps with olive oil thus transferring the growth essential to the olive oil.

Proteins.—Osborne and Mendel, using their "protein-free milk" as the source of unidentified dietary essentials, proceeded to study the dietary values of different purified proteins. Steady progress had been made in the discovery of new amino acids formed by the hydrolysis of proteins. A method was devised by Hausmann for differentiating quantitatively yields of ammonia and mono- and di-amino acids. By means of this method it was shown that proteins were very differently constituted as respects their yields of these constituents. E. Fischer

('06) in 1902 developed a much more elaborate method of analysing proteins for their content of individual amino acids. The method was applied between 1904 and 1909 by Osborne to the examination of many purified proteins. These studies clearly indicated that proteins should have very different nutritive values, but nothing was known about the significance of individual amino acids in nutrition. It was this field which was next explored by Osborne and Mendel. They brought to light the fact that one of the diamino acids, lysine, is the limiting factor in determining the growth value of a number of vegetable proteins. In others the first or second limiting factor was tryptophane, and in still others the sulfur-containing amino acid, cystine was regarded as the limiting factor. Their cystine was probably contaminated with the essential sulfur-containing amino acid methionine, then unknown. They demonstrated by their experiments the importance of the source and nature of protein in the diet, rather than its quantity, as a factor in nutrition. The field is still being explored to determine which of the 23 now known amino acids derived from the digestion of proteins are dispensable and which are indispensable in mammalian and avian nutrition.

Deficiencies of Individual Natural Foods.—McCollum and Davis ('15) turned their attention to determining the chemical nature of the limiting nutritive factors in individual natural foods. They found that wheat, maize, or oat grains were effectively supplemented for the production of growth in young rats by the addition of calcium, a purified protein (casein), and butter fat, which showed that these grains were deficient in calcium, in certain essential amino acids, and in the newly discovered nutrient in certain fats. The oat kernel, however, was less well supplemented by these additions than were wheat or maize. The cause remained unknown until Smith and Hendrick, in 1926, demonstrated that a heat-stable vitamin, soluble in water, which was hitherto unsuspected, is supplied by oats in smaller amount than in the other grains mentioned.

When they applied the same supplements to polished rice they found that the young rats immediately suffered nutritive disaster. The studies of Eijkman (l.c.) afforded a clue to the cause of these failures. When, in addition to calcium, casein, and butter fat, an alcoholic extract of wheat germ was added to polished rice, growth was secured. They were led by these results to assert that in addition to protein, carbohydrate, fats, and the essential inorganic salts, two unidentified nutrients "fat-soluble A" and "water-soluble B" were essential for growth and the maintenance of health.

The Antiscorbutic Vitamin.—Scurvy was for several centuries rightly regarded as a disease due to dietetic error. Apparently the first people to learn how scurvy can be cured were the Indians of Canada

(Biggar, '24), who must have been long familiar with the disease in winter. An Indian advised Jaques Cartier, who lost 26 of his party from scurvy while wintering on the St. Lawrence River in 1536, to give his sick men an infusion of spruce needles, a remedy which was immediately successful in restoring them to health. Bachstrom, in 1734, wrote on scurvy and attributed the disease to lack of "fresh fruits of the earth, and greens." James Lind (1753) experimenting on scorbutic sailors, clearly demonstrated the virtues of oranges and lemons in curing the disease. Yet failure of lime juice to prevent scurvy in the crews of the *Alert* and the *Discovery,* commanded by Sir George Nares on an expedition to the Polar Regions in 1875-1877, and in the British Army Garrison at Chitral, India, in 1895-1896, and in the party of Captain Scott while on their voyage to the Antarctic in 1902, tended to unsettle the view that scurvy was due to dietary deficiency (Medical Research Council, '32). There can be no doubt that in the above-mentioned cases the lime juice was inferior, perhaps adulterated, or heated to preserve it, thus destroying its antiscorbutic property.

The demonstration of a nutritive deficiency in scurvy was provided by Holst and Fröhlich ('07, '12), who showed that scurvy could be induced in guinea pigs by restricting them to a diet of grains and water. Supplementing the diet with fresh vegetables, fruits, or fruit juices resulted in the maintenance of health in the animals. Heating the antiscorbutic foods reduced or destroyed completely their protective value. The expressed juices of fruits or vegetables, they found, rapidly deteriorated in their antiscorbutic value on keeping, but citrous fruit juices and sorrel juice were more stable in this respect. It was evident that a specific antiscorbutic substance existed, and biochemists turned their attention to its isolation and identification.

Szent-Györgyi ('28), in a study of the nature of substances involved in the oxidation-reduction system in adrenal cortex, orange, and cabbage, isolated a specific substance and identified it as a hexuronic acid. Waugh and King ('32) isolated the antiscorbutic vitamin and identified it with Szent-Györgyi's compound. In the same year Svirbely and Szent-Györgyi ('32), confirmed the antiscorbutic value of the substance, which on further investigation proved to be not an uronic acid but a furane carboxylic acid. Its synthesis followed immediately (Hirst and coworkers). Tillmans and associates ('33) introduced the indicator 2:6-dichloro (or dibromo-) phenolindophenol as a titrant for the estimation of the vitamin, which was given the name ascorbic acid. The way was laid open for precise studies of the many interesting problems in the distribution, conditions of stability, and physiological requirements of the antiscorbutic substance.

The Antiricketic Vitamin.—Although cod liver oil had been recognized for a century or more as a specific remedy for the prevention or cure of rickets, the etiology of this disease remained a mystery. New ground was broken by E. Mellanby ('18, '19, '20), who induced experimental rickets in pups by feeding them diets consisting of bread or cereal, milk, linseed oil, yeast, and salt with or without orange juice. Ricketic pups were given various kinds of fats and from the response it was clearly evident that certain of these exerted remarkable effects in protecting the animals against rickets. Cod liver oil was far superior to all other fats tested. He was inclined to identify the antiricketic factor with vitamin A, but stated that the antiricketic value of certain vegetable fats, e.g. peanut oil, was greater than could be accounted for on the basis of its vitamin A content. Mellanby established the fact that there exists an antiricketic substance.

McCollum, Simmonds, Parsons, Shipley and Park ('21) undertook in 1918 an elaborate study of the characteristics of the diet which would cause the development of acute rickets. In 1920 they published a description of 5 diets, one of which contained cod liver oil, and described the histological appearance of the bones of young rats restricted to these regimens. They, as well as Sherman and Pappenheimer ('21), emphasized the importance of the ratios between calcium and phosphorus in the experimental production of rickets. The Baltimore group also emphasized the role of certain fats in rickets-prevention. More than 300 diet formulae were employed and histological studies were made on the bones of about 2500 rats. It was concluded that diets deficient in calcium but rich in phosphate, in the absence of certain fats or of sunlight, caused the rapid development of rickets complicated with tetany. Diets deficient in phosphate but rich in calcium, in the absence of certain fats or sunlight, caused rickets uncomplicated with tetany. In the prevention of rickets cod liver oil was the most potent of the fats tested. Butter fat, when fed to the extent of 20 per cent of the diet, exerted some protection as did a sample of coconut oil. The coconut oil was devoid of vitamin A since rats given this fat, in an otherwise A-free diet, developed xerophthalmia. Hopkins had shown that blowing air through heated butter fat destroyed vitamin A. The Baltimore Group of investigators (McCollum et al., '22) destroyed the vitamin A of cod liver oil by this means and observed that its antiricketic value was still retained, thus demonstrating that a second fat-soluble vitamin existed, which they named vitamin D. Rats kept on rickets-producing diets, they observed, did not develop rickets when exposed daily to sunlight, a confirmation of the observation of Huldschinsky ('19). Schmorl ('09) had described in detail the reformation of the provisional zone of calcification on the epiphyseal side of the metaphysis, which is the

first sign of healing of rickets in children. On the basis of this change McCollum, Simmonds, Shipley and Park ('22) described a delicate biological test for calcium-depositing substances, the so-called "line test" which still remains the most satisfactory method for assay of the antiricketic substances.

Relation of Vitamin D to Ultraviolet Rays.—The seasonal incidence of rickets and its geographical distribution had for years supported a vague opinion that rickets was in some way associated with sunshine, fresh air, and other hygienic factors in the child's environment. Huldschinsky (l.c.), working with German children during and after the World War, was the first to establish the fact that ultraviolet irradiation constituted a specific cure for rickets. For a time there appeared to be no way of reconciling the apparent contradiction of the existence of a specific antiricketic dietary factor as well as a specific antiricketic action of ultraviolet rays. The problem was solved simultaneously and independently by Hess and Weinstock ('24) of New York, and Steenbock and Black ('24) of Wisconsin, who announced in 1924 that antiricketic potency could be developed in various foodstuffs by exposure to the rays of a mercury vapor quartz or carbon arc lamp. Using the "line test," and the content of ash in the bones as criteria, it was soon demonstrated that purified protein, carbohydrates, fats, and mineral elements do not acquire antiricketic potency on irradiation. Cholesterol, and other sterols from plants, did show this property. Hess and Windaus ('26-27) reported that if cholesterol is purified by the formation of the dibromide, and the cholesterol is regenerated, the purified substance cannot be activated by irradiation. This pointed to an impurity in cholesterol as the provitamin D. Rosenheim and Webster ('26) compared the absorption spectrum of cholesterol purified by repeated crystallizations, with that which had been purified through the dibromide, and observed that certain lines were absent in the spectrum of the latter. They announced that the "impurity" in ordinary cholesterol, after many crystallizations, was ergosterol, a sterol abundant in oil of ergot. This sterol had been isolated and studied by Tanret. Ergosterol, after irradiation, protects rats against rickets when administered in doses equivalent to 0.0001 milligram daily. The presence of a provitamin D in the skin accounts for the cure of rickets by sunlight.

The B-Complex.—It was assumed for years that extracts containing "water-soluble B," later called vitamin B, owed their dietary significance to a single substance, the antineuritic factor. Smith and Hendrick ('26) repeated the experiment of McCollum and Davis of supplementing rolled oats with casein, butter fat, and a calcium salt. The latter workers had exhibited growth curves which were far below normal, showing that the supplementation by these additions was not

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complete. Smith and Hendrick demonstrated that the addition of yeast, or yeast extracts containing very little protein, exerted a remarkable improvement in growth of rats on the oat diet. Since yeast or yeast extracts which had been heated sufficiently to destroy the antineuritic vitamin were as effective in improving the condition of the animals as the unheated material, it was evident that the improvement of the diet was not due to supplementation with any recognized nutrient, but to a heat-stable factor distinct from the antineuritic substance. Goldberger (Goldberger and Wheeler '27) was led to believe, by his studies on human pellagrins and on dogs suffering from "blacktongue," a condition supposed to be the analogue of pellagra in man, that the heat-stable water-soluble vitamin of Smith and Hendrick was the antipellagra vitamin. There is still reason to believe that his surmise was correct, although it is now known that there are contained in such extracts, as were then used, several indispensable nutrients, one or more of which are concerned with the etiology of pellegra. To the heat-stable substance he gave the name P-P factor (pellagra-preventive). This substance now appears to be nicotinic acid. In England the factor became known as vitamin B_2, the antineuritic factor being called B_1. In America it was the custom to speak of the antipellagra factor as vitamin G, with occasional employment of the term P-P factor. The situation was, however, much more complicated than workers of that time realized.

Kuhn and coworkers ('33), while attempting to concentrate vitamin B_2, isolated from whey an orange-brown dye, now known as riboflavin. This substance was announced as representing the most active preparation of B_2 yet obtained, and probably identical with it. It has been shown that this flavin, which in solution gives a strong greenish fluorescence, is an essential nutrient. It appears to have been invariably a constituent of extracts containing the B-complex of vitamins. It is now known, however, that this dye is not the antipellagra vitamin and does not prevent the dermatosis produced by certain faulty diets in rats or chicks, which were formerly believed to be the analogues of pellagra in man. It was, therefore, misnamed vitamin B_2 or G.

Rat and Chick "Pellagra."—Several dietary regimens were known which induced in rats a dermatosis that was believed to be "rat pellagra." It remained for Birch, György and Harris ('35) to show that the inclusion of maize in these diets prevented or cured the condition. Human pellagra has been associated for many years with excessive maize eating, so it is evident that this cereal cannot have a preventive action in human pellagra. Molasses, which is known to be deficient in the antipellagra substance, is rich in the factor which cures the so-called rat pellagra. The disorders in man and the rat are,

therefore, distinct entities. Birch and coworkers designate the substance as B_6, a lack of which causes the characteristic dermatosis in the rat.

Ringrose, Norris and Heuser ('31) first observed a pellagra-like syndrome in chicks. The preventive factor, they reported, is distinct from the antipellagra factor. Lepkovsky and Jukes ('35) found that the dermatosis of dietary origin in chicks is due to deficiency of an entirely different factor (filtrate factor). These authors ('36) state, however, that deficiency of riboflavin causes in rats a dermatosis which is distinctly different from that described by Birch and coworkers as due to lack of B_6.

Dam and Schønheyder ('34) and Almquist and Stokstad ('35) described a deficiency syndrome in chicks which is characterized by subcutaneous, intramuscular and abdominal hemorrhage and by anemia, due to lack of the fat-soluble factor hitherto unknown. They designate this factor vitamin K. Administration of ascorbic acid does not relieve the condition; hence the hemorrhage is not due to a scorbutic state.

There is also a nutritional deficiency disease of chicks described by Pappenheimer and Goettsch ('34) as nutritional encephalomalacia, caused by lack of a fat-soluble factor which is present in various fats and oils. They have made a 20-fold concentration of the curative substance by dissolving a fraction of soy bean oil in 95 per cent alcohol.

These recent studies on the nutrition of the chick reveal that there are hitherto unsuspected differences in the nutritive requirements of the mammal and the chick. This is further illustrated by the fact that certain forms of vitamin D, which are highly potent in the prevention of rickets in the rat, are of little value for the chick.

Vitamin E.—A striking result of nutrition researches was the demonstration by Evans and Bishop ('22) that laboratory animals (rats, mice) could be successfully reared on certain simplified diets of proteins, carbohydrates, fats, mineral salts, and crude vitamins preparations, and though apparently healthy adulthood was reached, sooner or later such animals exhibited lack of reproductive ability. Their experiments showed that very small additions of certain kinds of foods, especially wheat germ or wheat germ oil, would prevent the loss of reproductive power. After carefully excluding all known nutrients as responsible for the disability, they announced the existence of a new 5th member of the vitamin class which they designated vitamin E. The early phases of the reproductive process in the female do not require vitamin E. When a curative dose of vitamin E is administered as late as the 5th day after insemination, a few hours before implantation of the blastodermic vesicles, the normal course of preg-

nancy is assured. In the absence of this vitamin, the fetus dies after a few days and is resorbed. Neither the ovaries nor ova are affected in this deficiency.

In the male, deficiency of vitamin E causes sterility due to degenerative changes in the germinal epithelium, which resembles those seen in the male deprived of vitamin A. The latter, however, is reparable but the former is irreparable. Not only vitamin E but each of the other well-known vitamins, exerts its own special influence upon reproduction.

Indispensable Unsaturated Fatty Acids.—Although it was learned early in the era of modern nutrition that the body can readily synthesize fat from certain nonlipid substances it was only within the present decade that the indispensability of certain types of unsaturated fatty acids became fully demonstrated. As shown by Burr and coworkers (Burr and Burr, '30) and by Evans and associates (Turpeinen, '38) rats cannot be reared in good health unless the diet contains linoleic acid, linolenic acid or arachidonic acid. These compounds appear to be mutually replaceable. That is, it is only necessary that the diet contain one or more of them. The significance of indispensable fatty acids is obscure as yet.

The Inorganic Elements in Nutrition.—That the body requires calcium and phosporus for the construction of bones was never questioned by physiologists. The appetite for sodium chloride and the richness of body fluids in this compound, the abundance of potassium in all the tissues, the occurrence of iron as a characteristic element in hemoglobin and the presence of sulfur as a constituent of proteins, constituted sufficient evidence that these elements were all essential to life. Magnesium, although relatively abundant in the tissues, played, if any, an unknown role in physiological processes until the present decade when its indispensability was clearly demonstrated by McCollum and Orent ('31). They prepared a diet which was exceedingly low in magnesium. When young rats are restricted to this food they pass successfully through stages of vasodilatation, hyperirritability, trophic disturbances, and fatal tonic-clonic convulsions. The content of magnesium in the blood falls to a low level. This deficiency has been observed in farm animals in several instances.

Iodine was first used in the treatment of goiter by Coindet, who painted goiters with tincture of iodine in 1820. Boussingault, in 1825, wrote "Until now iodine is the only specific known for goiter." In 1895 Baumann (1895) discovered that the thyroid gland contains iodine in amounts easily distinguishing it from other organs. Marine and Williams ('08) showed that deficiency of iodine results in characteristic histological changes in the thyroid gland. In 1916, Kendall ('19) isolated the iodine-containing hormone, thyroxin, from thyroid

glands. In 1917, Marine and Kimball ('17) demonstrated on school children the prophylactic value of iodine administered as sodium iodide in Ohio, where simple goiter is endemic. The prevention of goiter in man and in farm animals by the provision of iodine is now fully established.

Copper had been known as a constituent of the blood of snails since the study of hemocyanin by Harless (1847), but it remained for Hart and his coworkers ('25) to demonstrate that in experimental anemia produced in rats by restricting them to a diet of milk, the administration of pure iron is not effective in causing restoration of the hemoglobin content of the blood. A small addition of copper with iron resulted in prompt alleviation of the anemia. Copper is, therefore, an indispensable element in the diet.

The nutritional role of manganese has been demonstrated by means of diets very low in their manganese content. Studies with rats and mice have shown that a deficiency of this element in females, results in deficiency of lactation and loss of maternal solicitude for the young, and in male rats it induces complete degeneration of the germinal epithelium (Orent and McCollum '31). It produces congenital debility in second generation young (Daniels and Everson, '35). A definite relationship exists between manganese deficiency and perosis ("slipped tendon") in chicks (Wilgus and associates, '37).

The significance of zinc in nutrition has not yet been definitely established. Certain observations (Hubbell and Mendel, '27; Todd, Elvehjem and Hart, '34) suggest that zinc in the diet of animals stimulates growth and plays a role in the development of a normal coat of hair. Of interest are the findings that insulin, even in purest crystalline form contains zinc (Scott, '34). The indispensability of cobalt in the nutrition of sheep and cattle has been demonstrated (Marston, '35).

Present Concept of Nutrition.—In this brief review of some outstanding discoveries in the field of nutrition we have indicated that in recent decades the principal emphasis has been on the discovery of indispensable nutrients, or the primary components of an adequate diet. We now regard an adequate diet as composed qualitatively of many chemically discrete components provided in such states of combination that they are utilized efficiently. Moreover, the adequate diet must contain these essentials in approximately the proportions required by the body in order to promote optimum efficiency and the prolongation of physiological well-being. Also, the adequate diet must contain a minimum of injurious factors, e.g., selenium and fluorine. Since the necessary experimental procedures are well-defined it appears probable that the next one or two decades will witness an essentially complete solution of the present major nutritional problem.

This is the discovery of all the indispensable nutrients for man and some of the domestic animals. But the field of modern nutrition covers more than qualitative aspects. In addition it is concerned with the determination of the requirements for all nutrients and factors which affect their utilization. Moreover, it involves the study of interconversion and intermediary metabolism of foodstuffs, the chemical reactions concerned with the exchange of energy, and the economical production of nutrients in available form for man and animals. The present state of the newer knowledge of nutrition requires that this book shall be mainly devoted to discussions of specific nutrients as concerns their chemical nature, physiological roles, requirements in the maintenance of health, and the effects produced by their deficiencies. It is this concept of the present problem of nutrition that will determine the scope of the subject matter in the chapters to follow.

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CHAPTER

III

Carbohydrates in Nutrition

THE OUTSTANDING importance of sugar metabolism as a source of energy in the animal body, and great commercial importance of the fermentation industries, make the chemical processes brought about in biological transformation of the sugars one of the attractive fields for investigation by biochemists. This interest is heightened because of the frequency of perversion of sugar metabolism, as for example, diabetes in man. The result is an enormous output of papers dealing with a great variety of aspects of carbohydrate metabolism. No adequate discussion of this general subject is within the province of this book, but a selection of certain of the more interesting newly-discovered facts has been included because they are so fundamental to an understanding of physiological well-being as it is influenced by carbohydrate utilization.

Rate of Carbohydrates Absorption.—Cori and Cori ('28) state that different sugars are not absorbed at the same rate. Thus if the rate for glucose is taken as 100, the rate for galactose, fructose, mannose, xylose, and arabinose are, in the order given: 110, 43, 19, 15 and 9. All of these sugars, when introduced into the peritoneal cavity, are absorbed at the same rate. This shows that the intestine exerts a highly selective action on carbohydrates and that this action is not shared by another membrane, i. e., the one lining the peritoneum. When two sugars are in solution together in the intestine and are absorbed simultaneously, the rate for each is reduced. Thus when glucose and galactose are fed in mixture, the total amount of sugar absorbed is not larger than when each sugar is fed separately. This rule was found by Cori ('26) to hold true not only when two sugars were fed together, but for two amino acids, or one sugar and one amino acid. The rate of absorption of sugar is not increased, he states,

when increased amounts are ingested. Only a limited number of molecules seem to be able to pass through the intestinal mucosa in a given time. This does not hold for alcohol, which is absorbed the more rapidly the higher the concentration in the intestine. It seems probable, however, that the amount of sugar absorbed in a given time would be much greater when a larger volume of a sugar solution of a given concentration is taken, than in the case when a smaller volume is taken, since it would spread over a larger area of mucosa, each area absorbing at the normal rate. This assumption seems necessary since earlier observations demonstrated that different individuals showed decided differences in the concentration of glucose in the blood when 100 gm. of this sugar were taken in solution under comparable conditions. Groen ('37) studying carbohydrate absorption in man, found that each sugar has its individual absorption rate.

Intolerance for Carbohydrate after Fasting.—Goldblatt and Ellis ('32) state that after a period of some 40 hours of complete starvation in human subjects, the administration of a dose of glucose, normally easily tolerated, was followed by high and prolonged elevation of blood sugar curves, glycosuria, and a smaller rise in the R. Q. than that occurring in ordinary post-absorptive conditions. This intolerance, they find, is not due to either ketosis or acidosis. It is in line with the observation of Dann and Chambers ('30, '32) that in dogs, after a period of starvation, insulin does not completely reestablish normal carbohydrate oxidation. Goldblatt and Ellis ('32 l.c.) express the view that in the starving organism there is a temporary dissociation of the two mechanisms initiated by insulin, viz., the formation of peripheral glycogen, and the oxidaton of carbohydrates, the second effect being manifest only when the first has attained adequate proportions. But in view of the specific role of thiamin in carbohydrate metabolism (Chapters XVIII and XIX) it is plausible to suppose that in these experiments the subjects were suffering some degree of thiamin deficiency.

Glucose, Galactose, and Lactose as Glycogen Formers.—Deuel, MacKay, Jewel, Gulick and Grunewald ('33) found that when glucose was administered to female dogs previously fasted for 6 days the accumulation of glycogen was greater 6 hours after ingestion than in animals which retained a corresponding amount of galactose. However, the liver glycogen in animals killed after 12 and 72 hours after sugar feeding was higher in dogs which had taken galactose than in those which had taken glucose. After an interval of 6 and 12 hours from feeding, the muscle glycogen was higher in the glucose-fed dogs. In a long series of tests with rats, it was found that when the animals had been fed diets high in galactose, lactose, or galactose-glucose for 7 to 12 days, the liver and muscle glycogen were almost invariably

higher after periods of fasting up to 72 hours than they were when animals were fed a similar diet containing glucose only as the carbohydrate. They suggest that the glycogen formed from galactose is retained longer than that formed from glucose, although in experiments on dogs it appears that glycogen is formed faster from glucose. These results afford an explanation for the superior ketolytic effect which the authors had previously reported for galactose in human subjects as well as in fasting rats in which a mild ketosis is artificially induced by the oral or subcutaneous administration of diacetic acid. Feeding rats a high-galactose diet over a period of 3 months did not increase the well-known low tolerance for galactose above that of rats kept for the same period on a glucose-containing diet.

Bell ('35, '36) applied the method of "end-group assay" devised by Haworth and Percival ('32) to the glycogen from livers of rabbits which had been fed galactose. The "galactose glycogen" molecule consists of a chain of 18 glucose units whereas the glycogen from normally-fed rabbits which is derived from glucose consists of 12 glucose units. Young ('36), using the methods of Bell and Young ('34., found that the liver and muscle glycogen were identical. The specific rotatory power of glycogen from the two sources was likewise essentially identical. The manner of feeding the rabbits from which the glycogen was obtained was not stated, but it would appear that the glycogen from both of these sources was derived from glucose.

Deuel, MacKay and Gulick ('32) studied the quantitative relationships in glycogen storage in rats' livers and muscles upon feeding glucose, galactose, and lactose respectively, following fasting periods of 24, 33, 48, and 54 hours. The glycogen content of both tissues of galactose-fed rats far exceeded those of glucose-fed animals at the end of these fasts. Rats fed galactose after previous fasting periods had higher values for liver glycogen if they had been previously fed galactose than if they had been previously fed glucose. Lactose feeding gave intermediate values as compared with glucose and galactose. It appears, therefore, that notwithstanding the low tolerance of the body for galactose, and the tendency for this sugar to pass through the kidneys, it is a good glycogen former. The glycogen formed from it persists much more during fasting than is the case when glucose is fed.

Dann and Chambers ('30 l.c.) showed that a complete, though temporary, loss of the ability to burn glucose occurs in dogs after a fast of three weeks or more, with frequent exercise on the treadmill. During the first four hours after 50 gm. of glucose were ingested, the blood sugar rose to a high level and a part of the sugar was excreted in the urine. However, at least 30 gm. could not be accounted for either by excretion or oxidation. In a later study ('32 l.c.)

they determined the glycogen, glucose, and lactic acid in portions of liver and muscles of dogs subjected to fast and then given glucose. They found that the power to form glycogen was not seriously impaired after a fast sufficiently long to suppress almost all sugar oxidation. Absorption of 25 gm. of glucose from the alimentary tract was practically complete within 4 hours in these animals. MacKay and Bergmann ('33) conclude from similar studies on the rat that the variations in blood glucose tolerance curves caused by feeding glucose after 24-48 hours' fasting are due, in large part, if not entirely, to changes in the rate of glucose uptake by tissues other than the liver. These changes, they believe, are probably produced by impairment of their capacity to oxidize glucose. Here, also, the possibility should be considered that the animals were suffering some degree of thiamin deficiency.

Murlin and coworkers ('36) found that in human subjects, the ketosis produced by taking high fat diets was greatly reduced but never quite extinguished within 3 to 4 hours by feeding sugars. Sucrose is somewhat more effective in doses up to 60 gm.; glucose is more effective in doses of 100 gm. Fructose stands between the other two. Although the quantity of carbohydrate oxidized by these subjects was more than sufficient, judging by the Shaffer theory, which maintains that the combustion of one molecule of glucose will cause the combustion of two molecules of aceto-acetic acid, the ketosis was not completely controlled. In only one of nine subjects was the change in ketogenesis in exact agreement with the increased combustion of carbohydrate. Glycogen formation seems to be as important as combustion in the antiketogenic action of sugar. These investigators suggest that the presence of a certain minimal "normal" content of glycogen in the liver is a condition precedent to the normal oxidation of fatty acids. There is somewhat more evidence, however, that this normal content of glycogen prevents the formation of ketone substances in the liver.

Deuel, Gulick and Butts ('32) insist that galactose is markedly superior to glucose in preventing the formation of ketone acids, which result from fatty acid oxidation when oxidation of sugar is impaired. This is surprising in view of the well-known fact that galactose is more difficult to metabolize than glucose, sucrose, or fructose, and its ingestion is attended by greater tendency to hyperglycemia and glycuresis than is the case with the other sugars. Goldblatt ('25), working with human subjects subjected to a 36-hour fast of both food and water, concluded that glucose, fructose, sucrose, and maltose were ketolytic, but that galactose, mannose, lactose, and glycerol exerted no such effects. Deuel and coworkers question whether his tests for acetone bodies, which were qualitative rather than quantitative, af-

forded sufficient data; they also question whether his method of preparing his subjects for study gave a sufficiently uniform level of ketosis from which to start his experiments.

The Conversion of Carbohydrate into Fat.—Wesson ('27) and Wesson and Murrell ('33, '34) have reported the results of studies which appear to show that in the absence of an unidentified dietary factor there is, in rats, an abnormally rapid conversion of carbohydrate into fat. They restricted rats for 2 or 3 weeks to a diet deficient in fats and very rich in carbohydrate. In some experiments the animals were restricted to dextrin, calcium carbonate, and water. These rats were then given a definite amount of dextrin and their respiratory quotients measured. When carbohydrate alone is being metabolized the R. Q., $\frac{\text{vol. of } CO_2}{\text{vol. of } O_2}$, is I. It is well known that when carbohydrate is being converted into fat an oxygen-rich substance is being converted into an oxygen-poor one, and the R. Q. is greater than 1. They found respiratory quotients in their rats which had been long restricted to a fat-free regimen to be from 1.5 to as high as 2, which indicated that they were converting carbohydrate into fat at an abnormally high rate. The administration of small daily amounts (6.67 mg.) of either the liquid or solid fraction of lard prevented the metabolic abnormality although the stunting and emaciation were of about the same degree as in the control animals on the fat-free regimen. The liquid fraction was more than ten times as active as the solid fraction. This abnormal fat formation is not brought about by reason of a need for fat for catabolism but because of deficiency of some metabolic factor which is supplied by certain fats. Ethyl stearate was inactive. They were unable to secure the effects mentioned with any known vitamin or any of the essential amino acids. Lineoleic acid was not effective even when fed in large amounts and over long periods. The fatty acid fraction obtained from saponified lard was active, hence the hypothetical substance, in the absence of which abnormal fat formation takes place, is stable to saponifying agents.

Wesson and Murrell ('33 l.c., '34 l.c.) have extended their studies to include restriction of rats to (1) sucrose, (2) maltose, (3) lactose, (4) d-glucose, (5) d-fructose, (6) d-galactose, (7) d-mannose, (8) l-arabinose, (9) d-xylose. The sugars containing d-glucose (1-4) and d-xylose (9) gave R. Q. curves appreciably higher in the abnormal rats than in the normal ones. The remaining carbohydrates (5-8) gave values practically the same for both abnormal and normal rats.

Pentose Metabolism.—It has long been known that the tolerance of humans and of mammals, generally, for the pentose sugars is very low. Since the pentosans, which are very abundant in many vegetable foods, are indigestible in the alimentary tracts of the Omnivora and

Carnivora, and since pentose sugars occur in natural foods in but small amounts, these carbohydrates have been of little importance in the nutrition of man. Miller and Lewis ('32) call attention to the fact that in 1930 the U. S. Bureau of Standards announced a simple method for the preparation of xylose from cottonseed hull bran, which is cheap and abundant. Xylose is now obtainable in large amounts and at a price comparable with that of sucrose. The commercial possibilities of exploiting xylose did not escape the notice of the unscrupulous, who have brought it to the attention of the public as a "slenderizing sugar from cottonseed," and as a "non-fattening sugar." Miller and Lewis, as a result of their new studies on the nutritive value of pentose sugars, conclude that although xylose is absorbed fairly readily from the digestive tract, no evidence could be secured that it is at all utilized in the nutrition of the rat. Rhamnose, a-methyl pentose, is absorbed more rapidly than is d-xylose, but it is not utilized by the body.

Role of the Hypophysis.—The hypophysis has been shown to exert a regulatory action on the blood sugar and on glycogen formation. Thus, Russell ('36) found that hypophysectomized rats, when well-fed show normal levels for blood sugar, and liver and muscle glycogen. When fasted, these levels fall extremely rapidly as compared with normal animals. The figures in the following table represent the decrease in carbohydrate content as per cent of the average levels found in well-fed animals.

DECREASE IN CARBOHYDRATE LEVELS DURING FASTING OF NORMAL (N) AND HYPOPHYSECTOMIZED (H) RATS

	8-HOUR FAST		18-HOUR FAST	
	N	H	N	H
Liver glycogen	27	95	96	99
Muscle glycogen	8	24	6	41
Blood glycogen	20	49	32	54

Rats used as controls, which were subjected to operation in which one-third or more of the anterior lobe of the hypophysis remained, including several with various degree of brain injury, did not differ from the normal and were not included in the above table. In operated rats, after fasting, the feeding of starch causes a restoration of muscle glycogen and blood glucose to normal, but liver glycogen values increase much more slowly than in normal animals. The rates of digestion and absorption of ingested carbohydrate were shown to

be about 30 per cent below normal in the operated rats. Fisher and Pencharz ('36) found that a larger proportion of absorbed carbohydrate is oxidized by hypophysectomized than by normal rats following glucose feeding. This may account for the decreased amounts stored as glycogen in the liver.

Russell and Bennett ('36) found that intraperitoneal injections of hypophyseal extracts were very effective in preventing the rapid falling off in the glycogen stores of well-fed rats during early fasting. They tested an extract containing all the known hormones of the hypophysis, two extracts that were rich in the growth hormone and one which had a high content of the adrenotrophic hormone and containing a fairly large amount of growth hormone. All were effective. They used preparations from which the gonadotropic and mammotropic hormones had been removed and found these still effective in protecting the glycogen stores. One active preparation appeared to contain no thyrotropic hormone. It appears that these hormones may be ruled out in connection with the effect of the hypophysis on glycogen stores. Working with adrenalectomized rats these authors seem to have excluded the possibility that the hypophyseal extracts exert their influence through the adrenal cortex. Boiled preparations were without effect. Russell and Bennett state that muscle glycogen is the store principally affected. It has been shown by Fisher, Russell and Cori ('36) that rats in the hypophysectomized state, which were losing muscle glycogen had correspondingly higher respiratory quotients than normal animals. Treatment with the same type of extracts as were used by Russell and Bennett, which diminished the loss of muscle glycogen, restored the respiratory quotients to the levels observed in normal rats. The hypophysectomized rats excreted the same amount of nitrogen during the experimental period as the normal animals. It is concluded that hypophysectomized rats suffer a defect in the mechanism by which normally carbohydrate oxidation is depressed, and carbohydrate levels are thereby maintained during fasting, and that anterior lobe extracts are able to restore this function, apparently by exerting a depressing effect on carbohydrate oxidation.

Role of the Adrenal Cortex.—The adrenal cortex is also intimately associated with the regulation of the storage of glycogen in the tissues. But whereas the hypophysis is shown to exercise a regulatory function on the rate of oxidation of carbohydrate, the effect of the adrenal cortex seems to be largely concerned with synthesis of glycogen, a function which is lost in the adrenalectomized animal. Evans ('36) showed that fasting adrenalectomized rats failed to respond to lowered atmospheric pressure with increased deposition of glycogen in the tissues as is the case in normal animals. This has been traced in the adrenalectomized rat to failure to metabolize addi-

tional protein under reduced atmospheric pressure. The normal rat, when placed under one-half an atmospheric pressure, excretes an excessive amount of nitrogen which is derived from tissue destruction. The adrenalectomized rat does not do so. On the average, about 58 per cent of the carbon of protein can be converted into glucose and is so converted when there is a carbohydrate deficit. In phlorhizinized animals whose renal epithelium is altered so as to reduce the threshold of sugar elimination to zero so that sugar leaks away rapidly, the glucose and nitrogen excretion are profoundly reduced. This situation does not arise when the medulla of the adrenal alone is extirpated, hence the effect is referred to the cortex. Evans was unable to restore normal protein-metabolizing power to adrenalectomized rats by administration of extracts of adrenal cortex.

Buell, Anderson and Strauss ('36) have observed that in adrenalectomized rats liver glycogen formation from d-lactic acid is greatly reduced. Administration of the active principle of the adrenal cortex in the form of the charcoal adsorbate of Grollman, Firor and Grollman restored the power of forming glycogen from this acid. Butts et al. ('35-36) demonstrated that normal animals can readily form glycogen from d-alanine, and Samuels, Butts, Schott and Ball ('36-37) have shown that this amino acid is not well utilized for glycogen formation in adrenalectomized rats. It seems not to have been determined whether this failure is due to inability to convert alanine to sugar, but this is to be inferred since Samuels and coworkers found low blood sugar values. Butts has demonstrated that d-alanine, but not the l-isomer, is an excellent glycogen-forming substance. Barnes and Regen ('34) have found that dogs made hypersensitive to insulin by denervation of the adrenals or by hypophysectomy can be protected against two to four times the convulsion-producing dose of insulin by a simultaneous injection of epinephrine at a constant rate.

Sex Variation in Carbohydrate Metabolism.—Deuel and Gulick ('32) and Deuel and coworkers ('33) have called attention to the fact that normal women excrete much larger quantities of acetone bodies during fasting than do normal men, and they have also shown that similar discrepancies occur between male and female guinea pigs and rats during inanition when these animals are fed sodium acetoacetate. The characteristic behavior in this respect suggests that there must be a fundamental difference in the carbohydrate metabolism of the sexes. Greisheimer ('31) first pointed out that when fed various diets male rats had higher liver glycogen and lower liver fat content than did female animals on similar diets. Others have provided analytical data which confirm this fact. Stohr ('32) confirmed these findings with young, but not with old rats of unstated ages. Deuel and coworkers ('32, l.c.; '33, l.c.) have found that the liver glycogen

was significantly higher in male than in female rats 24, 48, and 72 hours after feeding glucose. The liver fat was higher in female than in male rats during the entire fasting period. When rats were fed a high carbohydrate diet and were killed without fasting there was no difference in the glycogen content of the livers of the sexes. The liver glycogen of female guinea pigs was higher after 48 hours' fasting than in males, but the liver fat content (lipid) in males and females corresponded with the relations found in rats. Deuel points out that the experiments on guinea pigs were made on animals of undetermined ages. The amount of diacetic acid which is oxidized in fasting male rats is considerably higher than that utilized by female rats when it is administered orally.

Gulick, Samuels and Deuel ('34) found double the amount of glycogen in the livers of ovariectomized rats as compared with normal females 48 hours after feeding comparable amounts of glucose based on estimated surface area. When theelin was administered to ovariectomized rats, lower values were obtained from them than from uninjected controls. They do not, however, believe that the sex difference in carbohydrate metabolism is solely dependent on the production of theelin.

Recent observations, therefore, demonstrate clearly that not only the pancreas, through its production of insulin, influences carbohydrate utilization, but that this is influenced by the hypophysis, adrenals, and sex glands as well. The influence of the thyroid gland on metabolic rate is, of course, well known.

For years it has been a matter of controversy whether the vagus nerve controls the activity of the pancreas in the secretion of insulin, or whether the response of that organ is regulated by the concentration of sugar in the blood. The discussion still continues, but it now seems to be fully established that the blood sugar level is the normal regulating mechanism. Gayet and Guillaumie ('27, '33) transplanted the pancreas of dogs to the neck by means of vascular anastomoses. Stimulation of the vagus nerve caused copious external pancreatic secretion, but there was no lowering or other change in the blood sugar. Injection of hyperglycemic blood into the artery of the transplanted pancreas caused a precipitous drop in the blood sugar. Kosaka ('33) showed by infusing a glucose solution into the pancreatoduodenal artery, the femoral artery, and the portal vein, that only in the first case was there a pronounced drop in the blood sugar. This appears to prove that the pancreas responds to an increased blood sugar concentration in its arterial blood with increased liberation of insulin.

The Nutritive Value of Carbohydrates.—Following the ingestion of starch there is almost as rapid a rise in blood sugar as when

glucose is taken, hence starch must be hydrolyzed to glucose very rapidly. Quite characteristically, however, there is no rise in the blood sugar content after taking even considerable amounts of milk sugar or lactose. The questions arising from these observations are: Is failure of lactose to elevate the blood sugar due to delayed absorption dependent upon slow hydrolysis, or to the rapid passage through the alimentary tract with consequent slow absorption? The fact is well established that a considerable amount of lactose may reach the colon, and because of its presence there lead to the establishment of *L. acidophilus* as the prevailing flora, and consequent depression of proteolytic types of bacteria. Lusk ('15) showed that lactose in the dog failed to produce the specific dynamic effect shown by glucose or sucrose and failed to materially increase the respiratory quotient. It has long been known that lactose injected intravenously or subcutaneously is excreted in the urine unchanged.

Deuel and Chambers ('25) found that in the phlorhizinized dog, after feeding lactose, about 50 per cent appeared in the urine as glucose. The rat appears to be more capable of utilizing lactose than is the dog. Greisheimer and Johnson ('30) demonstrated that glycogen is formed from lactose in the rat, but after 16 to 18 days on diets deriving 87.5 per cent of their caloric value from sugar, the liver glycogen amounted to 62 per cent of that found in animals fed glucose or sucrose. H. S. Mitchell ('27) found that growth on diets containing 30 per cent lactose was only about 50 per cent as great as that of rats fed other carbohydrates in comparable diets. She found that the amount of carbohydrate lost in the feces of these rats was only 3.12 per cent greater in the lactose animals than in those fed sucrose. Both the average food intake and voluntary activity decreased in the lactose period as compared with the glucose period, hence the decreased rate of growth was not due to expenditure of energy in work. The possibility that increased hydration of the tissues entered into the differences between lactose- and glucose-fed rats was ruled out by experiment. Koehler and Allen ('34) conclude from their experiments that of ingested lactose, approximately 40 to 50 per cent may be lost to the rat as far as weight or energy relationships are concerned. A part of the ingested lactose is converted through fermentation by *L. acidophilus* organisms in the gastrointestinal tract into lactic acid, but this when absorbed behaves like carbohydrate in metabolism. Subsequently it will be pointed out that the increased acidity in the intestine caused by the ingestion of lactose favors the absorption of calcium. In chick feeding it is generally believed that lactose in the digestive tract is effective, presumably because of increasing the acidity, in holding in check the development of coccidiosis.

H. S. Mitchell's ('27, l.c.) results on growth of feeding lactose

have not been confirmed by others. Thus Whittier, Cary and Ellis ('34) compared lactose fed to young rats at the level of 45 per cent of the diet, with sucrose and dextrin fed in similar amounts. They observed that during the early period rats given lactose increased in weight much faster than did those receiving sucrose. After reaching adult age the sucrose-fed rats became heavier than the lactose-fed animals. The former consumed more food than did the latter. The differences in weight of the adults was accounted for on the basis of the greater amount of fat in the bodies of the sucrose-fed rats. These results were confirmed with swine which accumulated fat faster when fed sucrose than when fed lactose. The lactose-fed rats lived longer than did those on the sucrose diet. Feeding excessive quantities of lactose to rats caused diarrhea for a short time; this retarded growth, but this disturbance was soon overcome. They conclude that the characteristic results obtained in their experiments were not the result of stimulation of acidophilic organisms in the lower intestine since dextrin accomplishes this result; but it does not have the same influence on growth as does lactose. These results are in harmony with the view expressed by Jarvis ('30) that "age-weight for age-weight, the lactose-fed infant possesses more living tissue than does the infant fed on vegetable sugar."

Feyder ('35) studied the nutritive value of dietary sucrose and glucose. Under the conditions of his experiment the sucrose diet resulted in more rapid gains in weight than the glucose diet due to a more rapid deposition of fat and also a markedly greater deposition of protein. The glycogen formation was practically the same in the two sets of animals.

H. H. Mitchell ('37) compared the value of glucose and sucrose, glucose and fructose, and glucose and lactose in the diet. He found that in case of sucrose, fructose, and lactose intake, the digestibility of the organic nutrients of the diets became impaired, being greater on the lactose, intermediate on the fructose, and least on the sucrose-containing diets. Growth was affected depending on the degree of impairment. The metabolic utilization of calcium was increased by sucrose, fructose, and lactose. Furthermore, lactose definitely furthered the utilization of phosphorus.

The Effects of Lactose in the Diet.—Dragstedt and Peacock ('23) were the first to call attention to the fact that the inclusion in the diet of 50 to 100 gm. of lactose daily would prevent the onset of tetany in thyroparathyroidectomized dogs. Feeding lactose has been shown to modify the intestinal flora toward the aciduric rather than the proteolytic type. Dextrin has been reported to possess this property in some degree whereas other carbohydrates seem to be without influence on the intestinal flora. Hudson and Parr ('24) found that

lactose, in contrast with other carbohydrates, produces an aciduric flora, and consequently an acid reaction in the intestine. The prevention of the onset of tetany in animals deprived of the parathyroid glands which exert a regulatory influence as a result of feeding lactose is ascribed to the increased facility of the intestinal mucosa owing to the acid reaction in the intestine in absorbing calcium, since, as is well known, tetany can be alleviated by the administration of soluble calcium salts.

Kline and his coworkers ('32) observed that 40 per cent of lactose included in the ration of chicks fed a rickets-producing diet had a favorable effect upon calcium absorption and skeleton building. There was maintained throughout the entire length of the intestinal tract an acid concentration greater than was produced by the basal ration containing other carbohydrate. When but 20 per cent of lactose was fed, calcification was improved to a lesser extent than with 40 per cent, and with 2 to 10 per cent the effect was slight. Feeding 40 per cent of maltose, or 5 per cent of citric acid, produced no effect on the intestinal reaction or calcium absorption. Inouye ('24) states that feeding galactose exerts an influence similar to lactose feeding on the prevention of the onset of tetany in dogs.

Barenberg and Abramson ('30) studied the effect of including large amounts of milk sugar in the diet of infants. The ingestion of 12 to 15 per cent of the diet in the form of milk sugar did not cause loose stools. Infants fed 15 to 17 gm. of lactose per kilogram of weight, in health or disease, did not excrete sugar in either urine or feces. Few acidophilic organisms were found in the stools. The infants receiving large amounts of milk sugar responded with a better rate of growth than did the control group. They conclude that milk sugar is a safe carbohydrate for the modification of cow's milk for infant feeding, and that it does not have a laxative effect in infants when used even in high concentrations.

Gerstley ('30) has reviewed the history of lactose in infant feeding and notes that every text-book on infant feeding reflects a fear by pediatricians that lactose as a sugar for modifying cow's milk for infants is attended with danger. He attributes this fear to clinical experience plus uncritical acceptance of the teachings of Escherich (1886). Following the favorable reports on the use of lactose in infant feeding by Barenberg and Abramson (l.c.), Gerstley employed this sugar in his practice and is convinced that where diarrhea and intestinal fermentation arise, they are due to some other element in cow's milk than lactose. He finds that lactose added to whole boiled cow's milk does not result in diarrhea with increase in the excretion of acids when given to normal infants. The amount of various acids excreted by the intestine does not depend directly upon the amount

of lactose in the diet but on various factors which are probably concerned in its absorption from the intestine. The relation of lactose to protein in the infant's diet is of great importance. In respect to certain chemical relationships in the stool, lactose, he believes, is preferable to maltose-dextrin preparation.

Kopeloff and Cohen ('30) express similar views. They point out that the normal nursling thrives on a lactose-rich diet. Lactose is as much a food for beneficial microbes as for man. In the intestinal flora of the breast-fed baby *Bacillus bifidus* predominates. When the baby is weaned to cow's milk, *L. acidophilus* becomes the predominating microbial species. Lactose, as is well known, is an excellent source of energy for this microbe. They emphasize that lactose stimulates the acidophilus bacilli native to the individual's intestinal tract which is preferable to the ingestion of alien strains. Its slow absorption, which enables much of the ingested sugar to reach the colon, where it serves as a pabulum for *L. acidophilus,* is a valuable property. Their experimental observations show an effective transformation of the colon flora to the *L. acidophilus* type in only about 20 per cent of their cases when 100 gm. of lactose were fed daily. The ingestion of 50 gm. daily appeared to be as effective as the larger intake.

The cause of the appearance of lactose in the urine of pregnant and lactating women has been studied by Watkins ('28). He states that during the last stages of pregnancy there is a more or less constant excretion of small amounts of lactose, and that this phenomenon seems to be in no way related to the activity of the mammary glands. During the last days before delivery there is a sudden very marked rise in lactose excretion which reaches its height on the day of delivery. After delivery the lactose excretion immediately drops to a low level for 2 to 5 days. There is then a sudden and often tremendous excretion of this sugar, and during the first few weeks of lactation, the fluctuations in its excretion are very great. By the end of the first month after delivery the lactose excretion has assumed a constant and lower level and slowly approaches the normal values as lactation progresses. The tolerance of most women for lactose is, in the intermenstrual period, the same as that of normal men, the tolerance dose being about 10 gm. Menstruation causes an increase in the tolerance of women for lactose so that many women at this time show no urinary responses to the ingestion of 20 gm. of the sugar. During pregnancy the tolerance for lactose is increased, reaching in some cases three times that of non-pregnant individuals. During lactation the tolerance for this sugar is about the same as in normal men and in most women in the intermenstrual period.

Galactose in the Diet.—Galactose originates almost entirely in the lactose of the diet and is, therefore, a more prominent digestive prod-

uct in infants than in adults. Galactose is absorbed slightly more rapidly than glucose. It is only a temporary blood component. According to Cori ('25) galactose does not play a significant role as a source of liver glycogen. However, galactose may perform an important function in the processes involved in the early growth of the child. This carbohydrate is a component of the glycolipins which are structural components of nervous tissue. It is believed that the adult is independent of galactose in the diet and can synthesize this sugar at a rate adequate to replace the losses incurred through wear and tear. This may not be true of the newly-born infant. According to Mathews ('25) a rapid production of glycolipins takes place during the first six weeks of postnatal life. He suggests that the large amount of lactose in human milk may be related to these synthetic changes. If cow's milk is used as a basis for the infant's diet, the supply of galactose will be ample. However, in the infant's dietary where modification of cow's milk is used, the presence or absence of galactose may assume great importance.

Cataract in Rats Fed High Lactose and High Galactose Diets.— H. S. Mitchell and Dodge ('35) made the observation that rats quickly develop cataract when fed a diet containing 70 per cent of lactose. Rations containing less lactose caused slower development or less pronounced lens changes. Cataracts never appeared in rats fed starch, maltose, dextrin, or sucrose, respectively, as the sole source of carbohydrate at the 70 per cent level. The developing opacities give the histological picture of human cataract. Blood, urine, and tissue analysis indicated some disturbance in both carbohydrate and mineral metabolism. Galactose was found in the urine. The calcium content of cataractous eyes was double the normal, but the total and diffusible calcium of the blood was not significantly altered. The inclusion in the diet of 35 per cent of galactose likewise induces cataract in rats. This corresponds to the amount of galactose in a diet containing 70 per cent of lactose. It is, therefore, the galactose part of the lactose molecule which causes the disturbance. P. L. Day ('36) has confirmed these observations. Seventeen of 18 rats receiving 60 per cent lactose developed cataract at an average time of 44 days. Three rats receiving the same amount of galactose exhibited cataract on the 11th day. Glucose, sucrose, and starch did not cause cataract. The mean level of blood sugar in rats receiving glucose or starch was 121 mg. per 100 cc. The mean level of 89 determinations of sugar in lactose-fed rats was 160 mg.; the highest value was 234 mg. The mean of 27 determinations upon galactose-fed rats was 372 mg., the highest value being 556 mg. These results are of great interest because of the frequent occurrence of cataract in human subjects with diabetes. As yet it is undetermined whether it is caused by persistent

hyperglycemia, by lipemia, or by some other factor. P. L. Day and others have described the experimental production of cataract in rats by depriving them of riboflavin. He states that cataract caused by these two procedures is easily distinguished with the ophthalmoscope. Since in the vitamin deficiency cataract there is no elevation of blood sugar, it is still an open question whether hyperglycemia is the causative factor.

H. S. Mitchell ('36) found that rats from different colonies fed the lactose or galactose cataract-producing rations showed variable susceptibility to cataract. Young rats were more susceptible than older ones. Yudkin and Arnold ('35) have confirmed these observations and pointed out that in young rats the changes take place in the growing areas of the lens, the nucleus being altered, whereas in older rats the periphery of the lens is involved. In these experiments the tolerance for galactose was greatly exceeded. There is no evidence that the amounts of lactose which are likely to be employed in modification of cow's milk for infant feeding would produce any untoward effects on health.

H. S. Mitchell, Cook and Merriam ('37) report that the cataract-producing action of lactose or galactose rations is not appreciably altered by:—inclusion as supplementary carbohydrate, starch, dextrin, sucrose, or glucose; substituting different proteins such as casein, dried meat, egg albumen, or lactalbumin; changing the source of fats by including respectively, Crisco, butter, mutton tallow, or cod liver oil; addition of excess of cholesterol; feeding different amounts of the salt mixture (0.4 and 10 per cent); including excess of calcium lactate (4, 6, and 8 per cent); shifting the acid-base balance (4.76% Na citrate or 3.4% ammonium chloride); the amount of water ingested; administration of vitamin C; deficiency or excess of B_1 (thiamin) and B_2; ingestion of large doses of dinitrophenol; or injection of lens antigen. Cataract-producing action was hastened by lowering the protein intake to 5 per cent and it was slightly delayed by addition of cystine.

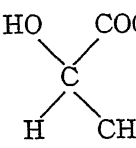
Relation of Carbohydrates to the Phenomenon of Refection.— Refection refers to a condition in experimental animals under which they are able to grow and reproduce, even in the second generation, on diets free from the vitamin B complex. The condition was first observed by Fridericia ('26) and independently by Roscoe ('27). Bliss ('35), Guerrant and coworkers ('35), and Guerrant and Brown ('37) have made important contributions to the understanding of this phenomenon. Bliss finds that refection can be easily produced by feeding diets containing raw potato starch as the carbohydrate. He carefully excluded the possibility that this starch is contaminated with the vitamins. Cooked potato starch does not cause refection. In

refection the feces are bulky, white, and gas-occluded. Feeding corn or rice starch did not produce such feces, nor did it promote nutrition such as is caused by refection.

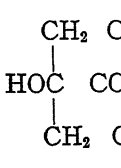
Guerrant and associates ('35 l.c., '37 l.c.) found that dextrinized corn starch exhibited the unusual dietary effect of producing refection. They have shown that the elaboration of the vitamin complex takes place in the cecum of the rat and suggested that this is due to the action of certain microorganisms, and is the result of delayed digestion of the dextrin so that it reaches the cecum in amounts sufficient to afford a medium for the growth of these organisms. They found that it was essential that the rats ingest their feces in order to obtain the full effect of the synthetic products referable to the flora of the digestive tract. Since yeast cells were found in enormous numbers in the ceca of refected rats, it is suggested that the synthesis of the vitamins is the result of their profuse growth in the cecum under conditions which provide a favorable medium for their proliferation.

Kelly and Parsons ('37) found that gelatinization of potato starch at temperatures not significantly destructive of thiamin prevented the occurrence of refection. On uniform intakes of a ration high in potato starch, rats tended to eliminate about three times as much total vitamin B in the feces when the starch was raw as when it was gelatinized. The non-extractable fat of the starch is not a significant factor in the phenomenon of refection.

The Nutritional Significance of Certain Organic Acids.—*Malic acid* occurs widely distributed in apples, pears, peaches, tomatoes, sugar beets, rhubarb, maple sap, alfalfa, plums, quinces, strawberries, citrous fruits, etc. A considerable part of the acidity of many fruits and vegetables is due to this acid. It serves as a source of glycogen, hence it is a source of energy to the body. It is essentially non-toxic and is a wholesome nutrient. It is easily oxidized in the body, combustion being complete to the end-products water and carbon dioxide.



Citric acid is even more widely distributed than malic acid. Indeed, it seems to be ubiquitous, occurring in cereal grains, milk, and other animal products, as well as in a great variety of fruits and vegetables. It is said to be absent in oysters, clams, crabs, shrimps, and scallops, but these may contain traces difficult to estimate. Milk contains 1.0 to 4.0 gm. per liter. The amount daily excreted in the urine of a man amounts to 0.2 to 1.0 gm. It has been



detected in almost every tissue and fluid in the body. It is so easily metabolized that little extra citric acid is excreted in the urine when large amounts of citrous fruit juices are taken. Blatherwick and Long ('22) found that the citric and other organic acids in 2400 cc. of orange juice taken in one day by human subjects was almost completely destroyed by oxidation. There is some evidence that citric acid may be converted into glycogen.

Sherman, Mendel and Smith ('36) confirm the findings of Ostberg ('31) that the amount of citrate excreted by an individual varies directly with the pH of the urine regardless of the cause of the alterations of this value. The addition of sodium bicarbonate (10 per cent of the dry mixture) to the basal citrate-low diet of rats caused a 100-fold increase in citrate elimination. They studied citrate excretion in dogs fed high-protein low-carbohydrate, and low-protein high-carbohydrate diets and found no consistent changes in citrate excretion. The ingestion of the basal, citrate-low diet generally produced an increase in the rate of citrate excretion, and in some but not all cases a slight rise in the blood citrate concentration in dogs. In dogs the administration of alkali increased citrate excretion as it did in rats. This was greatly favored by substitution of sucrose for casein in the diet, but the addition of sucrose without lowering the casein did not produce this result. They conclude that since animals excrete so much citrate when fed citrate-low diets during prolonged alkalosis, and since there are no stores of citric acid in blood, liver, muscle, or kidney, the body is capable of synthesis of citric acid.

Orten and Smith ('37) injected into dogs the disodium salts of malonic, succinic, fumaric, malic, and maleic acids while the animals were maintained on a constant, low citrate, basal diet. All of these compounds markedly increased the citrate excretion, although they produced only moderate increases in the urinary pH. Injection of sodium salts of acetic, glycolic, glycinic, lactic, and gluconic acids did not significantly increase citrate elimination. It appears, therefore, that the dicarboxylic acids named, or their metabolites, contribute to the synthesis of citric acid in the organism.

Oxalic Acid.—Oxalic acid is also very widely distributed in foods of vegetable origin. Small amounts are found in potatoes, beans, beets, tomatoes, cauliflower, onions, mushrooms, celery, currants, raspberries, grapes, pears, many other fruits and vegetables, tea, coffee, and cocoa. This acid is especially abundant in rhubarb and spinach among the vegetables ordinarily eaten. During the World War there were reported some cases of oxalic acid poisoning in England as the result of eating rhubarb leaves, which contain more oxalic acid than do the leaf stalks that are usually eaten. Children have been poisoned by eating sorrel, whose pleasant acid taste is due to its high content of

this acid. The ordinary "acid" fruits such as apples, oranges, lemons, tomatoes, pineapples, and strawberries, contain very little oxalic acid, their acids being mostly malic and citric.

Oxalic acid is very difficult to oxidize in the body; most of that which is ingested is excreted. The ingestion of oxalic acid or its salts tends to prevent absorption of calcium from the alimentary tract, since calcium oxalate is very insoluble under the conditions prevailing in the body. Calcium oxalate sometimes constitutes the calculi of renal or uretal origin. Götting ('09) cites changes in the bones indicating decalcification as a result of feeding oxalic acid. Fincke and Sherman ('35) found decreased utilization of calcium by growing rats fed a diet one-half of which was derived from spinach. Kohman and Sanborn ('35) found that feeding soluble oxalates caused excessive calcium excretion in the urine. On the other hand, Mackenzie and McCollum ('37) found that on a diet of purified foodstuffs containing optimal amounts of calcium, phosphorus, and vitamin D, and otherwise satisfactory, the presence in the diet of 0.9 per cent of potassium oxalate did not affect growth or the per cent of bone ash in the rat in a period of 10 weeks. Feeding 2.5 per cent of potassium oxalate did not affect growth, but did lower the per cent of ash in the bones. The presence in the diet of 0.06 to 2.5 per cent of potassium oxalate did not grossly influence calcium excretion. On the same type of diet, but deficient in vitamin D, the presence of 1.7 per cent of the oxalate hindered growth and bone formation. It was found that even when oxalate and calcium were fed in amounts of equal molarity, some of the calcium was absorbed and utilized, as shown by growth, per cent of bone ash, and calcium excretion.

There are many reported observations on the excretion of oxalic acid and oxalates in various diseases, but the results are not sufficiently conclusive to warrant discussion here. It is obvious that the presence of oxalic acid or soluble oxalates is undesirable beyond the content in which they are found in foods which provide but small amounts.

Aromatic Organic Acids in Foods.—Benzoic acid is present in cranberries and prunes to a greater extent than in any other common foods. Whereas malic and citric acids are easily oxidized in the body to carbon dioxide and water, and the sodium or potassium ingested as salts of these acids remain in the body fluids in the form of their bicarbonates, thus exerting an alkalizing action, benzoic acid is not capable of destruction in the body. It is mildly toxic and the body conjugates it with the amino acid glycine to form hippuric acid, which is excreted by the kidneys. The amounts of benzoic acid in these fruits is so small, however, that it is questionable whether it has any practical significance in nutrition, since these fruits are ordinarily eaten in relatively small amounts. These foods do, however, have the

property of contributing to the acidity in the acid-base balance. Since the glycine necessary for the formation of hippuric acid is synthesized by the body, the loss of this amino acid is of little or no importance.

Quinic Acid, or hexahydro-tetrahydroxy benzoic acid, has been shown by Kohman and Sanborn ('31) to occur in prunes and cranberries along with benzoic acid, the amount of quinic acid present being about 1 per cent. Quinic acid, when ingested, is converted into benzoic acid and is then transformed into hippuric acid.

Salicylic Acid.—Salicylic acid has about the same distribution as malic and citric acids in that almost all fruits and berries, grapes, etc. contain it in small amounts. Quick ('33) states that this acid is not converted into benzoic acid and only a small amount is conjugated with glycine to form "salicyluric acid." Most of the salicylic acid administered to human subjects seems to be excreted as the free acid.

Lactic Acid.—Lactic acid is an intermediary in carbohydrate metabolism and occurs in considerable amounts in the working muscle. It is ordinarily ingested in appreciable amounts when sour milk is taken or when lactose is eaten; lactic acid may also arise through fermentation of lactose in the alimentary tract. It has already been noted that the increased acidity in the intestine produced by feeding lactose facilitates the absorption of calcium. It is well demonstrated that when absorbed into the blood, lactic acid is capable of being converted into glycogen. It may, therefore, replace to a certain extent carbohydrate in the diet. Lactic acid is easily dealt with by the body in useful ways. Its formation in the colon, when lactose is fed liberally, may in most subjects bring about a transformation of the bacterial flora from the protein-decomposing types to a fermentative type, with consequent reduction of the extent to which unwholesome products of protein putrefaction are formed in the colon. There has been much discussion as to whether the latter products are of physiological significance. Certainly they are unwholesome, have no physiological value, and pollute the blood stream.

Succinic Acid.—This acid occurs in muscle. It is found in many vegetable foods in slight amounts. Annau and coworkers ('35) have proposed a scheme representing an essential catalytic system in the respiration of tissues, involving Warburg's respiratory enzyme (riboflavin-protein complex), cytochrome, and an unknown intermediate. According to their scheme, oxygen uptake is accomplished by fumarate which is oxidized to oxalo-acetate; the latter molecule oxidizes the activated hydrogen donators and undergoes reduction to succinate and to fumarate. Succinate is oxidized to fumarate which reestablishes the catalytic cycle. This hypothesis, which is supported by considerable experimental evidence, is interesting in that it attributes to certain four-carbon acids a role in intermediary metabolism.

The Effects of Vitamin Deficiency on Carbohydrate Metabolism. —Sure and Smith ('31) observed that in vitamin A deficiency so pronounced as to cause severe eye lesions, there is no significant change in the concentration of "true" blood sugar as compared with normal animals. The "apparent" sugar of the blood in the experimental animals was frequently considerably higher in the pathological animals than in the controls. They also report that in ricketic rats the blood sugar, alkaline reserve, and liver glycogen values are within the normal range. Similar results were obtained using vitamin G-deficient rats. Shimada ('34) found no differences in the carbohydrate tolerance and no disturbance of carbohydrate metabolism in scorbutic as compared with normal guinea pigs. On the other hand, Sigal and King ('36) found that successive stages of depletion of ascorbic acid (10, 15, and 20 days) induce a corresponding rise in fasting blood sugar level and a distinctly lowered glucose tolerance. The typical peak in the blood sugar curves moves characteristically upward and to the right with successive stages of ascorbic acid depletion. Administration of 10 mg. of ascorbic acid daily after 20 days' depletion induces a return to normal within 15 days.

The Effects of Inorganic Elements on Sugar Metabolism.— McQuarrie, Thompson and Anderson ('35), having observed that certain diabetic patients exhibited an abnormal craving for common salt, made observations which led to the discovery that the ingestion of between 1 and 2 gm. daily of sodium chloride per kilogram of body weight exerted a favorable influence on the carbohydrate metabolism of diabetic children taking simplified diets low in potassium. The effect was usually observable on the second or third day. High salt consumption caused significant elevation of both systolic and diastolic blood pressure levels. One of their patients required 60 to 90 gm. of sodium chloride daily to satisfy his craving for salt. Sodium appeared to be chiefly responsible for these effects, since other salts of this element likewise exerted these effects, but to less marked extent. Potassium chloride had diametrically opposite effects on both glycosuria and blood pressure. In terms of chemical equivalents, potassium completely antagonized the effects of sodium when given simultaneously in amounts as little as one-third that of sodium. The ingestion of sodium chloride caused a marked reduction in the degree of glycosuria. The fasting blood sugar was found to range at lower levels after a few days of high sodium chloride ingestion than during the foreperiod. In severe diabetes, ketonuria appeared earlier after withdrawal of insulin when the salt intake was low than when it was high. Ingestion of much salt changed the nitrogen balance from negative to positive.

Crabtree and Longwell ('36) carried out experiments, suggested

by the findings of Chaikelis ('34) that there is an inverse ratio between blood chlorides and blood sugar, to determine whether the amount of sodium chloride in the diet would exert an influence upon the deposition of glycogen in the liver and muscles of the rat. They found that a high salt diet almost doubled the glycogen content of the livers as compared with the content in the control and the low sodium chloride groups. Muscle glycogen seemed not to be affected. This phenomenon has not been further investigated.

Blatherwick, Bell and Hill ('24) observed no consistent change in the blood phosphate of humans upon oral administration of dextrose. Speirs and Sherman ('36) state that calcium and phosphorus utilization was not influenced by the inclusion of dextrose, dextrin, corn starch, or sucrose, respectively, in liberal amounts in the diet.

Keil and Nelson ('34) found in a study of the effects of copper on anemic rats that the oral administration of this element alone produces a different type of glucose tolerance curve than that obtained in the same animal before the mineral ingestion. A significant lowering of the maximum point demonstrates a role of copper in nutrition aside from its hemopoietic function. An increase in hemoglobin in anemic rats produces a proportional increase in sugar tolerance, coupled with a lowered glucose level in the blood after a 24 hour fast. In their experiments there was no change in the hemoglobin of the blood, which led them to attribute the effects of copper to some process other than ordinary oxidative processes. Pure iron alone did not improve glucose utilization in their anemic rats as did copper.

Nutritive Requirements.—Carbohydrate is the most easily available and most economical source of energy for the animal body. A certain minimum amount is essential for the metabolism of other foods, especially fats, to prevent the development of ketosis. A reasonable excess above this minimum is desirable in order to meet a portion of the requirements for energy without necessitating the intake of exceedingly large amounts of fat and proteins.

The nutritional requirement for carbohydrates varies according to the activity of the individual. It is believed that the carbohydrates furnish about 60 per cent of the fuel supply of the average normal adult. The minimum amount of carbohydrate necessary for maintenance of life and the prevention of ketosis is from 10 to 15 per cent of the total energy requirement.

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CHAPTER

IV

Lipids in Nutrition

BIOCHEMISTS have been increasingly attracted to researches relating to fats, fatty acids, glycolipids (compounds of fatty acids with carbohydrate, and containing nitrogen but not phosphoric acid), phospholipids, sterols, and hydrocarbons which occur in plant and animal substances. Much of this work deals with isolation and study of the chemical characteristics of lipids, their occurrence in different tissues, and the variation of their content under different conditions. The student of nutrition is primarily interested in the nutritive significance of these substances, particularly as respects the capacity of the body to synthesize them, whereas the biochemist is concerned with elucidating all the chemical transformations which these bodies undergo in metabolism. But the student of nutrition has a keen interest in learning what effects disturbances of nutrition may bring about in the behavior of any and all chemical substances with which the body has to deal. His inquiries must, of necessity, lead him to consider all phases of pathology which are influenced by the nutritional state. In this chapter a more arbitrary selection of topics is made for consideration since space does not permit of an extended discussion of all phases of lipid metabolism.

Effect of Diets Deficient in Fat.—Several investigators have studied the effects of diets which contained no fats. The observations show that rats fail in health when deprived of all fats. Burr and Burr ('29), using rats, first described the fat-deficiency syndrome. Owing to accumulation of dried secretion the eyelids tend to stick together, and there is loss of hair beginning on the head which may continue to almost complete denudation. Dermatitis develops on the ears, neck and upper part of thorax, forearms, backs of forepaws, shins, and backs of hind paws. There is a yellowish incrustation on

the reddened and thickened ears, and frequently a linear fissuring or ulceration at the angles of the mouth, and a lesion at the tip of the tongue, which first appears as a small, roughly circular grayish opacity or bleb or as an ulcer, which in some cases forms a yellowish slough. A striking symptom is necrosis of the tail.

This dietary deficiency disease is entirely distinct from what was formerly called "rat pellagra," but which is now known to have been misnamed, and is usually designated "rat dermatitis." It would be better named nutritional dermatosis, since it appears that changes in the skin involve factors other than inflammation. The rat dermatosis is produced by diets deficient in vitamin B_6 but containing such fats as cod liver oil and Crisco, either of which would prevent the fat-deficiency syndrome.

Fat-free diets regularly produce kidney lesions in the rat, and kidney degeneration probably causes the death of the animals in most cases. High protein feeding seems to increase the severity of the kidney damage, since it is accompanied by hematuria. A curious phenomenon is that the small emaciated rats on the fat-free diets drink twice as much water as their controls on the same diet containing fat. Ovulation is irregular or ceases entirely in these animals but is resumed on feeding them the essential fatty acids. When female rats on the diet do ovulate they mate and produce litters but these are inferior young, probably because of the poor nutritive condition of the mother. Male rats on the fat-free diet will generally not mate, while their controls receiving 10 per cent of lard, mate and sire normal litters. Fat-free diet males which do mate cannot sire young. This new type of sterility differs from that induced in males made sterile by vitamin E deficiency in that the fat-deficient animals quickly lose their sex response, while those lacking vitamin E retain it even after degeneration of the seminiferous epithelium.

When young rats are restricted to a fat-free diet, otherwise adequate, growth is not much retarded during the first 60 days, but after this time the rate of increase in weight falls off and they plateau at weights of 160-180 gm., which is about 100 gm. less than the controls on the same diet plus fat. A group of rats under these conditions die by the time they have reached the age of 240 days. Rats getting this diet with fat during the same interval are much larger and remain in good health.

Indispensable Unsaturated Fatty Acids.—This nutritional disease is curable by the inclusion of 2 per cent of fatty acids from certain sources. Glycerol and the non-saponifiable fraction of fats are ineffective. Storage fat can be almost entirely used up when rats are restricted to a fat-free diet. Burr and Burr suggested that the amount of body fat can be controlled over a wide range by the addition of minute

quantities of fat to the diet. After extensive investigations Burr and associates ('32) discovered that both linoleic and linolenic acids are effective in preventing or curing the fat-deficiency disease. These fatty acids seem to be of about equal value and can replace each other in the tissues. Contrary to the earlier findings of Burr and coworkers, Turpeinen ('38) found that arachidonic acid, $C_{20}H_{32}O_2$, which contains 4 double bonds, is superior to linoleic or linolenic acid as a growth restorative. He explains the apparently conflicting results in his studies and those of Spadola and Ellis ('36), who reported that the rat is able to synthesize arachidonic acid, by suggesting that this synthesis can be accomplished only from, or in the presence of, linoleic or linolenic acid. The need of the animal may be primarily for arachidonic acid, and linoleic or linolenic acid may be of value because of the capacity of the body to convert them into arachidonic acid. Oleic acid, stearic, palmitic, myristic, lauric, and lower fatty acids are ineffective, and alpha eleostearic acid, an isomer of linolenic acid, is of no value in preventing the disease. Also, as shown by Turpeinen (l.c.), erucic, ricinoleic, $\Delta^{12:13}$-oleic, and chaulmoogric acids are ineffective. Butter fat and tung oil contain little of the curative acids. Olive oil, lard, corn oil, linseed oil, egg yolk fats or lecithin from egg yolk, and poppy seed oil contain relatively large amounts of linoleic or linolenic acid or both, and are curative.

From these studies it is apparent that the rat, and presumably other mammals, require linoleic acid, linolenic acid, or arachidonic acid as nutrients. It is, of course, well known that the animal body is capable of synthesizing from carbohydrates, and from those amino acids which are glucose formers, all of the fatty acids which are commonly found in the body fats of animals. The body does not, however, possess the power to synthesize the unsaturated fatty acids of the linoleic or linolenic acid type and arachidonic acid. These are indispensable for some metabolic processes, or as structural units of protoplasm.

Brown and Burr ('36) state that it is not necessary to use highly purified food substances in order to produce the fat-deficiency syndrome. Crude casein, when substituted for purified casein, raises the weight of rats slightly at the time of cessation of growth. Decline and death are postponed, but hematuria, scaliness, and tail necrosis occur to almost the same extent. Substitution of fresh skim milk for casein and potato starch for part of the sucrose lead to results similar to those obtained with crude casein. Yeast oil and buttermilk lipids, rich in unsaturated fatty acids, are non-curative.

Sinclair ('36) points out that although the growth of rats on a diet devoid of triply unsaturated fats is distinctly subnormal, it is nevertheless very considerable. The source of the essential unsaturated acids

which must have been available has remained unsettled. He fed rats upon a diet of casein, salt mixture, yeast, and elaidin (elaidic acid is the trans-form corresponding to oleic acid which is the cis-form), supplemented with cod liver oil concentrate. The elaidin supplied 74 per cent of the calories. After 3 to 4 weeks, when the animals weighed 80 to 90 gms., growth ceased, and their weights remained constant for some weeks until decline set in, terminating in death at the age of about 4 months. When growth had been arrested for a few weeks, some animals were fed a diet in which sucrose replaced the elaidin. Immediately growth was resumed at the rate of about 20 gm. a week, the animals eventually reaching weights comparable to those of rats fed from weaning age on the same fat-free diet. Elaidin, therefore, caused cessation of growth earlier than would have occurred on a fat-free food. Replacement of 5 per cent of the elaidin by a mixture of cod liver oil and corn oil in equal parts resulted in excellent growth, showing that the elaidin was not toxic. Sinclair suggests that the high intake of elaidin—and presumably of other fats devoid of the essential unsaturated acids—almost completely abolishes the limited synthesis of linoleic or linolenic acid from carbohydrate, and thus prevents growth. His experiments suggest that the body is not without the power to synthesize these acids, but that they cannot be synthesized fast enough to meet the requirements of the animal for growth.

Sinclair ('32) found that the degree of unsaturation of the phospholipid fatty acids in the entire body of the rat, as shown by the iodine number, was about 102 for those fed a fat-free diet, and increased to 125 when 50 mg. of cod liver oil was fed daily. The value increased progressively to a maximum value of 140 when 100, 150, 200, and 250 mg. were fed daily. The corresponding figures for saturated fatty acids ranged from 63 to 72 approximately. The effect of feeding small amounts of cod liver oil on the degree of unsaturation of the fatty acids of the phospholipids was much greater in the liver phospholipids than in those of the skeletal muscles. Lard and coconut oil, when added to the fat-free basal ration, produced similar effects. Feeding cod liver oil with the fat-free basal diet does not prevent the development of the typical lesions caused by fat deficiency. It is also shown that these lesions do not develop when rats are kept in ordinary stock cages, but only when kept in false bottom cages which prevent access to the animals' feces.

Sinclair points out that since the maximum iodine value of phospholipids derived from oleic acid, which the rat can certainly synthesize, is 90, whereas that of animals fed the fat-free diet is 100 or over, the animals must have synthesized some more highly unsaturated fatty acids while on the latter diet. The fact that on the

fat-free diet they do grow, although more slowly than normal, points to this possibility.

The Metabolic Rate and Respiratory Quotients of Rats on a Fat-Deficient Diet.—Wesson and Burr ('31) subjected rats for some time to a fat-deficient diet and determined their metabolic rates and respiratory quotients. During the first hours after feeding carbohydrate to such rats the respiratory quotients were well above unity, which indicates that they were forming fat from the absorbed carbohydrate. The fat thus synthesized does not relieve the animals from the symptoms of their deficiency disease, because linoleic or linolenic acid are not formed, or, as seems plausible from the studies of Sinclair, are not synthesized fast enough to meet the nutritive requirements for them. The formation of fat from carbohydrate is a normal process of well fed animals whose glycogen stores are practically filled. After the stores of glycogen are depleted by fasting, fat synthesis would, on the basis of present knowledge, cease because of lack of carbohydrate reserves and because protein of the tissues in the normal individual is never drawn upon as a source of energy (part of which comes from carbohydrate synthesized from amino acids) beyond the actual immediate needs. It is a matter of common observation that fasted animals lose fat, and under conditions of energy deficit it has been assumed that fat formation is at a standstill.

The high R.Q. (respiratory quotient) of the emaciated animals, as they become progressively free from fat notwithstanding a generous intake of food, is remarkable. The administration of linoleic or linolenic acid causes an immediate increase in weight and an excessive gain in fat, which is shown by the R.Q. to be derived, at least in part, from carbohydrate.

Wesson ('27) fed rats a restricted diet consisting of dextrin, traces of iodine, calcium carbonate, sodium chloride, and small amounts of rolled oats. Rats taking this diet during a two weeks period had R.Q.s as high as 2, which indicates rapid formation of fat during the assimilation of a carbohydrate meal. When this diet was modified by the inclusion of casein high R.Q.s were still observed. If a small amount of lard, liver, or kidney fats was fed (0.4 to 1.0 gm.) the animals showed a day or two later R.Q.s of 1.0 or lower, i.e., normal assimilative respiratory quotients were obtained.

Wesson and Burr (l.c.) have studied further this interesting behavior of rats suffering from fat deficiency. The remarkable fact is that, whereas these rats can synthesize considerable amounts of fat under the experimental conditions, it does not relieve them of their symptoms or correct their aberrent metabolism; but the administration of very small amounts of linoleic or linolenic acid (in lard or other fats) at once changes the manner in which the body deals with

carbohydrate which is being assimilated. The basal and assimilatory metabolic rate in the case of rats showing early symptoms of the fat-deficiency disease was well above the normal value, while the metabolic rate in the later stages of the disease was normal or subnormal. The temperature of the rats in fat-deficiency disease was normal. Miyazaki and Abelin ('24) and Abelin ('26) reported abnormally high specific dynamic action of carbohydrate in patients suffering from hyperthyroidism and were able to reduce it by fat feeding. Wesson and Burr could not accomplish a reduction of the metabolic rate in their rats by this means. The cause of the high metabolic rate in their animals remains unexplained.

Finally, Wesson and Murrell ('34) have demonstrated that the partial hydrogenation of the alcohol-soluble fraction of lard, leading to the complete or almost complete conversion of the linoleic acid which it contains into a saturated fatty acid, does not diminish the effectiveness of the fat with respect to lowering the abnormal respiratory quotients of rats with fat-deficiency disease. They are, therefore, led to conclude that the factor influencing carbohydrate metabolism is not linoleic acid. The latter is, however, definitely preventive or curative for the fat-deficiency disease. The carbohydrate factor is still of unknown nature.

Effect of Fat-Deficiency on Humans.—Since a predominant symptom of fat-deficiency in rats is a scaliness of the skin, the possibility has been investigated that certain eczematous conditions in humans, particularly of infants, might be related to a deficiency of essential fatty acids, or a disturbance in their metabolism (Hansen, '33; Epstein and Glick, '37). As shown by the important studies of Hansen and his associates, the blood of eczematous infants tends to have less unsaturated fatty acids than normal. The liberal administration of fats yielding high amounts of unsaturated fatty acids raised the degree of unsaturation of blood fats and, in a gratifying degree, alleviated the eczema. Further investigation should clarify the relation of dietary fat to eczematous conditions, but at present there is scant basis for the extravagant claims, frequently seen, that the indispensable fatty acids should be rubbed into the skin as an aid to beauty. Exploiters of these nutrients refer to them as "vitamin F" or "skin vitamin." Obviously the purpose is to promote sales since designation of a nutrient as a vitamin increases its sales-appeal.

A normal adult man subsisting 6 months on a diet extremely low in fat experienced no evidence of disease but the serum fatty acids became more saturated and the content of serum linoleic and arachidonic acids decreased approximately 30 per cent (Brown, Hansen, Burr and McQuarrie, '38). The R.Q. rose to 1.14 following a meal of the diet, indicating fat synthesis. The evidence from this study

suggests that humans do require fatty acids in the diet, but further investigations are needed before definite conclusions can be drawn.

The Effect of Fats on Galactose and Lactose Utilization.—Schantz, Elvehjem and Hart ('38) have made important observations on the effect of fats in the diet on the utilization of milk sugar and galactose. Rats fed whole milk, supplemented with iron, copper, and manganese (in which milk is deficient) were very efficient in the utilization of the milk fats. A pig and a calf were studied with the same result. When, however, the animals were confined to a diet of skim milk, similarly fortified with minerals, galactose was excreted in the urine after a few days' feeding. As much as 35 per cent of the galactose of the milk sugar was recovered in the urine. When butter fat, lard, corn oil, coconut oil, palmitic and oleic acids were added to the diet to the extent of 3 to 4 per cent the loss of galactose in the urine was prevented. Glycerine, butyric, beta-hydroxybutyric, caproic, and lactic acids did not prevent the loss. The blood sugar in rats fed the mineralized whole milk was about 140 mg. per cent. Whereas that of rats fed mineralized skim milk rose to about 200 mg. per cent.

These experiments show that some relation exists between the utilization of the galactose part of the molecule of milk sugar and the presence of certain fatty acids. The glucose portion of the sugar is utilized readily without fat. Schantz and associates found that rats fed fat-free diets containing glucose did not excrete sugar as they do on a lactose—or galactose—containing diet. They suspected that the presence of fat in the intestine might serve to delay absorption of galactose and thus prevent accumulation of this sugar in the blood and tissues beyond the powers of the body to catabolize it. However, they found that the feeding of skim milk at the rate of 2 cc. per half-hour did not prevent an abnormal rise in blood sugar and excretion of galactose for a few days.

These observations have a special interest in connection with the findings of Deuel, Gulick and Butts ('32), and of Clark and Murlin ('36) who state that galactose is superior to other hexoses in ketolytic activity. Deuel et al. ('36) have pointed out that fatty acids containing eight or more carbon atoms are much greater producers of beta-hydroxybutyric acid and acetoacetic acid formers than are butyric acid and caproic acid which the Wisconsin workers find to be ineffective in increasing the utilization of galactose.

The Role of Fat in the Phenomenon of Refection.—Whipple and Church ('35) found thiamin in the feces of rats on a thiamin-deficient diet containing 10 per cent of lard with sucrose as the only source of carbohydrate. When the diet was made fat-free the feces were free from thiamin. But little of the vitamin was formed when corn starch was substituted for sucrose. The symptoms of thiamin-

deficiency (polyneuritis) were produced more quickly on fat-free diets than when fat was provided, and the larger the amount of fat fed the longer were the symptoms delayed. The feeding of fat alleviated the symptoms of polyneuritis in rats permitted to develop the disease on a fat-free diet, but they eventually succumbed to the avitaminosis. The amount of thiamin eliminated in the feces is directly correlated with the content of fat in the diet, which seems to be essential for the formation of the vitamin in the alimentary tract through the agency of yeast. This suggests that fat favors thiamin synthesis, probably by bacteria, in the lower intestine.

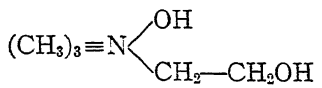
The observation of Whipple and Church is of interest in connection with the studies of Evans and Lepkovsky ('29) from which it was concluded that fats exert an important function in the metabolism of the animal other than supplying energy. Fats, they found, would delay the onset of the symptoms of thiamin-deficiency when this vitamin was fed in inadequate amounts. They raised the question whether feeding fats influenced in some manner the phenomenon of refection. Evans and associates ('34) have recently concluded that for fat to exert its optimal sparing action upon thiamin both protein and the heat-stable factor (their vitamin G), must be high. The sparing action of fats on thiamin requirements will be discussed in Chapter XIX.

The Phospholipids.—In animals three types of phospholipids are found: 1, the lecithins—substances consisting of two fatty acids, glycerol, phosphoric acid, and the nitrogenous base, choline; 2, the cephalins—substances similar to the lecithins except for the substitution of aminoethyl alcohol for choline; 3, the sphingomyelins—substances related to lecithins in that they contain phosphoric acid and choline, and to the cerebrosides in that they contain the nitrogenous base, sphingosine, and, in part, C_{24} fatty acids. Since these lipids are all capable of synthesis in the animal body they have been generally believed to be dispensable in the diet. They are extremely important as structural elements in most tissues, but a discussion of these roles would be out of place here. The observations of Best and Channon and their coworkers on the effects of administration of choline to animals have a nutritional bearing, however, and will be briefly considered.

Hershey and Soskin ('31) investigated the cause for the long known phenomenon that depancreatized dogs suffer impairment of assimilation of protein and fat, and since the condition is greatly ameliorated by feeding the dogs raw pancreas, the belief long prevailed that this benefit was due to the provision of the pancreatic digestive enzymes by this means. Various investigators have reported that depancreatized dogs did not survive more than 1 to 8 months on a meat and sugar diet, notwithstanding complete control of diabetic

manifestations by the use of insulin, unless raw pancreas was also provided. This was interpreted to mean that the external digestive secretions of the pancreas were essential in the alimentary tract for the survival of such animals. Hershey and Soskin found that depancreatized dogs could survive for a very long time, presumably indefinitely, on lean beef muscle, cane sugar, and insulin, without pancreatic enzymes being given by mouth, provided they were given frequent doses of egg yolk "lecithin." Lecithin is put into quotation marks because the preparations used were never pure chemical entities, but crude material containing various impurities. Without "lecithin" the animals fail because of impairment of liver function characterized by fatty changes in the liver. When depancreatized dogs develop the characteristic syndrome, the addition of "lecithin" promptly alleviates the signs and symptoms of this condition. Depancreatized animals fed beef muscle and sucrose, with insulin, accumulate much fat in their livers. The administration of lecithin to these animals greatly increases their excretion of sugar in the urine.

Best and Huntsman ('32) studied the components of the lecithin preparation in order to identify the substance to which this material owed its remarkable effect. Oleic acid, glycerophosphate, and aminoethyl alcohol, proved ineffective, but choline,



possessed the same protective action as raw pancreas or lecithin in improving the well-being of the animals, and in preventing the liver damage associated with the accumulation of fat.

That one function of the liver is desaturation of fatty acids is clearly shown by experiments of Best, Hershey and Huntsman ('32) who fed rats their stock diet with added fat equivalent to 40 per cent of the food. The average fatty acid content of the livers in a group of 10 animals after 22 days on the diet was 18.3 per cent. The livers of a group of rats similarly fed, but with the provision of lecithin contained 4.5 per cent. The iodine number of the food fat was approximately 40. In rats not receiving lecithin the iodine number of the fatty acids of the livers was approximately 100, whereas that of the fatty acids of livers from a comparable group receiving lecithin was approximately 132. The minimum effective dose of choline is less than 3 mg. per rat per day but the amount necessary to maintain livers at their normal lipid content was 190 mg. per day. Choline not only prevents accumulation of fats in the liver but of cholesterol esters as well. The effect on the latter was less than on fats, being only about 60 per cent,

but these results were obtained with diets containing 2 per cent of cholesterol.

It appears from studies of Best and of Channon ('35) that the action of choline, as revealed in these experiments, is not confined exclusively to this substance. Casein which was sufficiently purified as to contain entirely insufficient amounts of choline to produce the effects observed, had a choline-like action. It is estimated that 2 gm. of highly purified casein are equivalent to 1 mg. of choline. It seems necessary to conclude, provisionally, that some amino acid derived from casein, or perhaps a lipotropic factor such as betaine, is made available in the metabolism of the amino acids of casein. Gelatin has been shown to possess no choline-like action. That the lipotrophic action of choline is not specific is shown by the observations of Channon and Smith ('36) that the triethyl homologue of choline is effective in preventing deposition of fat in the liver, although its action is not so intense as that of choline.

The choline content of various substances of biological interest has been determined by Fletcher and associates ('35). Their data are assembled in the following table:

TOTAL CHOLINE CONTENT OF ANIMAL TISSUE

Tissue	*Average Choline Content mg./100 g.*
Rat:	
Spermatic fluid	514
Spinal cord	370
Brain	325
Adrenals	304
Cerebellum	296
Cerebral hemispheres	274
Liver	260
Pancreas	232
Pituitary	224
Kidneys	202
Thyroid	167
Lungs	164
Heart	158
Lymph glands	152
Stomach	152
Spleen	151
Small intestine	142
Salivary glands	131
Tongue	123
Thymus	113
Skeletal muscle	100
Uterus	74
Skin	64
Bone	44
Connective tissue	40
Fat	23
Blood-starved	22
Blood-fed	31
Ox:	
Liver	270
Pituitary, anterior lobe	259
Pituitary, posterior lobe	217
Pancreas	230
Muscle	76
Blood, defibrinated	13
Fat	0.5–2.6
Dog:	
Liver	230
Stomach	90
Blood, whole	34
Pig:	
Pancreas	280
Bacon (cured side)	44
Fat (from cooking bacon)	6
Lard	1
Codfish:	
Muscle	78

These studies on the physiological role of choline are of great interest since they indicate that choline is an essential dietary factor when certain sources of protein supply the amino acid requirements of the body. It appears that choline, or a substance resembling it chemically and physiologically, may arise through synthesis from certain amino acids. The pathological manifestations of animals on a choline-free diet is much more easily demonstrated when the experimental diet is poor in protein, than when the protein intake is liberal. With moderately high protein diets these manifestations may not occur. It seems clear that the phospholipids as a source of choline contribute an essential dietary factor. A point of interest is that such cereal products as white flour, skimmed milk powder, rice flour from polished rice, and possibly other refined cereal flours, which are very deficient in fats and other lipids, are, nevertheless, relatively rich sources of choline or other substance exerting a choline-like action.

The Lipids in Living Tissues.—The conception that lipids are essential components of protoplasm arose in the minds of the earlier investigators of fatty substances in brain and other tissues. This subject has in recent years interested workers anew, and since methods for identifying the individual component lipids have been greatly improved, some new and interesting facts have emerged relative to the role of these substances in the life processes.

Bloor and his associates ('30) have reviewed the older literature on this subject. Klem ('35) found that two rats after being starved to death, contained 1.54 and 1.58 per cent respectively of fatty acids. The value for starved mice was 2.0. Terroine and Trimbach ('34) have discussed the composition of the tissues in respect to lipids and hold that there is a constant content characteristic of each tissue which is not altered by extreme changes in the nutritional status of the animal.

Bloor and associates found the total lipid content, on the basis of dry weight of uterine mucosa, lung, kidney, and beef pancreas, to be approximately the same. There is a definite but not large increase in the percentage of lecithin in the uterine mucosa at the time of greatest elaboration of endometrium, which is accompanied by an increase in free cholesterol. The amount of cholesterol esters present in the endometrium is always very low. The percentage of phospholipid in the corpus luteum of the sow varies greatly with the activity of the tissue, being 2 to 3 times as high during the period of activity (previous to estrus and during pregnancy) as at the time of formation or after retrogression. Free cholesterol increases up to and during the period of active functioning but to a much lesser extent. Cholesterol esters vary inversely with the activity of the gland, a high content being characteristic of the degenerated organ. These results support

the hypothesis that the phospholipid content of this tissue is a function of its physiological activity. The relation of free cholesterol is less clear, but appears to be similar to phospholipid.

Bloor and Snider ('34) found the following amounts of phospholipid fatty acids and of cholesterol in animal tissues.

LIPID CONTENT OF MUSCLE OF WILD RABBIT
(in Per Cent of Dry Weight)

	PHOSPHOLIPID FATTY ACIDS		CHOLESTEROL	
	Average	Range	Average	Range
Heart	5.2	4.0–5.7	0.45	0.36–0.60
Jaw	3.2	2.6–3.8	0.36	0.23–0.39
Diaphragm	3.2	2.2–3.8	0.29	0.20–0.36
Neck	2.9	2.5–3.8	0.31	0.26–0.39
Front leg	3.1	2.6–4.2	0.27	0.18–0.35
Thigh	2.9	2.5–3.4	0.25	0.17–0.33
Loin	2.6	2.2–3.0	0.25	0.15–0.27
Stomach	2.6	2.5–2.8	0.50	0.45–0.65
Abdominal wall	2.5	1.6–3.4	0.24	0.12–0.40
Back	2.4	1.9–3.2	0.22	0.17–0.33
Forearm	2.36	1.8–2.7	0.27	0.08–0.28
Pectoralis	2.2	1.9–2.7	0.28	0.20–0.44
Gastrocnemius	2.1	1.9–2.6	0.22	0.15–0.28

LIPID CONTENT OF PECTORAL MUSCLES OF BIRDS
(in Per Cent of Dry Weight)

	PECTORALIS MAJOR		PECTORALIS MINOR	
	Phospholipid Fatty Acids	Cholesterol	Phospholipid Fatty Acids	Cholesterol
Pigeon	4.7	0.25	2.10	0.18
Owl	3.7	0.26	1.92	0.28
Rooster	1.37	0.17	1.10	0.18

These values indicate that the more used the muscle the higher is the content of phospholipid. Increased activity is accompanied by increase in phospholipid. The iodine number of the phospholipid fatty acids showed no difference in much used or little used muscles. The differences in cholesterol content, when they occur, are in the same direction, but not so great.

Sinclair ('34) in a review on the physiology of phospholipids, suggests that the functions of the phospholipids are (1) as possible oxygen-transporting substances due to the ability of their double bonded carbons to take up and probably to transfer oxygen; (2) as intermediaries in the metabolism of fats, as regards the early stages of fat absorption and transport, but doubtful in the later stages of combustion in the tissues; (3) as structural elements in tissues; and (4) in increasing transport fat in the cells owing to the solvent action on fat of the phospholipids.

In harmony with these views is the finding of Boyd ('35) that an inactive tissue, Wharton's jelly (gelatinous embryonic connective tissue of the umbilical cord), contains only about 1 per cent of phospholipids on the dry basis. The cholesterol ester content was insignificant, which is in accord with the general finding that a high content of these esters is characteristic of degenerating rather than active tissues. The generalization that phospholipids are abundant in highly active, and less so in inactive tissues, is further supported by analyses of tumors and of the mammary gland.

Boyd has reported interesting observations on the phospholipids and the associated variations in free and esterified sterols of the blood under different conditions. After surgical operation of any cause the phospholipids of the white blood corpuscles increase by 200 per cent if recovery sets in, and fall by a like amount if post-operative complications ensue. If recovery is normal after fever, the content of phospholipids in these cells increases, but falls if the illness proceeds fatally. There is a marked increase in phospholipids in the white cells of rabbit blood following parturition, the values returning to normal in three weeks. The phospholipid content of rabbit ovary increases from the beginning of pregnancy to 300 per cent of the normal value by the 15th day, and returns to normal just before parturition.

The Toxicity of Certain Fats and Fatty Acids.—It is a matter of common experience that cod liver oil, especially in the adult, may produce anorexia and gastric discomfort. This may well be attributable to its disagreeable taste and oily nature. Fats, when taken too liberally, inhibit gastric secretion and motility, but cod liver oil is more disturbing than most other fats. The effects may reasonably be attributed largely to its psychic influences, especially its flavor. Normal adults taking 2 ounces of cod liver oil daily have been reported to suffer from tachycardia and precordial discomfort. Agduhr ('26) found, however, that cod liver oil when given continuously to animals may produce toxic effects on the heart and the development of abnormalities such as pigment degeneration and fatty degeneration of the heart muscle, transformation of the muscle cells into connective tissue, and calcareous incrustations. Parallel experiments with olive oil, rape oil,

and coco fat, did not produce these changes. He did not include in his studies observations on other fish oils, which are, in general, characterized by a high degree of unsaturation. It seems probable that Agduhr's results were magnified by the conditions of his experiments, especially by the use of a diet composed in great measure of purified food materials and consequently by the probability that certain nutrients were not present in the diet in sufficient amounts. In other words, it seems probable that the basal diet was not such as would promote optimal nutrition.

This view is supported by the studies of Harris and Moore ('29) who found that supplementing the diet with the vitamin B-complex inhibits the toxic effect of cod liver oil. It has been shown by Harris and Innes ('31) that vitamin D in excessive doses is much more toxic when added to diets composed of purified foodstuffs than when added to diets of natural foods. The principle is further illustrated by the protective effect of a high protein diet in offsetting the effects of lack of choline and by the effects of amino acid imbalance. Experimental diets have been frequently shown to provide some one or more essential nutrients in amounts below the optimal requirements which bring animals into a condition of lowered tolerance for any deficiency or excess of another constituent of the diet which imposes a physiological burden upon the body. Most of Agduhr's experiments were made with calves and other herbivorous animals. These animals tolerate fats badly. Rabbits die soon after the addition of even small amounts of fat to the diet unless green vegetables are given (Mellanby and Killick, '26).

In studies by Madsen, McCay and Maynard ('32-33) 6 kids died after 133 to 315 days when given a diet consisting of cellulose, starch, casein, sucrose, yeast, lard, a salt mixture, and tomato juice, to which was added 12 cc. of cod liver oil per week. The kids died unexpectedly and without obvious symptoms but autopsy showed failing circulation, intermuscular edema of the legs, increased fluid in the body cavities, congestion and edema of the lungs, and severe hemorrhagic enteritis. There was fatty degeneration of the liver and kidneys. Histological examination revealed hyaline, waxy, or Zenker's degenerative changes in the muscles, with fibroblastic tissue tending to replace the degenerating muscle. The heart muscle was involved in all cases. Histologically, local well-defined grayish areas and hyaline and granular degenerative changes were seen, with necrotic fibers in process of absorption in some areas. There was increase in the amount of connective tissue, which tended to replace portions of the degenerated muscle. Rabbits and guinea pigs, when fed the "synthetic" diet, were injured in a similar manner by cod liver oil. No deleterious effects

were observed, however, when the cod liver oil was added to a stock diet. Madsen and associates tentatively suggested that the injurious factor is contained in the fatty acid fraction, and Golding ('28) found that the non-saponifiable fraction of cod liver oil was not injurious. In this connection it is of interest to note that Daniel and McCollum ('31) found that rats grew well and appeared healthy when fed a diet of ether-extracted menhaden meal, 14; cod liver oil, 2; butter fat, 3; agar-agar, 1; salt mixture, 51.2; dextrin, 77.9; plus the 70 per cent alcoholic extract of 10 grams of wheat germ. On the same diet modified by including 5 per cent of oleic acid, which replaced an equivalent amount of dextrin, rats were stunted in growth and were very inferior in appearance, thus indicating that fats other than cod liver oil may be injurious.

Norris and Church ('30) have shown that small doses of isoamyl amine, which is found in cod liver oil, and also choline, when given continuously, may produce paralysis, convulsions, and impairment of growth, and that these symptoms may be prevented by increasing the amount of yeast in the diet. It is suggested that these and perhaps other nitrogenous bases contained in cod liver oil may be the cause of its toxicity.

Madsen ('36) has recently reported further studies on the muscular dystrophy in rabbits and guinea pigs caused by feeding cod liver oil or a concentrate made from it. Lard or cottonseed oil fed with cod liver oil did not protect against dystrophy. With his "synthetic" diet, the replacement of cod liver oil by a concentrate fed in cottonseed oil solution, the results suggest that the cottonseed oil was protective. The basal diet without added fat, but with addition of concentrate containing the nonsaponifiable portion of cod liver oil, produced dystrophy to nearly the same extent as the same diet containing 6 per cent of lard. Substitution of cottonseed oil for the lard resulted in a high degree of protection against the muscle lesions. No such protection was afforded by this oil, however, when cod liver oil was fed with it, in place of the concentrate.

When the "synthetic" diet was supplemented with concentrate to provide vitamins A and D, and was fed with 6 per cent of either lard or cottonseed oil, the muscle lesions were seen in the lard group but the groups receiving either 3 or 6 per cent of cottonseed oil were in great measure protected. One in 10 receiving 3 per cent of this oil showed symptoms. Microscopically there were some scattered necrotic fibers of recent development but the muscles looked normal in color and consistency.

Goettsch and Pappenheimer ('31) observed degeneration of the skeletal muscles of rabbits fed a grain mixture consisting of oats 65,

barley 85, wheat 45, and wheat bran 5, supplemented with cod liver oil. Madsen employed this ration for guinea pigs, with and without cod liver oil. The grain mixture alone was inadequate and paralysis was seen in some of the animals, but the animals that received 0.5 gm. of cod liver oil daily all showed paralysis and severe muscle dystrophy.

From these studies it appears that the fatty acids of cod liver oil are toxic, and that the muscles are especially sensitive to injury from them. It is not improbable that the organic bases in this oil are partly responsible for its effects. But the grain mixture described, without cod liver oil, caused the condition in lesser degree. The feeding of cod liver oil with such natural foods as constitute an optimal ration apparently protects animals against this type of food injury. Possibly the toxicity of cod liver oil is caused by its autoxidation and consequent destruction of vitamin E (Chapter XXIII).

Fats in Infant Nutrition.—Holt and his associates ('35) have made extensive investigations on the fat metabolism of infants. They conclude that in the normal subject fats are completely hydrolyzed in the intestines. There does not appear to be a constant relation between total solids and fat in the feces. In infants on a low-fat diet the fecal fat is largely derived from the secretions. Calcium and magnesium in the food affect adversely the absorption of fat; other non-fatty foodstuffs have no apparent effect. Their observation that neither the fat globule size nor the melting point of dietary fats have any influence upon digestion and absorption in the infant is of special interest since emphasis on the importance of both these factors has been frequent by pediatricians. In a mixed fat, absorption is favored by fatty acids with one or more unsaturated linkages, and relatively short carbon chains. It is impaired by the long chain saturated fatty acids. They found that ketosis occurred just as quickly with odd-carbon as with even-carbon fatty acids.

Free fatty acids, they found, irritate the intestines and soaps increase the water requirement; hence hydrolyzed fats should not be used in diarrheal cases. Ethyl esters of fatty acids are inferior to triglycerides. They are saponified with difficulty, have a low percentage retention and lead to ketosis at unusually low levels of fat absorption. The absorption was found to be better with breast milk than with butter, but these were surpassed by olein, olive oil, and soy bean oil. Determination of iodine values indicated that there is selective absorption of individual fatty acids in mixed fats. On fat-poor diets infants tend to have loose, fermentative stools. In one case eczema and spasmodic bronchitis regularly appeared and were relieved by increasing the fat intake. Fat absorption in infancy increases slightly with age. Low fat retention in atrophic infants is not adequately explained by low

intake. It would appear that these infants have difficulty in absorbing fat.

Fat digestion (saponification) is less complete in the premature than in the full term infant. Retention of unsaponifiable material is also impaired. In premature infants olive oil and soy bean oil show much higher percentage absorption than butter fat, and substitution of these fats for milk fat is frequently followed by improvement of the weight curve. Linolein is better absorbed than olein, indicating that the presence of more than one double bond in a fatty acid chain favors fat absorption.

Sullivan and Fershtand ('35) utilized the observations on fat absorption as an index of liver function. One hundred grams of cottonseed oil were given orally to normal men, to diabetics, and to patients with various types of impaired liver function. After a 15 hour fast, blood was taken, the oil administered, and blood again drawn 3, 6, and 9 hours later. In the normal subject the total lipids rose to 65 per cent above the fasting level at 6 hours and returned to normal at 9 hours. In diabetics the same level was reached at 6 hours but there was a lag in the fall, while patients with liver disease showed but a small rise and there were no signs of a decrease even at 9 hours. Defective absorption is held responsible for this behavior.

The Effect of the Nature of Food Fat on the Composition of Body Fat.—Many studies have been reported on the effects of various feeding-stuffs on the melting point and iodine number of the fat of hogs. This work was stimulated because of the frequent occurrence of so-called "soft pork," a term applied to the hog carcasses which remain soft and flabby after being exposed in the chilling room. Such pork is unsalable. The literature has been reviewed by Hankins and Ellis ('26). Ellis and Isbell ('26) analyzed the fats of hogs fed various combinations of feeds. They found that the percentages of saturated fatty acids, oleic acid, and linoleic acid of lard from peanut- and soy bean-fed hogs were similar to the content of these acids in peanut and soy bean oils. The linoleic acid showed the most striking changes, increasing from 1.9 per cent in the lard from hogs fed brewer's rice, to 30.6 per cent in oily fat from hogs fed soy beans. There is a close relation between the food fat and the fat laid down by the hog. Anderson and Mendel ('28) have shown that the rat behaves in respect to food and body fat as does the hog. When the body fats were synthesized from starches or dextrin they were hard (i.e., they had a low iodine number and high melting point).

Absorption of Fatty Acids from the Intestine.—Experimental evidence seems to prove that the fatty acids liberated from fats in the intestine, through the agency of enzymes, are absorbed into the epithelial cells of the small intestine. Jeker ('36) has recently reinvesti-

gated this subject. By employing stains which differentiate between neutral fats and fatty acids, he has shown that fatty acids can be demonstrated in the tips of the epithelial cells of the villi within 10 to 20 minutes after feeding fat to rats. By the 6th hour the cells are completely filled with fat droplets which are composed of neutral fats, free fatty acids being absent. This indicates a resynthesis of neutral fat in the cells. Artom and Peretti ('35) administered iodized fat to rabbits and found that after a short time the iodized fatty acids in the intestinal mucosa are present in the neutral fats, but that after 3 days a considerable portion of these are to be found in the acetone-insoluble fraction, i.e., in the phosphoaminolipids, the lecithins, and kephalins. They have also shown that during absorption a portion of the saturated fatty acids is desaturated (e.g., stearic acid converted to oleic acid). Verzár and McDougall ('36) have thoroughly discussed fat absorption.

The Origin of Milk Fats.—The composition of the milk fat of animals is different from the stored fats in the same animal. It contains representatives of practically all the saturated fatty acids from butyric acid, which is C_4-, to cerotic acid, which is C_{26}- and possibly higher. The content of short-chain fatty acids is higher than in any other fat in the body. There is in milk a great complexity of unsaturated fatty acids, which, like the saturated ones, are present in the form of triglycerides. In the case of Carnivora and Omnivora, food fats may appear promptly in the milk. But in Herbivora, food fat has little effect on the composition of milk fat, which appears to be formed largely from carbohydrate. Meigs, Blatherwick and Cary ('19) made elaborate studies of the source of milk fats. Their findings are: 1. That normal blood plasma contains no phosphorized proteins, and probably no phosphorus compounds at all except phospholipids and inorganic phosphates. 2. The precursor in plasma of milk fat and milk phosphorus is either lecithin or some related body.

Since about 20 per cent of the total fatty acids of milk fats consist of fatty acids with 14 or less carbon atoms in their chains and these are notably rare in blood fats and food fats other than milk, the mode of formation of these fats is of great interest to biochemists and physiologists. A recent series of investigations by Graham, Jones and Kay ('36) has thrown new light on this question. They state that volatile fatty acids cannot be detected in blood, which is the source of all the constituents of milk. Parry and Smith ('36) made an elaborate study of the fats of cattle and reported the following values for fatty acids:

	Ox Depot Fat %	Ox Blood Fat %	Ox Liver Fat		Pig Liver Fat %
			Phosphatide %	Glyceride %	
Saturated acids					
Lower acids . . .	6	*	Trace	Trace	1
C_{16}	27	10	12.5	25	14
C_{18}	22	13	27	20	19
Higher acids . . .	Trace	3	Trace	Trace	2
Total	55	26	39.5	45	36
Unsaturated acids					
C_{16}	..	*	5	9	1.5
C_{18}	45	26	27	37	33
C_{20}	..	33	18	8	20
C_{22}	..	10	10.5	1	7.5
Total	45	69	60.5	55	62

* There was not sufficient material available to make possible the detection of small amounts of lower acids.

Hilditch and Paul ('36) give the following data relative to the component fatty acids of cow milk fat:

Component Fatty Acids of Cow Milk

Acid	Weight Percentages			Molar Percentages		
	(a)	I	II	(a)	I	II
Butyric	3.7	3.7	3.7	9.8	9.8	9.7
n-Hexanoic	2.0	2.0	2.0	4.1	4.1	4.2
n-Octanoic	1.3	1.0	0.9	2.1	1.6	1.5
n-Decanoic	2.7	2.6	2.6	3.7	3.5	3.5
Lauric	4.0	1.7	2.7	4.8	2.0	3.2
Myristic	7.9	9.3	8.6	8.2	9.6	8.9
Palmitic	23.8	25.4	26.2	21.9	23.4	24.0
Stearic	10.7	10.7	10.7	8.9	8.9	8.8
As arachidic	0.5	0.4	0.5	0.3	0.3	0.3
As decenoic	...	0.2	0.5	...	0.3	0.6
As tetradecenoic . . .	...	1.2	0.7	...	1.3	0.8
As hexadecenoic . . .	...	5.0	4.9	...	4.6	4.5
As oleic	38.3	32.4	31.9	31.9	27.0	26.5
As octadecadienoic . .	4.7	4.0	3.9	4.0	3.3	3.3
As C_{20-22} unsaturated. .	0.4	0.4	0.2	0.3	0.3	0.2

(a) Without allowance for unsaturated acids below C_{18}.

From a study of oxidation products of the unsaturated fatty acids of milk Hilditch and Paul conclude that in the principal unsaturated fatty acids in the glycerides of milk fat the position of the ethenoid bond in the three acids below oleic is the same as in that acid:

$CH_2 : CH \cdot (CH_2)_7 \cdot COOH$	Decanoic acid
$CH_3 \cdot (CH_2)_3 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Tetradecenoic acid
$CH_3 \cdot (CH_2)_5 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Hexadecenoic acid
$CH_3 \cdot (CH_2)_7 \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$	Oleic acid

These investigators suggest that the lower-saturated glycerides of milk, which contain the volatile fatty acids, are formed in the mammary gland by shortening the carbon chains of preformed oleo-glycerides, and the minor lower-unsaturated components just mentioned may represent degradation products of oleo-glycerides which have escaped complete saturation to lower-saturated groups. This hypothesis, which is supported by much experimental data, supposes the shortening of the carbon chain by oxidative removal of the terminal CH_3 group with subsequent reduction of the new terminal carbon atom. It is implied that the oxidation and reduction of the fatty acids occur while in combination with glycerol, thus offering an explanation for the high content of fully saturated glycerides in milk fat.

The Behavior of Certain Fatty Acids in the Mammary Gland.— Hilditch and Thompson ('36) have reported some instructive studies on the effects of feeding certain fatty acids (in the form of glycerides) to lactating cows, with the objective of identifying the components of cod liver oil which cause lowering of the fat content of milk.

Linseed oil has a higher mean unsaturation than most cod liver oils, this being due to glycerides which contain linoleic and linolenic acids,

$CH_3 \cdot (CH_2)_5 \cdot CH : CH \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$ Linoleic acid
$CH_3 \cdot (CH_2)_3 \cdot CH : CH \cdot CH : CH \cdot CH : CH \cdot (CH_2)_7 \cdot COOH$ Linolenic acid

each of which forms about 40 per cent of the total fatty acids of linseed oil. These are 18-carbon acids. Rape seed oil contains nearly half its weight of erucic acid glycerides. Erucic acid contains 22 carbon atoms in its chain:

$CH_3 \cdot (CH_2)_7 \cdot CH : CH \cdot (CH_2)_{11} \cdot COOH$ Erucic acid

Cod liver oil contains a high percentage of tetra- and penta-ethnoid unsaturated fatty acids of 20- and 22-carbon acids.

The effect of these fatty acids on the composition of the fats of milk is shown in the following table:

COMPONENT ACIDS OF COW MILK FATS AS AFFECTED BY FOOD FATS

ACID	MOLAR PERCENTAGES			
	Control	Linseed	Rape	Cod Liver
Butyric	11.5	11.2	9.9	5.7
n-Hexanoic	4.3	4.1	3.4	1.2
n-Octanoic	3.7	2.1	2.3	1.6
n-Decanoic	5.0	3.1	2.2	1.9
Lauric	5.0	3.6	3.4	5.1
Myristic	10.8	8.6	9.1	7.4
Palmitic	20.5	20.0	17.0	22.8
Stearic	10.1	8.2	11.7	7.3
As arachidic	0.5	0.4	0.4	0.5
Oleic	23.3	32.8	34.0	38.3
As octadecadienoic	4.5	5.0	3.2	4.1
As C_{20-22} unsaturated	0.8	0.9	0.8	4.1
As erucic	...	...	2.6	...

From the above table it is apparent that feeding cod liver oil reduced the lower-saturated fatty acids of the milk fat by one-half; the content of oleic acid is greatly increased, and the content of unsaturated acids of C_{20-22} is five times the normal. It is suggested that selective adsorption of the highly unsaturated fatty acids by the enzymes responsible for the elaboration of typical cow milk fats retards the normal enzymatic function. These newer researches greatly extend our knowledge of the types of chemical reactions which take place in the mammary gland, while milk secretion is in progress. Since a relatively small amount of the total milk obtained is in the turgid gland before milking starts and the gland forms most of the casein, fats, and milk sugar of milk during the short interval required to remove it in the milking process, the rapidity with which the formation of milk takes place in a high producing cow (yielding as much as 40,000 lbs. of milk a year) is truly phenomenal. A cow yielding 100 lbs. of milk daily, in three milkings, would transform more than half of the pound or more of fats yielded in one milking into modified fatty acids during an interval of a few minutes.

The Effects of Feeding Cholesterol to Animals.—When cholesterol, dissolved in oil, is fed to rabbits or guinea pigs there is a deposition of cholesterol esters (cholesterol-fatty acids) in the aorta, producing a condition simulating atherosclerosis as seen in human subjects. This has not been observed in most laboratory animals. Duff ('35) affords good experimental evidence that there is marked change in the permeability of the arterial walls and that anatomical damage and deposition of lipids, especially cholesterol esters, results from this rather

than from hyperlipemia and hypercholesterolemia. Thyroidectomy causes similar changes in the arterial walls. There is no correlation, however, between the blood cholesterol values and the degree of atherosclerosis in human accident cases, which strongly indicates that the level of this substance is not the primary cause of the disease but it may be a contributing factor. Elliot and Nuzum ('36) and Page and associates ('36) have shown that there is no apparent relationship between the concentration of the various blood lipids and the occurrence of essential hypertension in man. Sperry and Schick ('36) report that certain cases of xanthomatosis (a new growth in the skin consisting of connective tissue undergoing partial fatty degeneration) are improved by excluding from the diet cholesterol-rich foods. Other cases were not benefited. It is of interest that dogs deprived of magnesium have a high cholesterol ester content in their blood (Kruse, Orent and McCollum, '33).

Best and Ridout ('36) have recently reported the effects of choline feeding on the cholesterol ester content of the liver in rats in which fatty liver has been produced by feeding cholesterol. They found that the addition of choline causes a very definite fall in both glyceride and cholesterol ester content of the liver. Such fatty liver is produced by feeding a diet containing 2 per cent of cholesterol. When this high intake of cholesterol exists, choline administration may cause a fall in the glyceride level while the cholesterol esters are increasing, but later the effects of choline come into prominence.

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CHAPTER

V

General Nature of Proteins and Amino Acids and Their Significance in Nutrition

IT SHALL BE the purpose in this chapter and the one to follow to review and summarize the most pertinent evidence concerning the nutritional significance of proteins and amino acids, but it will not be possible to furnish a comprehensive treatment owing to the lack of space.

Investigations in the field of protein nutrition have had as their objectives the solution of many problems, some of which are: (a) the number and nature of the amino acids contained in proteins; (b) the amounts of each of these yielded by different proteins; (c) the various functions of proteins and their amino acids in the animal body; (d) the mechanisms of protein metabolism; (e) the mechanisms of protein formation in both the animal body and plant; (f) the factors affecting protein digestion and utilization; (g) the ability of tissues to synthesize amino acids; (h) the precursors of amino acids synthesized in the body; (i) the protein and amino acid requirements for various life functions, e. g., growth, maintenance, reproduction, and lactation; and (j) the most economical and physiologically satisfactory dietary combination of proteins and amino acids to promote the desired nutritional status.

General Functions of Proteins in the Body.—It is difficult to state a definite time in history when anyone first became concerned about the nature of proteins and their biological functions. Mulder (1839), a Dutch chemist, definitely recognized the general similarity between plant and animal proteins and indicated some appreciation of the rôles of proteins in bodily functions. He had the distinction of coining the name protein (from the Greek word, proteios, meaning primary). Gradually the concept developed that proteins are indispensable dietary factors which are necessary for the formation of tissue protein

and the replacement of nitrogenous compounds lost in metabolism. This is clearly evident in the theories of Liebig, Pflüger, and Voit concerning the mechanism of protein metabolism, which will be considered subsequently.

As early as 1866 it was learned that proteins are not essential as a source of energy if carbohydrate and fat are furnished in sufficient amount (Pettenkofer and Voit), but a realization that the functions of protein cannot be satisfied without regard to the amino acid composition of dietary protein was not appreciated before 1872. At that time Carl Voit found that gelatin could not be substituted for meat protein in the diet of dogs without the loss of body protein. By the beginning of the twentieth century the view had become generally accepted that the dietary functions of protein could not be served without regard to the "nutritive quality" of the constituent amino acids.

During the past thirty or forty years, and particularly the last fifteen years, we have witnessed marked progress in the chemistry and physiology of proteins. At present it appears that all enzymes and several of the hormones are protein in composition. Even viruses and bacteriophage, as yet mysterious indeed in function, have been shown, in some instances, to have the general nature of protein. Thus, in addition to the well-recognized role of proteins in the structure of various tissues, particularly muscle, the maintenance of fluid balance, and the mechanism of respiration and neutrality regulation, proteins are components of specific bodily activities which include those of enzymes, hormones, and viruses.

At present the following enzymes are known to be simple proteins: urease, pepsin, trypsin, chymotrypsin, and carboxypeptidase. In addition, Kunitz and Northrop ('33) have prepared crystalline chymotrysinogen and pepsinogen, and have shown that they are protein in nature. Also, Theorell ('34) has crystallized the yellow oxidation enzyme of Warburg and Christian, and found it to be a protein with adsorbed riboflavin. Certain enzymes, as for example catalase, are composed of a protein combined with a prosthetic group.

The definition of an enzyme as a catalyst, which is elaborated by living cells and which may act independently of the life processes of the cells, still holds in the light of recent researches, but the older theory that the proteins acted as carriers of the actual enzymes, must, it seems, be discarded. One cannot say at present, however, that all enzymes are protein, since emulsin is not inactivated by pepsin or trypsin.

Theories of Protein Metabolism.—It is appropriate to give some attention to protein metabolism, since one can scarcely contemplate

any nutritional aspect of proteins and amino acids without reference to their metabolism. The subject is deeply grounded in history and it is helpful to review certain aspects of it here.

Definite ideas of protein metabolism have existed ever since the time of Liebig. Voit's theory, which replaced the outmoded views of Liebig, was derived from metabolic experiments with dogs. He observed that a well-fed dog, subjected to fasting, excreted, at first, an abundance of nitrogenous waste products, but as the fast continued very little nitrogenous matter was excreted in the urine. This suggested to Voit that a marked destruction of circulating protein occurs for a time, during fasting, until this reserve is reduced to a low level. The nitrogen excreted after reaching this low level was believed to have its origin in the organized tissues. Voit attributed to the cells the function of utilizing proteins, believing that the circulating protein is drawn into the cells for transformation into degradation products which later appear in the urine.

Voit's theory, first formulated in 1867, was criticized by Pflüger in 1893, who presented a new one in its place. Pflüger held that protein must become living protoplasm before it can be utilized for the needs of the body. His pupil, Schöndorff, perfused the blood of a starving dog through its hind limbs, and the liver of a well-fed dog, and found an increase in the excretion of urea. But when the blood of a well-fed dog was perfused through the same channels as those of the starving dog the output of urea was not increased. This indicated that the determining factor in protein destruction is the state of nutrition in the tissue cells and not the circulating protein. Physiologists were not satisfied with Pflüger's hypothesis, since it was clear from Voit's experiments that very large quantities of protein may be metabolized with great rapidity, as shown by the large output of urinary nitrogen following the ingestion of a high-protein meal. It was difficult to believe that living protoplasm could be synthesized so rapidly, and be almost immediately destroyed.

Folin ('05) possessed the genius to solve the problem in its main outlines, and his interpretation of the mechanism of protein metabolism is almost universally accepted. He devised analytical procedures which were less time-consuming and more accurate than any hitherto available for the estimation in small amounts of all the principal constituents of urine, together with methods for the estimation of three types of sulfur compounds which it contains. By means of these methods complete analyses were made of urines from subjects (human) given a high protein diet, followed by a period of low protein consumption. The differences, as influenced by the protein intake, between the daily urines of two individuals (A and B) are illustrated by the following record:

	High-protein Diet		Low-protein Diet	
	A	B	A	B
Total nitrogen	14.8 gm.	18.2 gm.	4.8 gm.	8.0 gm.
Urea nitrogen	86.3%	89.4%	62.0%	80.4%
Ammonia nitrogen	3.3%	5.1%	4.2%	11.4%
Creatinine nitrogen	3.2%	4.5%	5.5%	11.1%
Uric acid nitrogen	0.5%	1.0%	1.2%	2.4%
Undetermined nitrogen	2.7%	5.3%	4.8%	14.6%
Total sulfur	3.11 gm.	3.73 gm.	1.04 gm.	1.65 gm.
Inorganic sulfur	89.4%	87.2%	60.6%	83.6%
Ethereal sulfur	6.5%	7.2%	11.5%	8.4%
"Neutral" sulfur	4.1%	5.5%	27.9%	8.0%

On the basis of his studies, Folin deduced the following generalizations:

1. The absolute amount of creatinine eliminated by a man on a meat-free diet (meat contains creatinine) is a constant quantity, different for different individuals, but independent of the amount of protein metabolized, and hence, of the nitrogen eliminated.

2. When the total amount of protein metabolized is greatly reduced, the absolute quantity of uric acid excreted is diminished, but not nearly in proportion to the diminution in total nitrogen eliminated, and the per cent of uric acid nitrogen in the urine in terms of the total is much increased.

3. When the amount of protein metabolized is small, and consequently the output of nitrogen in the urine is greatly reduced, there is usually, but not necessarily, a decrease in the output of ammonia. A pronounced reduction of the total nitrogen is, however, always accompanied by a relative increase in the ammonia-nitrogen, provided the food does not yield an alkaline ash.

4. With pronounced diminution of the total nitrogen eliminated, there is a corresponding reduction in the per cent of the total nitrogen of the urine represented by urea. When the total daily nitrogen excretion is reduced to 3 to 4 gm., about 60 per cent of it only is in the form of urea.

5. The inorganic sulfate excretion, in percentage of the total, falls when there is diminution of the total sulfur output.

6. The neutral sulfur elimination is analogous to that of creatinine. It represents products which are, in the main, independent of the total amount of sulfur eliminated, and hence, of protein metabolized.

7. Ethereal sulfates in the urine represent a form of sulfur metab-

olism which becomes more prominent when the diet provides little or no protein.

On the basis of his data Folin reasoned that protein metabolism must be of two different kinds. One is extremely variable in quantity, whereas the other remains nearly constant. The first yields chiefly urea and inorganic sulfates, no creatinine, and probably no neutral sulfur. The second, or constant catabolism, is largely represented by creatinine and neutral sulfur, and to some degree by uric acid and ethereal sulfates. As the total catabolism of protein is reduced, the less prominent becomes the type represented by urea and inorganic sulfates. While urea and inorganic sulfates represent chiefly the variable catabolism, the possibility is not precluded that they also represent to some extent the constant type of metabolism.

Folin distinguished the constant type of protein catabolism as the "endogenous," in contrast with the "exogenous" type. The former represents the protein transformations involved in metabolism, chiefly oxidative, of living tissues; the latter represents the hydrolytic cleavage and rapid degradation of protein digestion products into urea, inorganic sulfates, etc.

The physiological state represented by the constant type of protein in metabolism will remain nearly unchanged only when sufficient carbohydrate is ingested to maintain energy equilibrium. In the absence of sufficient carbohydrate for this purpose, protein degradation, at the expense of tissue protein, will take place, about 58 per cent of the carbon being converted into carbohydrate.

Therefore in the absence of sufficient carbohydrate, as was shown by Ringer (1912), an additional amount of protein is essential, above the "wear and tear" quota. This portion of dietary protein can be spared by carbohydrate, but not by fats, since all efforts to demonstrate the formation of glucose from fats have thus far failed.

Folin and Denis (1912) applied their analytical methods for the determination of urinary constituents to the elucidation of the mechanism of protein metabolism. They clearly demonstrated that, as amino acids are absorbed from the gut, their concentration first rises in the portal blood, then in the liver, and as the blood passes from the liver into the systemic circulation, the latter shows a high tide of amino acids. The high level in the blood soon falls, while that of the muscles and organs rises, showing that these tissues remove amino acids from the blood. Simultaneously with the flooding of the liver, systemic blood, and tissues with amino acids, the formation of urea begins, and continues until the blood and tissue content of these acids returns to the fasting level, after absorption from the gut is completed. Van Slyke and Cullen confirmed Folin and Denis' findings in every

detail, using Van Slyke's direct method for estimating amino acids.

The significance of the "deposit protein" which is retained for a time after high-protein feeding, and which puzzled the older investigators, is now clearer. It is not protein, but amino acids, held in the tissues, which form a reservoir. The lag extending over several days, when extreme changes are made in the protein intake, is explained by the existence of this reservoir.

The origin and significance of "neutral sulfur" and creatinine are still obscure. It seems necessary to conclude that they arise through certain reactions in the body which proceed at constant velocity, and are not associated with the rate of metabolism of ingested foodstuffs. Urea and sulfate sulfur, on the other hand, are proportional to the amount of catabolism, both of ingested protein which never becomes involved as part of the structure of living tissues, and of tissue proteins as well.

General Properties of Amino Acids.—When proteins are subjected to appropriate hydrolytic action, amino acids may be isolated in pure form from the hydrolysate. It is uncertain how many amino acids exist in nature, combined as protein, but the number is certainly greater than 20.

The following table, modified in the light of recent researches, is taken from the extensive monograph of Vickery and Schmidt ('31). It shows the names of the known amino acids, derived from protein, and the dates and names of their discoverers.

Amino Acid	*Earliest Observation of the Amino Acid as a Product of Hydrolysis of Proteins*	
Glycine	Braconnot	1820
Leucine	Braconnot	1820
Tyrosine	Bopp	1849
Serine	Cramer	1865
Glutamic acid	Ritthausen	1866
Aspartic acid	Ritthausen	1868
Phenylalanine	Schulze and Barbieri	1881
Alanine	Weyl	1888
Lysine	Drechsel	1889
Arginine	Hedin	1895
Iodogorgoic acid	Drechsel	1896
Histidine	Kossel, Hedin	1896
Cystine	Mörner	1899
Valine	Fischer	1901
Proline	Fischer	1901
Tryptophane	Hopkins and Cole	1901
Oxyproline	Fischer	1902
Isoleucine	Ehrlich	1903
Thyroxine	Kendall	1915
Hydroxyglutamic acid	Dakin	1918
Methionine	Mueller	1922
Threonine	Rose	1935

Several other amino acids have been reported to be derived from proteins but they have been studied by a single investigator only. These include the following:

Dibromotyrosine	Mörner	1913
Norleucine	Thudichum, Abderhalden and Weil	1901, 1913
Hydroxyaminobutyric acid	Schryver and Buston	1926
Hydroxyvaline	Schryver and Buston	1926
Hydroxylysine	Schryver and Buston	1925
Protoctine	Schryver and Buston	1926
Dihydroxyphenylalanine	Guggenheim	1913
Citrulline	Wada	1930
Djenkolic acid	Van Veen and Hyman	1935
Canavanine	Kitagawa	1929

The structure of the amino acids is shown by the following formulae:

I. Glycine, or amino-acetic acid: $CH_2(NH_2)\cdot COOH$

Alanine, or α-amino-propionic acid:

$$CH_3\cdot CH(NH_2)\cdot COOH \qquad (d-)$$

Valine, or α-amino-isovalerianic acid:

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CH\cdot CH(NH_2)\cdot COOH \\ \diagup \\ CH_3 \end{matrix} \qquad (d-)$$

Leucine, or α-amino-isocaproic acid:

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CH\cdot CH_2\cdot CH(NH_2)\cdot COOH \\ \diagup \\ CH_3 \end{matrix} \qquad (l-)$$

Isoleucine, or α-amino-β-methyl-β-ethyl-propionic acid:

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CH\cdot CH(NH_2)\cdot COOH \\ \diagup \\ C_2H_5 \end{matrix} \qquad (d-)$$

Norleucine, or α-amino-caproic acid:

$$CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH \qquad (d-)$$

II. Amino dicarboxylic acids:

Aspartic acid, or α-amino-succinic acid:

$$HOOC\cdot CH_2\cdot CH(NH_2)\cdot COOH \qquad (l-)$$

Glutamic acid, or α-amino-glutaric acid:

$$HOOC\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH \qquad (d-)$$

III. Hydroxy-amino acids:

Serine, or α-amino-β-hydroxy-propionic acid:

$$CH_2OH \cdot CH(NH_2) \cdot COOH \qquad (l\text{–})$$

Threonine, or α-amino-β-hydroxy-butyric acid:

$$CH_3 \cdot CHOH \cdot CH(NH_2) \cdot COOH \qquad [d(-)]$$

Hydroxyglutamic acid, or α-amino-β-hydroxy-glutaric acid: $HOOC \cdot CH_2 \cdot CHOH \cdot CH(NH_2) \cdot COOH$ (d–)

IV. Amino acids with aromatic nuclei:

Phenylalanine, or α-amino-β-phenyl-propionic acid:

$$C_6H_5 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \qquad (l\text{–})$$

Tyrosine, or α-amino-β-p-hydroxyphenyl-propionic acid: $HO \cdot C_6H_4 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$ (l–)

Iodogorgoic acid, or 3, 5-diiodotyrosine:

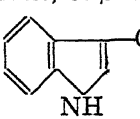
$$HO \cdot C_6H_2I_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \qquad (?\text{–})$$

Thyroxine, or β-[3, 5-diiodo-4-(3′, 5′-diiodo-4′-hydroxyphenyl)phenyl]-α-amino-propionic acid:

$$HO \cdot C_6H_2I_2\text{–}O\text{–}C_6H_2I_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \qquad (l\text{–})$$

V. Amino acid with an indole nucleus:

Tryptophane, or β-indole-α-amino-propionic acid:



(l–)

VI. Amino acids containing sulfur:

Cystine, or dicysteine, or di-(β-thio-α-amino-propionic acid):

$$HOOC \cdot CH(NH_2) \cdot CH_2 \cdot S\text{–}S \cdot CH_2 \cdot CH(NH_2) \cdot COOH \qquad (l\text{–})$$

Methionine, or α-amino-γ-methylthiol-butyric acid:

$$CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \qquad (l\text{–})$$

Djenkoic acid, or methylene-di-[β-thiol-α-amino-propionic acid]:

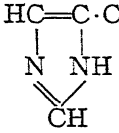
$$CH_2[S \cdot CH_2 \cdot CH(NH_2) \cdot COOH]_2$$

VII. Basic amino acids:

Lysine, or α, ϵ-diamino-caproic acid;

$$H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH \qquad (d\text{–})$$

Histidine, or β-imidazole-α-amino-propionic acid:



(l–)

Arginine, or δ-guanidine-α-amino-valeric acid: (d–)

$H_2N \cdot C(: NH) \cdot NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

Ornithine, or α, ε-diamino-valeric acid, (not derived from proteins):

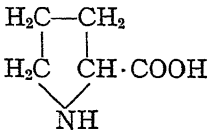
$H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

Citrulline, or δ-carbamide-α-amino-valeric acid, (isolated from melon juice):

$H_2N \cdot CO \cdot HN \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$ (?–)

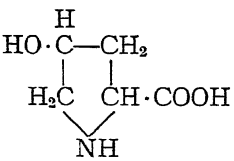
VIII. Amino acids with pyrrole nuclei:

Proline, or α-pyrrolidine carboxylic acid:



(l–)

Hydroxyproline, or γ-hydroxy-α-pyrrolidine carboxylic acid:



(l–)

It is evident from the tables that the general formula, R-CH (NH_2)COOH, is common to all the amino acids except proline and hydroxyproline. Since all amino acids contain both acidic and basic groups, they are amphoteric substances. Certain of the amino acids have rather unique structures, as for example, tyrosine, which is a substituted phenol. Thus it is relatively easy to isolate some of the amino acids in pure form, but most of them are difficult to isolate and purify owing to the lack of sufficient differences between them in chemical properties.

Emil Fischer demonstrated that the monoamino acids, after conversion into their esters, can be fractionally distilled *in vacuo*. The use of butyl alcohol as a means of isolating the monoamino acids from

other protein hydrolysates was discovered by Dakin. The method is of considerable advantage since it permits the separation of amino acids into three groups: monoamino acids insoluble in dry butyl alcohol; proline and hydroxyproline soluble in dry butyl alcohol; and the basic and dicarboxylic acids insoluble in wet butyl alcohol.

Various other procedures are employed for the isolation of these substances. They include the use of heavy metals, as precipitants, and electrolytic methods. The separation of the basic amino acids by means of electrolysis was made practicable by Foster and Schmidt. Methods have been devised for the synthesis of all the amino acids but even the best procedures for certain ones are too expensive for their production in sufficient amounts to be useful in nutritional research. Moreover, all the amino acids, except glycine, are optically active. The synthetic products are racemic mixtures and, therefore, generally unsuitable for many types of nutritional investigation as well as general dietary uses. This is owing to the almost complete inability of the body to utilize amino acids which are optically unnatural. The development of economical methods for the isolation of natural amino acids in pure form will constitute an important contribution. Without such methods nutritional and physiological investigations of amino acids will continue to be greatly hampered.

Structure of the Proteins.—Since the amino acids may be formed by acid hydrolysis of proteins, it is inferred that the former are linked together in chains through abstraction of a molecule of water from an amino group of one, and a carboxyl group of another:

$$\underset{\text{Glycine}}{H_2N\cdot CH_2\cdot COOH} + \underset{\text{Glycine}}{HHN\cdot CH_2\cdot COOH}\cdots\cdot \rightarrow \underset{\text{Glycylglycine}}{H_2N\cdot CH_2\cdot CO\cdot HN\cdot CH_2\cdot COOH}.$$

The general correctness of this view is shown by the fact that methods have been developed whereby such complex *peptides,* di-, tri-, tetra-, etc., can be prepared from amino acids. These are hydrolyzed, or digested, into their constituent amino acids on treatment with appropriate enzymes. Mild hydrolysis of proteins with acids leads to the formation of peptides, and they have been many times isolated from digestion mixtures where the hydrolyzing agent is an enzyme. There can be no question about the general nature of protein structure being represented by such polypeptide patterns.

On estimating quantitatively the yields of different amino acids from different proteins, such as gelatin, blood fibrin, casein, zein, gliadin, etc., it is found that these vary greatly. Only fifteen amino acids have been found in gelatin, other proteins yield twenty or more but in very different amounts. They consist of long chains of

amino acids in peptide union. Studies of proteins by physical methods demonstrate they they exist in the form of polypeptide rows, folded so as to make the molecules compact. Apparently these rows are held together by secondary valences.

In this brief introduction to a discussion of proteins and amino acids in their relation to nutrition, we are especially concerned with the yields of amino acids, since these constitute the "building stones" for the construction of tissue proteins. It is largely upon these yields that the nutritive value of proteins depends. Students who desire to learn the technic by which present-day investigators are making progress in determining the structure of protein, viz., the order in which individual amino acids are linked in the protein molecule, are referred to the recent papers by Bergmann and his coworkers (1936). However, in view of the recent fundamental progress in protein structure it is profitable, even in a textbook of nutrition, to include some of the information available at present concerning the subject.

The classical studies of Emil Fischer on the synthesis of polypeptides demonstrated that protein molecules are essentially peptide chains composed of many amino acid units. The differences in solubility, temperatures of coagulation, and yields of the various amino acids, show that proteins from diverse sources are of different composition. Serological studies have shown that each species synthesizes characteristic and specific proteins from the heterogenous mixture of amino acids which are absorbed from the digestive tract after the digestion of "foreign" food proteins.

The possible number of combinations of the 22 known amino acids in peptide chains is astronomical in magnitude. It is desirable that we understand the properties of protein molecules from different sources, as expressed in molecular weight, amino acid composition, arrangement of the amino acids in the peptide chains, and configuration of the molecules. Significant advances have been made in recent years in these directions.

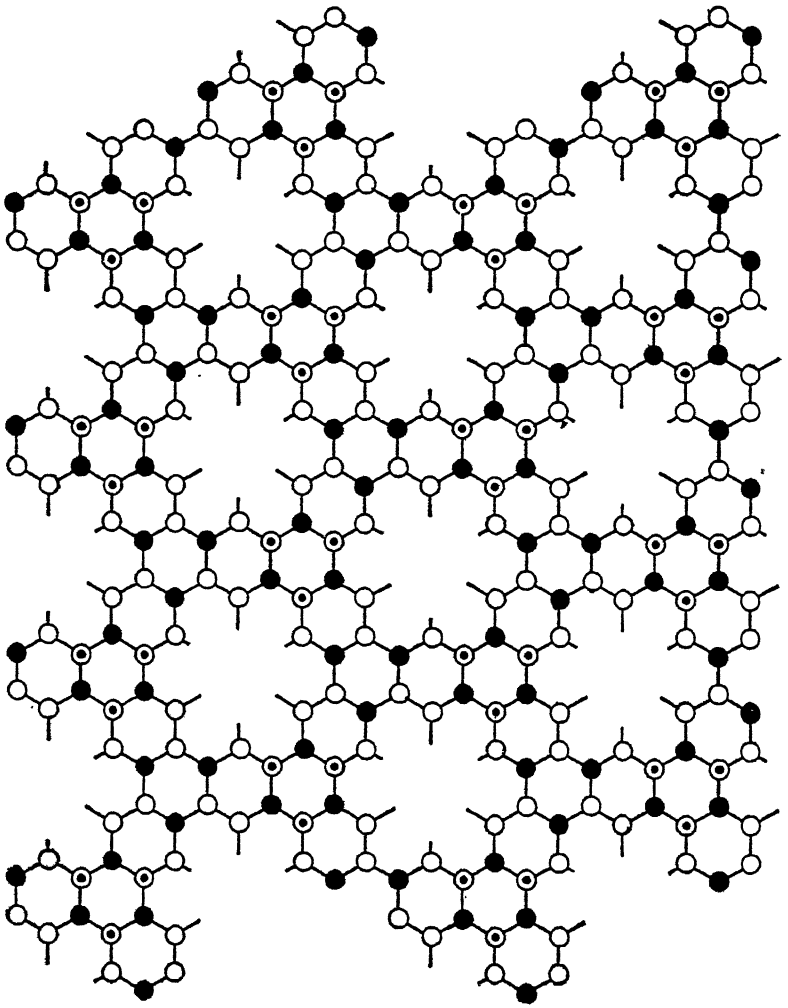
Svedberg ('34) perfected the technique of determining the molecular weights of proteins by observing the rate at which they sediment in centrifugal fields. With the ultracentrifuge, which he perfected, solutions can be subjected to forces up to 400,000 times gravity. Colloidal systems, like gold sols, are by this procedure shown to contain a continuous series of particles of varying mass and form. A protein solution, on the other hand, is either homogeneous with regard to particle mass and shape, or contains a limited number of particle species of different mass and shape. Each species proves to be homogeneous in these respects. The measurement of molecular sedimentation in centrifugal fields shows that most of the native proteins are homogeneous as regards molecular weight within certain pH ranges.

Each protein has a characteristic pH stability region. When the borders of the stability ranges are exceeded, disintegration or aggregation takes place. At pH 11 and beyond, disintegration of the protein molecule occurs. At a certain degree of acidity in the solution denaturation and aggregation to large particles occur. Many proteins possess, in the pH range of stability, molecular weights which are very great, but when brought to a certain degree of alkalinity characteristic of the protein, they dissociate into fragments having a molecular weight of about 34,500. Thus the hemocyanin, i. e., copper respiratory pigment, of the horseshoe crab exists in four components of molecular weights of 2,600,000; 1,300,000; 325,000 and 108,000, within the range from pH 5 to 10. Amandin, a globulin from the date, which has a molecular weight of 208,000 under conditions of stability, decomposes into fragments of about 34,500, or one-sixth the molecular weight at pH 12. The molecular weight of ovalbumin is 34,500, and most of the homogeneous proteins have been shown by the sedimentation method to possess molecular weights of 34,500, or simple multiples of this number. Gelatin is not homogeneous but consists of particles varying from 10,000 to 75,000 in molecular weight. Wyckoff ('36) found the molecular weight of Felton pneumococcic antibody to be 500,000. Beard and Wyckoff ('37) found the molecular weight of the heavy protein from virus-inducing rabbit papilloma to be in excess of 20,000,000. Virus proteins appear to have extraordinarily high molecular weights (as shown by Stanley and Wyckoff, '37). Svedberg views the protein molecule as globular in form.

That proteins are not made up of polypeptides of every conceivable length is shown by their molecular weights. The fact that different proteins contain, in peptide linkage, amino acids of different molecular weights, accounts for the observed deviation from 32,000 to 35,000 for the characteristic dispersed fragments in the upper pH region of instability.

Wrinch ('37) has proposed the cyclol theory of protein structure to account for the observed properties of proteins brought to light by Crowfoot's ('35) X-ray analysis of these substances, and by the structure of unimolecular films, multilaminar proteins, and globular structure proposed by Svedberg. According to this theory each space-enclosing molecule of protein consists of a piece of cyclol fabric composed of polypeptides folded into ring structures, and held together by bonds, the nature of which, i. e., whether hydrogen bonds, "cyclol links," or hydroxyl bonds, has not been decided. She conceives this network to lie on the surface of an imaginary truncated tetrahedron, and to possess a configuration with four triangular faces and four hexagonal faces. This polyhedral configuration is in agreement with Svedberg's "globular" molecule, and accounts for its globularity. Wrinch illustrates the

cyclol fabric pattern of insulin, which consists of a molecule containing 288 amino acid residues, by the following picture.



Bergmann and coworkers have made some progress in determining the order of arrangement of certain of the amino acids in peptide chains. The following table shows the molecular weights of four proteins, and the numbers of amino acids which are present in their molecules. All the amino acids included in this table can be estimated with considerable exactness.

THE NUMBER OF AMINO ACID RESIDUES IN THE MOLECULES OF CATTLE HEMOGLOBIN, CATTLE FIBRIN, CHICKEN EGG ALBUMIN AND SILK FIBROIN*

AMINO ACID	NUMBER OF AMINO ACID RESIDUES PER MOLECULE			
	Cattle Hemoglobin	Cattle Fibrin	Chicken Egg Albumin	Silk Fibroin
All amino acids	$2^6 \times 3^2$	$2^6 \times 3^2$	$2^5 \times 2^2$	$2^5 \times 3^4$
Arginine	$2^2 \times 3^1$	$2^5 \times 3^0$	$2^2 \times 3^1$	$2^2 \times 3^1$
Lysine	$2^2 \times 3^2$	$2^4 \times 2^1$	$2^2 \times 3^1$	$2^2 \times 3^0$
Histidine	$2^5 \times 3^0$	$2^2 \times 3^1$	$2^2 \times 3^0$	$2^0 \times 3^2$
Aspartic acid	$2^5 \times 3^0$	$2^5 \times 3^0$	$2^4 \times 3^0$	...
Glutamic acid	$2^4 \times 3^0$	$2^3 \times 3^2$	$2^2 \times 3^2$	...
Glycine	...	...	...	$2^4 \times 3^4$
Alanine	...	...	...	$2^3 \times 3^4$
Tyrosine	$2^2 \times 3^1$	...	$2^3 \times 3^0$	$2^1 \times 3^4$
Proline	$2^2 \times 3^1$	$2^5 \times 3^3$	...	...
Tryptophane	...	$2^1 \times 3^2$	...	...
Cysteine	$2^0 \times 3^1$	$2^0 \times 3^2$	$2^2 \times 3^0$	...
Methionine	...	$2^2 \times 3^1$	$2^2 \times 3^1$	...

* Bergmann and Niemann (l.c.).

In the case of cattle hemoglobin the molecule contains 576 or $2^6 \times 3^2$ amino acid residues. Of this total there are 12 arginine, 32 histidine, 36 lysine, 32 aspartic acid, 16 glutamic acid, 12 tyrosine, 12 proline, and 3 cystine residues.

They show that the frequencies of the individual amino acid residues $\left(F_i \frac{N_t}{N_i}\right)$ that are contained in a molecule of protein can be expressed by the following equations:

(1) $N_t = 2^n \times 3^m$ where n and m are positive whole numbers.
(2) $N_i = 2^{n'} \times 3^{m'}$ where n' and m' are either zero or positive whole numbers.
(3) $F_i = 2^{n''} \times 3^{m''}$ where n'' and m'' are either zero or positive whole numbers.
(4) $n = n' + n''$, and $m = m' + m''$.
(5) $N_t = N_i' + N_i'' + N_i''' + \cdots\cdots\cdots\cdots N_i^x$.

N_t = total number of individual amino acid residues.
N_i = number of the individual amino acid residues.

It is significant that the experimentally determined values of N_i', N_i'', N_i''', etc., and of F_i', F_i'', F_i''', etc. have led to values of N_t that are whole number multiples of 288 or $2^5 \times 3^2$, and it appears that the molecules of many proteins which do not show reversible

dissociation contain 288 amino acid units or a whole multiple thereof. Since this stoichiometrical law is of general validity it follows that every amino acid residue in the peptide chain of the protein molecule recurs at constant intervals. As an example, from the deductions of Bergmann and Niemann ('37), each glycine residue, G, in silk fibroin is separated from the adjacent glycine residue by an amino acid residue other than glycine, e. g.,

–G–X–G–X–G–X–G–X–G–X–G–X–G–X–G–X–

Each residue of alanine, A, is separated from the adjacent residues of alanine by 3 other residues, e. g.,

–A–X–X–X–A–X–X–X–A–X–X–X–A–X–X–X–A–X–X–X–A–

Each tyrosine residue, T, is separated from the adjacent tyrosine residues by 15 other residues, e. g.,

–X–T–X–X–X–X–X–X–X–X–X–X–X–X–X–X–T–X–X–
X–X–X–X–X–X–X–X–X–X–X–X–X–X–T–X–

On combining the above configurations the structure of a segment of the silk fibroin molecule is obtained, i. e.,

G–A–G–X–G–A–G–X–G–A–G–T–G–A–G–X–G–A–G–X–
G–A–G–X–G–A–G–T–G

* To the older demonstrated fact that the linkage of the amino acid residues is through the medium of the peptide bond (—CO—NH—CHR—), to form long polypeptide chains, are added the new concepts: (1) that the amino acid residues recur in a characteristic and periodic manner throughout the entire peptide chain, and (2) that these long chains fold upon themselves to form cyclic patterns, which are held in a fabric structure. The latter, in turn, is folded into space-enclosing geometrical figures (Wrinch). The physico-chemical and physiological properties of a protein are based on the frequencies with which its constituent amino acid residues recur within its peptide chains. Since many of the amino acids contain radicals having very different properties, e. g., the hydroxyphenyl group in tyrosine, the guanidine complex in arginine, the pyrrole nucleus in proline and hydroxyproline, the —SH group in cystine, —OH group in serine and in hydroxyglutamic acid, and the indole nucleus in tryptophane; and since, with a single exception (glycine), each of the amino acids contains an asymmetric carbon atom, and only one of the optical isomers of each plays a biological rôle, the fabric pattern, conceived of as representing the structure of the molecule of protein, must be

visualized as having various chemically active groups oriented in space on either side of its median plane.

Monomolecular Films.—A new technic which offers extraordinary possibilities for studying proteins has been described by Blodgett ('35) and by Langmuir, Schaefer and Wrinch ('37). By means of Blodgett's methods, which cannot be described here, monomolecular films, or multi-layer films of protein molecules, can be applied to a solid surface such as chromium-plated brass plate. The plate is first coated with many layers of monofilms of barium stearate. A monomolecular film of the protein (egg albumin or pepsin) is spread upon the surface of a tray of distilled water. There is then an upper, protein-air surface and an under, protein-water surface. If the plate is lowered vertically through the film the latter becomes applied to its surface, the protein-air surface being next the barium stearate surface. While the plate is submerged the protein layer is removed from the surface of the tray, and the plate then lifted out. It comes out wet, whereas if the protein film had not been placed on the barium stearate surface it would have come out dry, since the stearate surface does not wet with water. After evaporating the surface film of water the protein film of one molecule thickness is attached to the plate. Examination of the surface with polarized light, at angles near the grazing incidence, shows that the color differs markedly from the original barium stearate film. The thickness of the protein film is about $\frac{20}{100{,}000{,}000}$ cm.

If, on the other hand, the plate is lowered into the water, and a monomolecular layer of protein is then spread upon the water surface, and the plate then lifted out, edge up, a protein film is applied to its surface, but in this case it is the protein-water surface of the film which is adjacent to the barium stearate surface. Again the plate comes out wet, and after evaporating the water the protein film comes into contact with the barium stearate. The film prepared in this way is called a B-film. By applying a film, drying the plate, and repeating the dipping process, a second monofilm may be applied over the first. Blodgett designates the plate surface as P, the barium stearate surface as R, and the protein films prepared by the two methods, as A film and B film respectively.

When a PRA system has been prepared and the plate is dipped into water having a monomolecular film of protein on its surface, a B film is applied giving a system PRAB. When this is dried and is lowered into pure water or water having a protein film on its surface the B film is ejected. If zinc chloride is added to the water the B film is not ejected on dipping the plate, and AB layers can be applied successively, giving a system PRABABAB.....indefinitely.

Without adding zinc salts to the water a succession of B films can be applied, giving a system PRBBB.....indefinitely. After a single B film has been applied, and dried, the plate takes on an A film when it is lowered through a protein film, giving PRBA, and on this a B film can be applied, giving PRBAB. In order to apply more layers it is necessary to add zinc salt.

A B layer which lies upon an A layer is ejected when the plate is dipped, whereas a B layer which lies upon a B layer is not ejected. From the method of applying the films it is evident that A layers are inverted, or turned upside down in being applied, whereas the B layers are not inverted. Since the protein molecules are free to orient themselves when spread upon water, the hydrophylic groups on the molecules orient to the water surface, and the hydrophobic groups orient toward the air. The fact that A and B layers preserve their identity after immersing in water indicates that they cannot readily turn over. This, it is pointed out, supports the theory that the films consist of a two-dimensional network rather than polypeptide chains. "The most striking evidence that the outer surface of A and the inner surface of B are predominantly hydrophylic is furnished by the ejection on to the water of a B layer, which rests upon an A layer. This action is undoubtedly caused by the affinity of this hydrophylic interface for water."

The employment of the supercentrifuge for the separation of proteins by sedimentation, and the perfection of a technic for crystallizing proteins, by Stanley, Wyckoff, and others, together with the methods mentioned in this chapter, gives promise of steady advances in our knowledge of these substances and the rôles which they play in physiological processes. There is good reason to expect useful advances in the discovery of special reagents for the quantitative determination of the amino acid content of proteins, and it is highly probable that within a few years the composition and structure of many of the more readily obtainable proteins will be known with considerable exactness. The known rôles of proteins as enzymes, and as viruses, greatly heighten the interest of investigators in this field. Further developments along these lines no doubt will be of use in the field of nutrition.

Classification of the Proteins on the Basis of Their Chemical Composition.—The classification of most proteins has been based upon their solubilities and other physical properties. Certain ones are easily distinguished by their characteristic prosthetic groups, e. g., mucoproteins, phosphoproteins, nucleoproteins, hemoglobins, etc. The difficulty of perfecting analytical procedures for the accurate estimation of amino acids delayed the accumulation of sufficient data to serve as a basis of classifying proteins according to their amino acid

content. Block and Vickery ('31) made analyses of a number of the highly insoluble proteins, usually grouped together as keratins, in which they determined the content of the basic amino acids, arginine, histidine, and lysine, and the sulfur-containing amino acid cystine. Their data show that the keratin of human hair, sheep's wool, snake epidermis, goose feathers, *Gorgonia flabellum* (the horny skeleton of Gorgonian coral), *Plexaurella dichotoma* (a related species of coral), and silk fibroin, all yield histidine, lysine, and arginine in such quantities that the molecular ratios of these amino acids are respectively approximately as 1:4:12. The cystine content varied greatly, being in the order given above: 16.5, 10.0, 5.3, 6.4, 5.5, 3.2 and 0.0. Block ('33a) confirmed these ratios for the basic amino acids in keratin from finger nails and cattle horn.

The older view that the albumins and globulins of blood serum constitute individual proteins has been disproved by Block ('33b), who showed that when the total protein of the serum of cattle is fractionally precipitated by addition of different amounts of magnesium sulfate or of ammonium sulfate, the various protein fractions obtained do not have the same chemical composition based upon the determination of arginine, histidine, and lysine. The more soluble the protein in solutions of these neutral salts, the greater was the content of lysine. This seems to prove that the serum proteins are constructed of a large number of non-dissociable components. The proteins obtained by the usual physiochemical methods are not of constant amino acid composition. They are composed of two or more unstable coprecipitation systems in mutual equilibrium. The amino acid composition of the total coagulable serum protein is, however, constant in respect to the basic amino acids. Block ('34a) proposed the term *orosin* to designate the total coagulable protein of the serum.

Block, Darrow and Cary ('33) determined the basic amino acids in the serum of a patient suffering from nephrosis, in which the total protein (orosin) was 3.35 per cent as against the normal value of 7.0 per cent. The molecular ratio of arginine to lysine was in both cases 10:17. Normal blood serum contains about 4.3 per cent of "albumin" and 2.7 per cent of "globulin." The serum derived from the nephrotic patient contained but 0.44 per cent of albumin and 2.91 per cent of globulin. The urinary protein in nephrosis resembles serum protein in that the ratio of arginine to lysine is 10:18. Block et al. point out the fallacy of the interpretation of clinical results in terms of the changed ratio of albumin to globulin. The basic amino acid content of the orosins from human, dog and cow is the same. The work of these investigators emphasizes the fact that protein fractions derived from tissues, even by the mildest chemical procedures, are not necessarily present as such in those tissues during life.

Block ('34a, l.c.) determined the basic amino acids in the orosins of hen, turkey, and duck. The molecular ratio of arginine to lysine was, in all cases, 10:11. He states that the average ratio between these amino acids in 8 mammalian orosins is 10:18, and finds that, like the more soluble protein fraction of serum protein (albumin), the more soluble protein fraction of egg white protein contains a greater content of lysine than does the less soluble fraction (globulin). The proportions of the basic amino acids yielded by hen orosin, and by levitin, the non-vitellin protein fraction of egg yolk, are the same. From these studies it appears that the protoplasm of a specific organism, tissue, or cell mass, or certain derivatives thereof, is composed of a labile nitrogenous chemical aggregate, tissue protein which yields arginine, histidine, and lysine in molecular ratios that are approximately fixed, and are characteristic for the organism or tissue as it exists in various classes of animals. This observation stresses the primary importance of the basic amino acids in the genetic and embryological development of tissue protein.

Vickery and White ('33) found the cystine content of crystalline hemoglobins from horse, sheep, and dog to be 0.41, 0.61, and 1.16 per cent respectively, and the sulfur content 0.39, 0.73, and 0.57 per cent respectively. The iron content of the three kinds of hemoglobins was identical, viz., 0.33 per cent. The nitrogen content was 16.70, 16.83, and 16.48 per cent respectively. The atomic ratios of these hemoglobins was, in the order named, 2.05, 4.00, and 3.02. The cystine content of the hemoglobins from three species of mammals is shown to be quite distinctive, and this amino acid is in a different status as compared with the basic ones, arginine, histidine, and lysine. Vickery and White suggest that the yields of other amino acids from hemoglobins of different origin may likewise vary widely.

Block ('34b, l.c.) has extended the study of the composition of the hemoglobin of horse, sheep, and dog. He found that they yield iron, arginine, histidine, and lysine in the molecular ratios of approximately 1:3:8:9; while the molecular ratios of iron, sulfur, and cystine were 25:50:7, 25:100:14, and 25:75:21 respectively. He thus confirms the constancy of the molecular ratios of the basic amino acids, and the content of iron, in these crystalline hemoglobins. The cystine content, on the other hand, varies over 300 per cent among them.

Synthesis of Proteins in Living Matter.—The idea that enzymatic action, which is manifest through hydrolysis in the alimentary tract, might be reversible, and that proteolytic enzymes might be able, when in the presence of a concentrated system of digestion products, to synthesize protein, is not new. It was first put to experimental test in 1886 by Danilewski, who observed the formation of a precipitate of

protein when an extract of stomach containing pepsin was added to a concentrated solution of the products of peptic hydrolysis. Others have reported the synthesis of protein by means of trypsin and pepsin. The history of these investigations is reviewed by Wasteneys and Borsook ('30) who have defined the conditions under which the maximum amount of synthesis of protein-like material known as "plastein" is formed. They showed that it is of protein nature. Levene and Van Slyke ('08) carried out an amino acid analysis of plastein, and found it to contain at least 13 amino acids. Beard ('26) demonstrated by feeding experiments that plastein prepared from digestion products of egg white satisfies the nitrogenous needs of growing mice. It is obvious that an understanding of the minute structure of these synthetic proteins of the plastein type, and a comparison of them with the structure of the proteins from which they were derived, after digestion, is not possible until the amino acids can all be determined quantitatively, and until some method is worked out for determining the order of arrangement of the amino acid residues in the peptide chains which constitute the protein molecules. Distinct progress has been made in both directions.

Alcock ('36) has also discussed plastein formation, and expresses the belief that "it is probably safer to describe the reaction as peptone polymerization than as a synthesis of protein." This view is difficult to harmonize with the observation of Henriques and Gjaldbäk ('11), who furnished unequivocal evidence that there is a decrease in the free amino nitrogen in a digest after plastein formation. As a protein is digested (hydrolyzed) the amount of free amino nitrogen steadily increases. A decrease must be accepted as evidence of anhydride formation, i. e., formation of peptides. When plastein is digested by pepsin, the free amino nitrogen in the system increases just as is the case with protein.

The view that proteins are synthesized by the piecing together of their constituent amino acids, with multiple anhydride formation between each pair, would appear to rest upon convincing evidence, especially that of Cary and Meigs ('28). In the cow, while secreting milk, the concentration of amino acids in the blood of the vessels afferent to the gland is greater than in the efferent. It is assumed that casein, or other milk protein, is formed from the amino acids which have disappeared during passage of blood through the gland, since they are not present as such in the milk. If this is not the source of the casein, from where does it come?

Notwithstanding the general acceptance, by biochemists, of the synthesis of proteins from amino acids in living tissues by putting them together into chains by anhydride formation, this view is re-

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The view that proteins are synthesized by the piecing together of their constituent amino acids, with multiple anhydride formation between each pair, would appear to rest upon convincing evidence, especially that of Cary and Meigs ('28). In the cow, while secreting milk, the concentration of amino acids in the blood of the vessels afferent to the gland is greater than in the efferent. It is assumed that casein, or other milk protein, is formed from the amino acids which have disappeared during passage of blood through the gland, since they are not present as such in the milk. If this is not the source of the casein, from where does it come?

Notwithstanding the general acceptance, by biochemists, of the synthesis of proteins from amino acids in living tissues by putting them together into chains by anhydride formation, this view is re-

jected by Alcock, who suggests an alternative origin of the parenchymatous and tissue proteins. He postulates that the animal starts with something simpler than the amino acids derived by stripping down the molecules of the essential ones, and the polymerization of this substance, or substances, into a homogeneous molecule. These, he believes, undergo a process of internal differentiation during which the amino acids are formed. He points out that at no stage in protein synthesis in the animal is there a high concentration of the likely intermediates, the polypeptides, as evidence against the theory of a stepwise building of proteins—a view which has never been championed. He conceives the rôles of the non-essential and essential amino acids in a new light, illustrating the methods by which the body deals with a deficiency of the members of each class, using glutamic acid (dispensable), and tryptophane (indispensable), as examples. He writes, "It is necessary to picture the state of affairs in an animal living on a diet lacking in some one amino acid; say glutamic acid. During the shortage of glutamic acid the animal continues to build up proteins (and glutathione); continues, in fact, to lay down glutamic acid, and it is possible to maintain an animal in this way so long that it may lose appreciable quantities of the amino-acid in its shed hair and skin. Where does the glutamic acid come from? There are two possibilities. It may be synthesized in some special organ, say the liver, or it may on the other hand be produced locally at the site of protein synthesis. If the former, then it would seem that some mechanism exists, compensating the shortage of glutamic acid by a specific synthesis. The same must apply for all other dispensable amino acids. The possibility, which implies an extremely complex mechanism, which is only called into play under the most abnormal conditions, must be dismissed as highly unlikely."

In the case of deficiency of an essential amino acid, say tryptophane, "Deprival results in a syndrome resembling that following a vitamin lack; in particular, there is failure to grow, or even a definite loss of weight." The orthodox view is that limitation to protein synthesis is imposed by lack of tryptophane. Alcock suggests that tryptophane has some other function which accounts for its indispensability, and that the observed adverse effects of its deficiency are due to this function. He points out that Berg and Rose ('29) have shown that the demand for tryptophane is immediate, that feeding large doses at relatively long intervals is not equivalent to feeding smaller doses at shorter intervals. If a rat is starved for tryptophane for 24 hours it shows the effects. Allusion is made to the reports that when tryptophane is injected into an anemic animal, the return of the blood to normal composition is accelerated; and that its injection into a normal animal causes hyperemia; but his

own observations show that recovery from anemia is possible without tryptophane. Alcock believes that while tryptophane has some definite function other than as a component of the protein molecule, it is still a mystery. In animals without tryptophane, he reports prolonged survival with some blood formation, hair, and skin growth, and secretion of digestive fluids containing protein (and tryptophane). These observations are cited as evidence that the failure of animals to put on weight in tryptophane deficiency is definitely to be referred to the interference with the "essential" function of that amino acid, and that protein synthesis (and tryptophane synthesis) would go on unimpaired if that function could be restored. Alcock has observed in animals deprived of tryptophane, and injected with anterior pituitary growth hormone, sufficient increase in weight to indicate growth and protein synthesis. The other essential amino acids are, like tryptophane, believed to owe their indispensability, not to inability of the body to synthesize them in the process of protein synthesis, but to their "essential" functions.

The crucial part of the theory is nicely summarized by Du-Vigneaud, Sealock and Van Etten ('36), who state: "Alcock suggested that the limiting factor to growth on a tryptophane-deficient diet does not arise from the demand of the tissues for amino acids to be used for protein synthesis but that some material essential to life was made in the liver from tryptophane absorbed from the intestinal tract, whereas tryptophane delivered elsewhere than in the portal vein was not available for the 'essential' purpose. He also concluded that the animal can synthesize its proteins while receiving no tryptophane and that for this particular purpose the body can make its own tryptophane. He further pointed out that this conclusion implied that the synthesis of a protein probably starts at a lower stage than the fully formed amino acid and that the amino acid groups actually found in the proteins have been formed there, not assimilated from the blood stream as such."

Alcock, therefore, rejects the idea that proteins are synthesized by an enzymatic condensation of amino acids. Instead he offers the suggestion that a common attribute of living creatures is the synthesis of a primitive type of protein from relatively simple materials. The blood amino acids are taken up by the tissues and broken down to some simple unit. This unit, by polymerization, provides a basis on which the protein is built. Within this molecule of primitive protein, or "urprotein," a process of differentiation sets in and a definite proportion of each amino acid is formed, the protein so produced being similar in composition to the probable ancestral protein. The subsequent differentiation of the molecule, involving addition or elimination of amino acids, or the addition of prosthetic groups, follows a

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course determined by the nature of the tissue in which it is being produced.

A cogent argument against Alcock's theory of protein synthesis would seem to be the behavior of the animal in fasting. It is well known that during fasting the muscle proteins undergo autolysis to an extent sufficient to maintain the amino acid content of the blood at a constant fasting level. The orthodox view that this mechanism serves to maintain an adequate nutrient medium, as respects amino acids, for the nutrition of the organs and nervous system, and thus permit survival of the organism for a time at the expense of the more dispensable tissues, seems to argue convincingly against the possibility that all amino acids can be synthesized by the tissues.

Moreover, DuVigneaud, Sealock and Van Etten (l.c.) have recently reported that tryptophane injected subcutaneously can be utilized. Since the decisive point in Alcock's theory is his failure to obtain growth of rats injected with tryptophane on a tryptophane-deficient diet it is evident that the theory, although interesting and provocative, is based upon very questionable evidence.

Rôle of Enzymes as Specific Organizers in Protein Synthesis.— In discussing plastein formation it was pointed out that proteolytic enzymes appear to be able to produce polypeptides from amino acids by anhydride formation. This view is opposed to the older concept that the only function of these enzymes is the hydrolytic degradation of proteins into polypeptides, dipeptides, and amino acids. It is obvious that some mechanism exists for the synthesis of proteins in the body, for it is well established that proteins are degraded in the digestive tract into amino acids and perhaps some simple peptides, which enter the blood and serve as the pabulum for protein nutrition. Bergmann and Niemann (l.c.) conceive that since protein molecules are assembled from hundreds or thousands of amino acids according to an unvarying pattern, the conclusion is unavoidable that these gigantic molecules are synthesized under the control of some specific organizer. This organizer must, of course, be able to induce anhydride formation to produce the peptide bond (—CO—NH—CHR—). It must also be able to select, from all the available amino acids and peptides, a particular amino acid or peptide at the correct moment, so that the unequivocal pattern of the complete protein molecule is adhered to in every stage of the synthesis. This involves the promotion of hundreds of consecutive reactions, each highly specific, and each of which alters the structure of the substrate and thereby the character of the subsequent specific reactions. They deem it likely that the intracellular proteinases are in reality the protein organizers. They state: "The intracellular enzymes are able to degrade proteins through hydrolysis of the peptide bond, and recently it

has been demonstrated that this splitting proceeds so far that lower molecular weight peptides and even amino acids are formed. On the other hand, these same intracellular enzymes are capable of promoting the synthesis of the peptide bond, thereby forming higher order peptides from the lower molecular weight protein hydrolysis products. It appears that the reason that the intracellular enzymes are able to promote hydrolysis and synthesis under the same experimental conditions, with the result that these two reactions are in competition with each other, is that small differences in the structure of the substrate coupled with the very exactly tuned specificity of the individual intracellular enzymes determines whether or not and to what degree synthesis or hydrolysis shall occur. The organizing ability of the individual intracellular proteinases is concomitant with the possession of the above properties."

An interesting example of the synthetic action of an enzyme is brought to light by Bergmann and Fraenkel-Conrat (Bergmann and Niemann, l.c.). The enzyme papain acts upon two very similar peptide-like compounds, benzoylglycine amide and benzoylglycine anilide. Benzoylglycine anilide is not only hydrolyzed by the enzyme, but is synthesized by the enzyme in the presence of benzoylglycine and aniline:

$$C_6H_5CONHCH_2CONH_2 \xrightarrow{\text{enzymatic hydrolysis}} C_6H_5CONHCH_2COOH + NH_3$$

$$C_6H_5CONHCH_2CONHC_6H_5 \xleftarrow{\text{enzymatic synthesis}} C_6H_5CONHCH_2COOH + C_6H_5NH_2$$

In this "model" Bergmann points out that the amide plays the rôle of the extracellular proteins, and the anilide that of the intracellular proteins. Bergmann and Niemann suggest that since the proteolytic enzymes are themselves proteins (Sumner, Northrop), then there must exist other proteinases which have the ability to synthesize replicas of their own structural pattern and are able, therefore, to "multiply" in suitable surroundings. The tobacco mosaic virus of Stanley, which is a protein with an extraordinarily high molecular weight, would seem to be an example of this property. When placed in the nutrient medium afforded by the host organism, it would utilize the circulating amino acids, and induce the continuous production of its replica, a foreign protein. This process would account for the known behavior of viruses and the normal growth of animals using amino acids from entirely different proteins.

Amino Acid Composition of Tissue in Relation to Composition of Dietary Proteins.—Owing to the apparently complete degradation

of dietary protein to amino acids, or very simple peptides, before the synthesis of such nutrient principles into tissue proteins, there is considerable reason to doubt that the amino acid composition of tissue proteins is influenced by dietary factors. However, some investigators, particularly Schenck and Wollschnitt ('33), claim, for example, that the amounts of tryptophane in liver, kidney, and muscle protein of rats is influenced by the composition of the dietary protein. The question has been studied by Lee and Lewis ('34), who report essentially opposite conclusions. It is significant, however, that in their young rats receiving an adequate supply of dietary cystine there was a higher content of sulfur in the tissues than in similar rats receiving a diet deficient in cystine. This is attributed, with justification, to the presence of larger amounts of non-protein sulfur in animals ingesting the cystine-adequate diet.

The effect of diet on the protein or amino acid content of milk and eggs is also of interest. There seems to be a very inadequate amount of evidence regarding milk, and it is not yet clear whether the composition of egg proteins is affected by diet. For example, no differences could be found in the composition of proteins of eggs from white Leghorn pullets raised and kept on diets having entirely different sources of proteins, namely, wheat middlings, ground corn, corn gluten, and soy bean oil cake meal (Calvery and Titus, '34). Even the hatchability of eggs, as influenced by diet, apparently is not related to the content of total nitrogen, total amino nitrogen, tyrosine, tryptophane, and cystine (McFarlane, Fulmer and Jukes, '30). But the recent report of Patton and Palmer ('36) is of special interest in this regard. Although they found that "optimum" versus "deficient" protein rations caused no significant changes in the tryptophane, tyrosine, histidine, arginine, cystine, lysine, and glycine of egg protein, there was a significant difference between the glycine contents of normal and chondrodystrophic chick embryos. Since the content of glycine in foodstuffs seems to bear some relation to their efficacy in preventing the occurrence of chondrodystrophic embryos it may be tentatively assumed that the amino acid composition of dietary protein does have some influence on the amino acid metabolism of eggs during embryonic development. It may be concluded that further study is definitely needed. But, as suggested in the discussion of organizers in protein synthesis, there is scarcely any basis for the assumption that protein factors in the diet may influence the composition of tissues.

Amino Acid Composition of Protein in Relation to its Nutritive Value.—Kossel, Fischer, and Osborne, pioneers in protein analysis, made it clear that very pronounced differences exist in the composition of proteins from various sources. Although their technics were

never perfected so as to give results approximately quantitative, except in the case of less than a third of the known amino acids, it was shown in these few cases that there are great variations in the proportions of amino acids obtained from proteins from different sources. Thus, the proteins of the muscle tissues of several species of animals yield between 12 and 14 per cent of glutamic acid. This amino acid is present in the two principal proteins of the wheat kernel to the extent of about 40 per cent. Other equally great differences were shown to exist in the composition of proteins of common foods.

Since nine or more of the amino acids are, apparently, totally incapable of being synthesized by the body, it is necessary that they be provided in the diet in such amounts as are essential to the various bodily structures and functions. It is obvious, therefore, that the practical problem in protein nutrition is the provision of foodstuffs whose proteins furnish amino acids needed by the body, and in the requisite amounts. In the present state of our knowledge it appears that the problem may be illustrated by comparing the digestion products of the protein molecule to the letters of the alphabet. The proteins of the food and of the body tissues, which are unlike each other, may be regarded as made up of the same letters arranged in different orders and present in different proportions. During growth the ingested food proteins are resolved into the constituent amino acids and perhaps some simple peptides. These, after absorption, are reassembled in new order, and in new proportions to form the tissue proteins and promote bodily functions. For example, let the muscle tissue of an animal be compared to a block of printer's type so arranged as to print the rhyme "Jack Spratt could eat no fat, and his wife could eat no lean." Then allow the proteins of which the muscle consists to be represented by the individual words, and the protein digestion products, i. e., amino acids, by the letters of which the words consist. Now let the animal ingest food proteins which correspond to a block of type that would print the jingle "Peter Piper picked a peck of pickled peppers." It is easy to understand that when the proteins of the food are resolved into their constituent letters, namely, amino acids, and an effort is made to form the bodily proteins of the new and different printer's type from the letters supplied by the food, the transformation cannot be made.

The significance of this illustration is shown concretely by the nutritive behavior of the protein of corn, zein, and the amino acids, lysine and tryptophane, in which zein is particularly deficient. Muscle tissue protein contains considerable amounts of lysine and tryptophane. If these should correspond to the Jack Spratt jingle and zein should correspond to the Peter Piper jingle it is obvious that

lysine and tryptophane (Jack Spratt) could not be furnished for muscle tissue growth since zein (Peter Piper) does not supply those amino acids.

It is thus of importance in nutrition to consider the actual amino acid composition of proteins and protein-containing foods since the nutritional value of such foods is absolutely dependent upon it. Of course such factors as digestibility of protein, absorbability of amino acids, and ratio of absorbed amino acids to body amino acid requirements, must be considered in the nutritional evaluation of chemical data on proteins and protein-foods. Since these factors are only ascertainable by means of biological assay it is not sufficient to know only the amino acid content of such foods. Nevertheless such data obviously are of practical value and it is well to consider briefly some of the evidence regarding this subject.

There is considerable variation in the amino acid content of essentially similar foodstuffs as is indicated by Csonka's ('37) data on the amino acid content of different wheat varieties. This is shown in the somewhat modified table taken from his paper.

PROTEIN QUALITY AS SHOWN BY RELATIONSHIP BETWEEN AMINO ACIDS AND TOTAL NITROGEN CONTENT

VARIETY	MG. OF INDICATED AMINO ACID PER GM. OF TOTAL NITROGEN					
	Cystine	Tryptophane	Tyrosine	Arginine	Histidine	Lysine
Marquis	81	28	262	154	85	456
Tenmarq	65	26	173	146	43	475
Fulhio	67	34	217	152	34	373
Casein (for comparison)	20	130	405	236	156	475

Cereal proteins in general contain considerably less lysine and tryptophane than do animal proteins. This is indicated for tryptophane in the above table but in this case no very significant difference seems to exist between the lysine content of wheat and casein. As a rule cereal and vegetable proteins are relatively deficient in lysine. But, the real significance of this table is the marked variety differences in the composition of proteins. Hence it should be recognized that tables on protein composition provide only a general indication of the actual amino acid content of a given protein or protein food.

A useful amount of data on the amino acid composition of principal purified proteins is furnished by the table that follows:

AMOUNTS OF AMINO ACIDS ISOLATED FROM PROTEINS

	Ovalbumin	Lactalbumin	Casein	Vitellin	Hemoglobin	Gelatin	Silk Fibroin	Wool Keratin	Salmin	Wheat Gliadin	Wheat Glutenin	Edestin (Hemp)	Legumin (Pea)	Amandin (Almond)
Glycine	0.0	0.0	0.0	0.0	..	25.3	36.0	0.6	...	0.0	0.9	3.8	0.4	0.5
Alanine	8.4	2.5	1.5	0.8	4.2	8.7	21.0	4.4	..	2.0	4.7	3.6	2.1	1.4
Valine	..	0.9	7.2	1.9	..	0.0	0.0	2.8	4.3	3.4	0.2	..	..	0.2
Leucine and isoleucine	15.2	19.4	9.4	9.9	29.0	7.1	1.5	11.5	..	6.6	6.0	20.9	8.0	4.5
Aspartic acid	6.1	1.0	4.1	2.2	4.4	3.4	..	2.3	..	0.6	0.9	4.5	5.3	5.4
Glutamic acid	14.0	10.1	21.6	13.0	1.7	5.8	0.0	12.9	..	43.7	23.4	6.3	17.0	23.2
Serine	..	..	0.5	..	0.6	0.4	1.6	0.1	7.8	0.2	0.7	0.3	0.5	..
Hydroxyglutamic acid	1.4	..	10.5	..	..	0.0	..	..	..	..	..	..	..	..
Phenylalanine	5.2	2.4	3.2	2.6	4.2	1.4	1.5	0.0	..	2.4	2.0	2.4	3.8	2.5
Tyrosine	3.2	0.9	4.5	3.4	1.3	0.0	10.5	2.9	..	1.2	4.3	2.1	1.6	1.1
Tryptophane	..	..	1.7	..	..	..	..	..	..	1.0	..	..	..	..
Cystine	0.4	..	..	..	0.3	..	..	7.3	..	0.5	0.02	..	..	..
Methionine	2.5	..	1.4	..	..	..	..	..	..	..	..	..	..	..
Lysine	5.0	9.2	6.0	5.4	8.1	5.9	0.3	2.3	0.0	0.2	1.9	2.2	5.0	0.7
Arginine	5.4	3.2	3.8	7.9	3.3	9.1	0.7	7.8	87.4	3.2	4.7	15.8	11.7	11.9
Histidine	1.4	2.1	2.5	1.2	7.6	0.9	0.1	0.7	0.0	0.6	..8	2.1	2.4	1.6
Proline	4.2	4.0	8.0	4.2	2.3	19.7	0.3	4.4	11.0	13.2	4.2	1.7	3.2	2.5
Hydroxyproline	..	..	0.3	..	1.0	14.4	..	..	..	..	..	2.0	..	..
Ammonia	..	1.3	1.6	1.3	..	0.4	..	..	..	5.2	4.0	..	2.1	3.7
Total	72.4	57.0	87.8	53.8	68.0	102.1	73.5	69.0	110.5	84.0	59.72	61.4	63.1	59.2

Plimmer in Harrow & Sherwin, *Biochemistry*. Courtesy, W. B. Saunders Company.

It will be observed that gelatin and zein are especially deficient in certain amino acids. This chemical evidence is supported by the marked failure of these proteins to promote growth or even maintenance unless supplemented with the missing essentials, either as protein or the free amino acids. Edestin of hemp seed is noteworthy in that it contains sufficient amounts of all the indispensable amino acids to promote growth. It is somewhat unique in this respect since most of the vegetable and cereal proteins appear to be deficient in one or more of the essential amino acids. The known high nutritive value of casein and lactalbumin is indicated by their amino acid composition.

The following table furnishes more recent information on the content of certain amino acids in some proteins and protein-containing foods of general importance. In the table no distinction is made between values based on colorimetric procedures and those obtained by isolation of the amino acids. Also, no distinction is made between cystine and cysteine in the cystine column.

THE CONTENT OF CERTAIN AMINO ACIDS IN PROTEINS AND PROTEIN-CONTAINING FOODS

PROTEIN OR PROTEIN-CONTAINING FOOD	INVESTIGATION	PER CENT OF THE AMINO ACIDS									
		CYSTINE	METHIONINE	TRYPTOPHANE	TYROSINE	PHENYLALANINE	ARGININE	HISTIDINE	LYSINE	GLUTAMIC ACID	PROLINE
Casein	(1)	..	0.41	..	..	..	3.85	1.83	6.25	..	..
Casein	(2)	..	..	..	..	..	7.77	1.22	5.38	..	..
Highly purified egg albumin	(3)	1.33	..	1.28	4.21	..	5.03	2.44	6.41	13.96	4.15
Vitellin of hen's egg	(4)	1.19	..	1.24	5.01	..	8.22	0.62	1.93	..	..
Thyroglobulin	(5)	..	..	..	..	..	9.10	14.29	1.80	..	5.26
Globulin of sunflower seed	(6)	..	..	..	..	..	..	..	..	..	3.94
Protein of soy bean	(7)	..	..	..	3.82	5.21	..	..	..	..	..
" " "	(1)	..	0.08	..	..	..	..	..	..	..	..
Protein of Cocksfoot (forage grass)	(8)	..	..	..	..	..	1.36	1.66	5.27	..	..
Wheat bran	(9)	0.25	..	0.28	..	..	0.85	0.18	0.54	..	..
Wheat endosperm	(9)	0.14	..	0.12	..	..	0.36	0.20	0.23	..	..
Water-soluble baker's yeast protein by heat coagulation	(10)	0.44	..	2.66	4.79	..	..	..	..	..	..
Alkali-soluble baker's yeast protein	(10)	0.69	..	1.79	3.11	..	..	..	..	..	..
Water-soluble brewer's yeast protein by heat coagulation	(10)	0.49	..	2.67	4.11	..	3.50	1.38	4.50	..	..
Alkali-soluble brewer's yeast protein	(10)	0.31	..	1.68	3.93	..	2.82	trace	4.53	..	..
Muscle protein of sardine	(1)	..	0.52	..	..	..	..	..	..	..	..
Muscle protein of fresh cod fish	(11)	0.6	0.3	2.1	2.0	1.1	6.8	..	..	7.5	..

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The Dispensable and Indispensable Amino Acids in Nutrition.— It has long been recognized that the only method of obtaining unequivocal evidence of the role of individual amino acids in nutrition is to observe the response of animals to diets in which the protein moiety is replaced entirely by mixtures of amino acids, the composition of which can be varied at will. In 1912 Abderhalden attempted such a study, using amino acid mixtures prepared by the digestion of meat with appropriate enzymes. Dogs fed this diet made large growth gains but the removal of tyrosine and tryptophane from the mixture caused marked failure in both growth and maintenance. Since 1912 various workers have made some useful contributions to our knowledge but owing perhaps to the large cost of such investigations

relatively slight advances had been made until the recent work of W. C. Rose and his collaborators. The subject has been reviewed by Rose ('38).

Glycine. On the basis of various types of evidence it is concluded that glycine is dispensable. Animals fed large amounts of benzoic acid excrete more glycine in the form of hippuric acid, a condensation product of benzoic acid and glycine, than can be derived from the proteins catabolized. It is generally regarded that the synthesized glycine may be formed from ammonia and non-nitrogenous materials, or from other amino acids. It should be stated, however, that precursors of glycine do not appear to be readily available in protein metabolism (Griffith, '34). Aside from other secondary indications of dispensability the crucial evidence was furnished by McCoy and Rose ('37), who found that a glycine-free mixture of amino acids, fed with an appropriate basal diet, promoted good growth. The inclusion of glycine in the diet did not influence growth.

Alanine. There is no proof that alanine is essential. Rose's feeding experiments indicate dispensability, as do those of Abderhalden ('22).

Serine. Like other hydroxyamino acids, i. e., hydroxyglutamic acid, hydroxyproline, and tyrosine, there is no certainty that serine is essential. Definite indications of its dispensability were furnished by McCoy and Rose (l.c.) simultaneously with their studies of glycine.

Valine. The only definite evidence of the role of this amino acid is furnished by Rose ('37), who claims that it is indeed indispensable since its absence from the stock amino acid mixture causes a profound failure in growth accompanied by a hypersensitiveness to touch and a lack of coordination in movement. A study of the pathology of valine deficiency should be made since it appears probable that the nervous system is directly affected by its absence from the diet.

Leucine. Again the only worker to furnish very clear-cut information is Rose. Womack and Rose ('36), using the Rose procedure, found that leucine is decidedly an indispensable amino acid for growth.

Isoleucine. As in the case of leucine, the only dependable data are those of Rose. Womack and Rose (l.c.) showed that growth cannot continue in the absence of isoleucine. Therefore that amino acid is essential. It should be noted that valine, leucine, and isoleucine have one common structural similarity, namely, a secondary carbon linkage which is in either the beta or gamma position.

Norleucine. Apparently norleucine is not needed in the diet but final conclusions must await further confirmation (Womack and Rose, l.c.).

Aspartic acid. This amino acid appears to be dispensable as shown by Bunney and Rose ('28). Likewise Hopkins ('16) obtained apparently successful nutrition in the absence of aspartic acid.

Glutamic acid. As in the case of aspartic acid, Bunney and Rose found glutamic acid dispensable. Various studies of the detoxication of phenylacetic acid by means of glutamine, probably the only naturally occurring form of glutamic acid, suggest the capacity of the body to synthesize glutamic acid. Also, Hopkins' (l.c.) feeding experiments with glutamic acid-low diets indicate its dispensability.

Hydroxyglutamic acid. Abderhalden's ('22, l.c.) early studies pointed to its dispensability and the work of Windus, Catherwood and Rose ('31) confirmed that conclusion.

Arginine. The status of arginine in nutrition has been in question for many years. In 1916 Ackroyd and Hopkins reported that rats were able to grow when the arginine- and histidine-free protein hydrolysate, furnishing the dietary amino acids, was supplemented with arginine and histidine, either together or singly. It was concluded, therefore, that arginine and histidine are interconvertible in nutrition. The plausibility of this view is supported by the similarity in structure of these two compounds, c. f. amino acid formulae. But neither Abderhalden ('22, l.c.) nor Rose and Cox ('24) were able to confirm the replaceability of histidine by arginine. Stewart's ('25) results failed to confirm those of Ackroyd and Hopkins, as well as those of Rose et al. Owing to present events, Stewart's observations probably are significant. Arnold and associates ('36), working with chicks, seem to have demonstrated that arginine is definitely indispensable for rapid growth. This work is of importance especially in view of their basal diet. It was:

Dextrin	64	Crude liver extract	2
Purified casein	18	Autoclaved liver extract residue	2
Water-extracted lung	5	Brewer's yeast	2
Salts	5	Cod liver oil	2

Chicks on this basal ration weighed about 335 gm. at the end of 6 weeks but when arginine was added at a level of 1 per cent the weight was about 390 gm. after a similar period of 6 weeks. These findings have been confirmed by Klose, Stokstad and Almquist ('38), who found that increasing the dietary casein to 30 per cent provided sufficient arginine for maximum growth. Heretofore it has been assumed with some justification that all the amino acids, with the possible exception of cystine and methionine, are furnished in adequate amounts by a diet containing 18 per cent of casein. Arnold et al. have not yet submitted any evidence regarding the relation of arginine to histidine in growth.

In view of the evidence from chick nutrition and the observa-

tions of Scull and Rose ('30) that rats on an arginine-free diet grow at only about three-fourths of the normal rate—whereas addition of arginine to the food remedies the dietary defect—it must be concluded that arginine probably is synthesized in considerable amounts in the body, but during rapid growth an exogenous source of the amino acid must be furnished. Hence arginine may be tentatively regarded as indispensable because synthesis is too slow for rapid growth.

Lysine. Although the necessity of lysine was conclusively demonstrated by Osborne and Mendel, and others, during the period of 1914-1920, by means of its ability to induce growth when added to gliadin rations or to zein rations supplemented with tryptophane, there has been no considerable attempt to augment those studies with purified amino acid mixtures. Abderhalden's ('22, l.c.) work with amino acid mixtures, since proved to be faulty in some respects, indicated the indispensability of lysine. According to Rose's ('37, l.c.) preliminary evidence the minimal amount of lysine necessary to support normal growth of rats is 1.0 per cent, i. e., the diet must contain 1.0 per cent of lysine. This value is higher than that for any other amino acid thus far reported. Since this amino acid tends to be low in vegetable proteins the above observation may be of more than ordinary practical significance.

There have been some studies of the replaceability of lysine in the diet by related hydroxy compounds. McGinty, Lewis and Marvel ('24) found that natural l-lysine promoted somewhat better growth than did synthetic dl-lysine. This has been verified by Berg and Dalton ('34), whose work indicates that the unnatural d-lysine of the dl-mixture could probably not be utilized at all for growth. Direct proof of this has been furnished by Berg ('36) and Rose ('37, l.c.). In this respect lysine is comparable to cystine but different from tryptophane, histidine, and methionine.

Histidine. Owing to its close structural similarity to arginine, as indicated previously, the nutritive role of histidine has been studied, in part, with reference to its interchangeability in the body with arginine. The early work of Ackroyd and Hopkins ('16), which suggested such a relationship, became questionable when Rose and Cox ('24, l.c.) showed that rats on an arginine and histidine-deficient diet were unable to grow or even maintain body weight when only arginine was added. However, the addition of histidine alone, or with arginine, caused resumption of growth. On the basis of this study it has been definitely concluded that histidine is indispensable.

Histidine, however, is replaceable by certain closely related compounds, as was first demonstrated by Cox and Rose ('26) when it was shown that dl-β-4-imidazole lactic acid added to a histidine-defi-

cient diet caused resumption of growth. Amino-N-methyl histidine also replaces histidine for growth of rats (Fishman and White, '36). Finally, as shown by DuVigneaud, Sifferd and Irving ('37), l-carnosine (β-alanyl-l-histidine) can replace histidine. According to these authors, injected histidine can be utilized for growth purposes.

Tryptophane. The establishment of tryptophane as an essential dietary factor, by Willcock and Hopkins in 1906, constituted the first definite evidence of the indispensability of an organic substance whose chemical nature was known at the time. Since then the various studies of Osborne and Mendel ('12), Abderhalden ('13), and Berg and Rose ('29, l.c.) have conclusively demonstrated its indispensability. In view of Alcock's (l.c.) radically different concept of protein metabolism, based considerably upon the failure of Jackson ('27) and himself to observe utilization of tryptophane injected subcutaneously, there has been some interest in the utilization of this amino acid as affected by the route of intake. But DuVigneaud, Sealock and Van Etten's (l.c.) conclusive evidence of the satisfactory utilization of tryptophane injected subcutaneously leaves Alcock's contention without basis. The fact that utilization of tryptophane is much more efficient if the injections are made at several intervals per 24 hours, as shown by Berg and Rose ('29, l.c.) and DuVigneaud et al. ('36, l.c.), is to be expected since all substances behave that way.

Of particular interest to considerations of the intermediary metabolism of tryptophane is the work on compounds which replace this amino acid in growth. Those which function rather efficiently in this regard are 3-indole-pyruvic acid (Jackson, '29; Berg, Rose and Marvel, '29), acetyl-l-tryptophane (DuVigneaud, Sealock and Van Etten, '32), and amino-N-methyltryptophane (Gordon and Jackson, '35). But, whereas, optically unnatural d-tryptophane can be utilized for growth, the acetyl form, acetyl-d-tryptophane, is not utilized, in contrast to the effect of acetyl-l-tryptophane (DuVigneaud, Sealock and Van Etten). There is need of further investigation concerning the basis for these facts.

Thus far no definite evidence is available with respect to any special nutritive functions which might be possessed by tryptophane. The symptoms of its deprivation are failure of growth, loss of weight, loss of hair, hardening of the skin, and depletion of fat stores. There seems to be no conclusive evidence that tryptophane is the source of the pyrrole group of hematin.

Phenylalanine and tyrosine. The early studies of Abderhalden ('15) indicated that tyrosine is a nutritive essential but Totani's ('16) observations, which appeared to be more convincing, indicated the dispensability of that amino acid. Owing to the very close similarity in chemical configuration between phenylalanine and tyrosine it was

early suggested that they may be mutually interchangeable in metabolism. This idea was given considerable credence owing to the observation of Embden and Baldes ('13) that the perfusion of the surviving liver with blood containing phenylalanine leads to the production of small amounts of tyrosine. But according to Shambaugh, Lewis and Tourtellotte ('31), the two amino acids when administered in moderate doses, do not yield identical intermediates.

Apparently this question concerning these compounds has been finally answered. Womack and Rose (l.c.), by means of a mixture of amino acids free from phenylalanine and tyrosine, adduced convincing evidence that phenylalanine is a growth requisite which cannot be successfully replaced with tyrosine. Tyrosine deficiency alone permitted good growth and its addition to the diet provided no indication that this amino acid is needed. Therefore phenylalanine is indispensable and tyrosine is dispensable.

Proline and hydroxyproline. In view of St. Julian and Rose's ('32) convincing studies which demonstrate the dispensability of both proline and hydroxyproline the older questionable evidence for the indispensability of proline (Sure, '24) is no longer tenable.

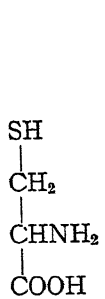
Threonine. It is generally recognized that the most laudable investigation in the modern era of protein nutrition is that of Rose and associates concerning the discovery of evidence for, and the eventual isolation and identification of, a hitherto unrecognized and indispensable amino acid, namely, α-amino-β-hydroxy-n-butyric acid. The common name assigned to it by Rose is d-threonine, inasmuch as its spatial configuration is exactly analogous to that of the sugar d-threose. Space does not permit the extensive account of this work that its importance deserves. Some of the original papers are: Rose ('32), McCoy, Meyer and Rose ('35), and Meyer and Rose ('36). It seems probable that the discovery of threonine was only through Rose's fortitude in the preparation and use of a diet in which the proteins were replaced entirely by mixtures of highly purified amino acids. This is indicated because the chemical properties of threonine are so similar to those of the mono-amino monocarboxy acids, particularly the leucines, that it probably would have escaped detection by the ordinary procedures for investigation of amino acids in nutrition.

Attention should be called to the fact that the work on this amino acid apparently makes it possible for the first time to rear animals on diets containing mixtures of highly purified amino acids instead of proteins.

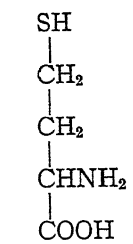
Apparently the only evidence regarding the nutritional role of threonine is the fact that its absence from the diet causes a marked failure of growth.

Nutritional Relationships between Sulfur-containing Amino Acids.—It is generally accepted now that methionine is definitely indispensable and that in its absence animals die even though an abundance of cystine is furnished. Nevertheless, under conditions of low methionine intake, the addition of cystine to the diet greatly improves growth (Rose, '37, l.c.). Thus, it is possible that methionine is essential for certain vital functions which cystine cannot satisfy and that cystine can spare methionine for certain functions. For example, in animals which normally produce large amounts of wool, hair, or feathers, it would seem that dietary cystine in abundance might be of considerable advantage since these structures contain large amounts of cystine and that it would obviate the necessity of synthesizing the cystine from methionine or such other substances as might be convertible into cystine.

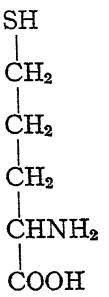
In the light of recent researches, the following compounds related to cystine and methionine respectively, are attracting attention:



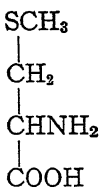
Cysteine



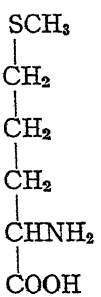
Homocysteine



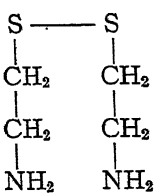
Pentocysteine



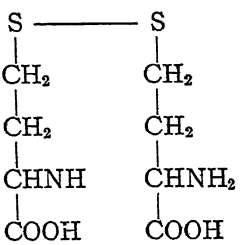
Methyl cysteine



Homomethionine



Cystamine



Homocystine

Of these only cystine and methionine have been isolated from protein. Dyer and DuVigneaud ('35) found that homocystine could support growth on a cystine-deficient diet. But pentocystine and homomethi-

onine could not be utilized for growth in lieu of cystine, thus demonstrating that the availability of homocystine and methionine for this purpose is not a general property of either disulfide or methylthiol amino acids. However, they were able to show that di-N-methylhomocystine and N-methylmethionine are able to support growth on a cystine-deficient diet. Cystamine appears to be without growth-promoting properties (Jackson and Block, '36). Glutathione, a tripeptide of cystine, glutamic acid, and glycine, is able to replace cystine in the diet.

It seems evident, as indicated previously, that the sulfur-containing amino acid of preeminence is methionine (Jackson and Block, '32). Apparently, that compound alone can satisfy the entire requirement for sulfur-containing amino acids. The dietary value of other such compounds seems to depend upon the capacity of the body to convert them to methionine, or cystine for certain purposes.

The ability of homocystine to support growth probably is due to its transformation to methionine by the body (White and Beach, '37). Compounds which are convertible into cystine, under conditions of low-cystine intake, may be regarded as of dietary value if the methionine intake is not sufficient to furnish adequate amounts of cystine. The nutritional role of sulfur-containing amino acids is a fertile field for investigation.

The effect of various compounds upon the utilization of cystine and methionine is also of interest. Certain substances conjugate with these amino acids, thus rendering them unavailable to the body and causing them to be excreted. Cholic acid and bromobenzene are particularly effective in this respect (White, '36). Recently, White ('37) has shown that iodoacetic acid, when fed to rats restricted to a low protein diet, show symptoms of sulfhydryl compound deficiency, as indicated by the resumption of growth by the addition of l-cystine or dl-methionine to the diet already containing iodoacetic acid.

Sulfur-containing Amino Acids and the Growth of Epidermal Tissues.—That large amounts of utilizable sulfur-containing amino acids are necessary for certain animals is indicated by the fact that the percentage of sulfur in clean, dry goose feathers and of sheep's wool is respectively 3.2 and 4.5. The problem of diet in the production of wool is indeed urgent since the quality and amount of wool growth are quite dependent upon the food of the sheep. Bosman ('34-35) states that, provided the feed is kept constant, variations in climatic conditions produce no effect on the dry weight of wool-cuttings or in fibre fineness. He claims, however, that underfeeding reduced the scoured fleece by 31.8 per cent, and reduced fibre diameter by 36 per cent. Subsequent good feeding restored the original

fibre diameter. The necessity of adequate sulfur-containing amino acids in the diet is further indicated by Marston's ('35) work. He estimated the growth of wool on merino ewes, on shaved areas, and compared the growth when the ewes were fed a diet of oat straw and lucerne hay, with that secured when cystine was added to the basal ration. The provision of 1 gm. of cystine daily per animal yielded a 14 per cent increase in the growth of wool. It is stated that the feeding of methionine gave doubtful results. This is somewhat puzzling since it appears, on the basis of observations of growing rats, that methionine is capable of meeting the entire need for sulfur-containing amino acids. It is possible that species differences might account for these apparent discrepancies. Certainly it suggests that further studies must be made of nutritional relationships between cystine and methionine.

Since wool contains about 13 per cent of cystine, whereas most proteins contain less than 1 to 3 per cent, Marston and Robertson ('28) advanced the important hypothesis ". . . that the carrying capacity of any country for sheep may very probably be determined by the capacity of its pasture plants to produce cystine."

If wool-producing animals are dependent, in any degree, upon methionine as a source of wool sulfur, present analytical data do not indicate that methionine deficiency in the diet might be a limiting factor in wool production. Barritt ('34) found that the methionine content of wool amounts to 0.44 to 0.66 per cent of its dry weight and methionine sulfur represents only 2.4 to 4.8 per cent of the total sulfur content. Methionine deficiency does not seem likely to occur in animals or in man if the diet is composed of natural foodstuffs.

Several studies have been made on the relationships between dietary cystine and the growth of hair in rats. Rats of Smuts, Mitchell and Hamilton ('32) on a cystine-deficient diet had a subnormal amount of cystine in the hair and the medullated fibres were abnormal. Also, hair growth was definitely inhibited. A diet deficient in lysine inhibited body growth and hair growth, but did not reduce the content of cystine in the hair.

Of interest here is the study of Martin and Gardner ('35) on the effect of sulfhydryl compounds on the growth of hair in genetically hairless rats. Five milligrams of cystine administered daily to such rats stimulated the growth of a hairy coat within one month, but it was lost during the next two weeks. Higher doses failed to initiate a regrowth of hair. However, hair growth stimulated by cysteine continued as long as the experiment lasted, which was six weeks. Glutathione had no stimulative action. It is unfortunate that these experiments were not continued longer, since the effects of

sulfur-containing amino acids, under such conditions, are of considerable significance regarding the genetic mechanism of hypotrichosis. However, these brief observations do suggest that hereditary hypotrichosis might be regarded as an "inborn error of metabolism" as in the case of pentosuria, cystinuria, and alkaptonuria.

The possible relationship of dietary deficiencies of sulfur-containing amino acids to loss of hair in humans is indeed problematic. As emphasized by Brown and Klauder ('33), loss of hair is not necessarily associated with low sulfur in the diet. At this time there is not sufficient information to warrant unequivocal conclusions regarding sulfur-containing amino acids and hair growth in humans.

Sulfur-containing Amino Acids and Tissue Growth.—It has been postulated by Hammett ('33) that the natural regulation of growth by increase in cell number is brought about by the essential chemical equilibrium between the sulfhydryl group, –SH, and its partially oxidized derivatives. It is claimed that the sulfhydryl group accelerates growth and the partially oxidized derivatives of such compounds retard it. Hammett has employed as sources of sulfhydryl, reduced glutathione, parathiocresol, thiophenol, and thio-glucose.

Voegtlin, Johnson and Thompson ('36) have confirmed earlier observations showing that deficiency of cystine or methionine, or both, inhibit the growth of mice. The growth of neoplasm, i. e., mammary carcinoma, is retarded in mice restricted to a diet deficient in cystine and methionine. However, as in other cases of tumor inhibition by dietary deficiencies, the growth of the mice also is inhibited. The addition of cystine or glutathione to the deficient diet causes a stimulation of tumor growth, concomitant with resumption of growth by the host. In no case has it been demonstrated that tumor growth can be retarded or controlled by dietary measures without causing simultaneous retardation of normal tissue growth and function. This does not imply, however, that diet is without significance in tumor growth and tumor susceptibility.

Sulfur-containing Amino Acids in Processes of Detoxication.—Cysteine and glutathione play a prominent role in the bodily processes of detoxication. The former compound, although not a significant constituent of the normal diet, may be easily produced in the body by reduction of cystine. Detoxication may possibly occur by means of several different reactions involving cystine, glycine, and glutamic acid, all of which are components of glutathione.

Sullivan and Hess ('34) are of the opinion that sulfur-containing amino acids are especially important in arthritis and perhaps other diseases of a degenerative nature. A basis for this opinion is the low cystine content of finger nails. They found low values in 65 per cent of patients with arthritis. The normal range is reported to be 10.2

to 13 mg. per cent; the average being 11.8 mg. In patients with arthritis the values were 7.2 to 13.1 mg. per cent, with an average of 9.8 mg. per cent. Since the amounts of arginine, histidine, and lysine are normal in these cases, the low values for cystine are interpreted as an indication of the existence of an inadequate mechanism for detoxication, suggesting, therefore, a significant relationship for the arthritic state of the patients. Further support of this view is the report that sulfur therapy is followed by an increase in the content of nail cystine.

In view of the present evidence it appears that deficiency of sulfur-containing amino acids, due perhaps to inadequacy of the diet, failure in absorption, or increased metabolic requirement, is a contributing factor in degenerative diseases such as arthritis. However, the present evidence is merely presumptive and should be regarded as such unless further proof is obtained.

Glutathione.—The tripeptide, glutathione, was isolated by Hopkins in 1921 and its structure was established by Harington and Mead in 1935 by synthesis. However, previous investigations of the isolated substance had indicated its composition and structure. Several workers contributed to the latter discovery.

The structure of the reduced compound is:

$$\begin{array}{l} HOOC\text{–}\underset{\displaystyle NH_2}{CH}\text{–}CH_2\text{–}CH_2\text{–}CO\text{–}NH\text{–}\underset{\displaystyle CH_2\text{–}SH}{CH}\text{–}CO\text{–}NH\text{–}CH_2\text{–}COOH \end{array}$$

Glutathione may exist in the body as the reduced (sulfhydryl) or oxidized (disulfide) compound. Its participation in enzymatic processes is definitely proved but the specific roles of this reversible sulfhydryl-disulfide substance are not known. In all probability the tripeptide is synthesized in the body from its constituent amino acids. This is especially indicated by the work of Rose whose basal diet for the study of amino acids is entirely devoid of glutathione except for the small amount contained in the vitamin B concentrate.

Specific Functions of the "Indispensable" Amino Acids.—One of the fields for future research in nutrition is the detailed investigation of the effects of specific deficiency of each of the indispensable amino acids. At present there is relatively little definite knowledge to record. The question is still an open one whether starvation for a simple amino acid produces a unique syndrome.

Some indication of what might be observed in specific amino acid deficiencies is revealed in Weichselbaum's ('35) study of cystine and methionine deficiency in the rat. It was observed that rats on a Sherman-Merrill (1925) cystine-methionine deficient diet ". . . began to sicken and die during the sixth week. They refused food and became

sluggish; within the next two or three days they exhibited a marked curvature of the spine, showed clear signs of icterus, cyanosis of the feet, ears, and nose, became cold and had a partial paralysis of the throat which prevented swallowing. Death occurred usually within three days of the first obvious signs. Postmortem examination showed no signs of pneumonia but definite haemorrhages throughout the liver."

Also, the important observations of valine deficiency by Rose suggests further the need of investigations of specific amino acid deficiencies. As pointed out by Rose ('37, l.c.), "Its (valine) absence from the diet induces a profound failure in growth accompanied by the development of symptoms which are unlike any we have encountered in other types of deficiencies. Animals deprived of valine become sensitive to touch and manifest a severe lack of coordination in movement." The study of specific amino acid deficiency will become interesting and profitable indeed as soon as it becomes possible to prepare basal diets containing smaller amounts of interfering amino acids and to secure pure amino acids at lower cost.

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CHAPTER

VI

Nutritional Value of Proteins and the Effects of Variations in the Level of Dietary Protein

THAT THE NUTRITIONAL value of proteins varies greatly, depending upon their amino acid composition and physical condition, was not clearly realized until it was emphasized by Rubner in 1897. Shortly thereafter the work of several investigators, particularly Willcock and Hopkins in England, and Osborne and Mendel in America, demonstrated, for instance, that while rats could not live on diets in which zein, the chief protein of maize, was the principal source of protein, they could do so if tryptophane were added. It was soon shown that several purified proteins could be greatly enhanced, as respects their nutritive value, when they were supplemented with certain amino acids. Furthermore, it was learned that combinations of certain proteins in the diet produced better effects than the same proteins when fed individually and in similar concentrations. It has therefore become of practical as well as academic importance to estimate the nutritional value of purified proteins and protein-containing foods. This has necessitated an understanding of the various needs for protein in the body, particularly in relation to growth, maintenance, reproduction, and lactation.

For these reasons it is necessary to consider the physiological status of the organism, whose protein requirements are being considered, if the nutritional value of protein used in promoting that status is to be reliably assessed. This is of some importance since, for example, it has not been satisfactorily determined whether the amino acid requirements for maintenance of an animal are identical with, or different from those for growth. With respect to maintenance one must regard protein nutrition as involving both the provision and the utilization of such amino acids as cannot arise in the body through synthesis, and are needed for the replacement of tissue

proteins which undergo degradation in the chemical processes of life.

But in addition to these needs, certain amino acids are apparently required for the special purpose of serving as precursors from which hormones are synthesized. Thyroxine and epinephrine are almost certainly derived from tyrosine or phenylalanine. It is not known what happens to the proteins resulting from the endogenous degradation of tissue nitrogenous compounds. Clarifying information is needed as to whether molecules are completely degraded by hydrolysis, or whether some other type of chemical change occurs. It is known that when an animal is restricted to a diet which provides sufficient energy in the form of carbohydrates and fats, and all the essential mineral elements and vitamins, it continues to excrete hourly and daily a certain irreducible quota of nitrogenous end-products, two representative ones being creatinine and neutral sulfur compounds. But regardless of the uses made of protein in maintenance and other functions, in a practical sense, it is only necessary to ascertain the degree to which given proteins function in meeting those needs considered as a whole.

The Nutritional (Biological) Value of Proteins.—In order to determine the nutritional value of a particular source of protein, either as isolated protein or a naturally-occurring mixture, it is logical to suppose that it is only necessary to determine the minimum amount of such protein which will just serve to meet the endogenous need, and keep the animal in nitrogen equilibrium. This food protein would replace any protein degraded in the tissues, that used to form epidermal structures and that secreted in the digestive fluids as mucus, which, in part at least, escapes reutilization and is lost in the feces.

McCollum and Steenbock ('12) studied the utilization of zein and of gelatin by young swine which were fed starch, a salt mixture, and water. In one pig, for example, the nitrogen excreted in the urine was about 2.6 gm. daily, after the value had become reduced to an almost constant daily output. When the pig was fed this amount of nitrogen daily, in the form of zein, the urinary nitrogen output during eight successive days was 2.48, 2.75, 2.98, 2.62, 2.56, 3.87, 3.11, and 3.85 gm. respectively. In a succeeding period of 5 days the animal was fed only the basal ration of starch, salts, and water. In this period the output of urinary nitrogen was 3.07, 2.37, 3.69, 2.43, and 2.94 gm. per day. Since the fecal nitrogen output remained almost constant during these periods it appears that the animal utilized a considerable part of the nitrogen of this "incomplete" protein for repair or maintenance purposes. These studies were made when little, if anything, was known about vitamins and some other dietary factors. Hence the diet was inadequate with respect to several factors. Nevertheless, it seems very doubtful whether this fact invalidates the

conclusion that incomplete proteins can serve, to some degree, as tissue sparers when given to an animal as a sole source of amino acids. It is not possible to decide whether the effect of feeding an incomplete protein under these conditions is due to the mass action effect of keeping up the amino acid concentration of the blood and tissues, even with certain ones lacking which cannot be synthesized. But on the basis of this consideration, and others which might be given, it is obvious that the nutritional value of a protein, or protein mixture, for growth cannot be estimated by feeding at the endogenous plane of protein metabolism.

Without further consideration here of protein metabolism, which is fundamental in regard to the estimation of the nutritional value of proteins, it suffices to state that two general procedures can be used. They are usually referred to as the nitrogen balance method and the growth method. Each of these will be considered in as much detail as space will permit. However, it is important to realize that a protein, or mixture of proteins, might not satisfy the requirements for lactation, as an example, in the same degree that it satisfies the needs for growth. There is no definite evidence in opposition to this premise, but theoretically it is valid since, for example, the amino acid mixture required by the mammary gland to synthesize milk might not be similar to that required in the young animal to promote growth.

There are no very satisfactory procedures for determining the nutritional value of proteins except for growth and even in those there is no general agreement. The factors involved have been extensively discussed. Mitchell and Hamilton ('29) have canvassed the entire subject and Boas Fixsen ('34-35) has succinctly reviewed the methods applicable to this problem.

Nitrogen Balance Methods for Determining the Nutritive Value of Protein.—In 1909 Thomas devised a procedure wherewith the value of a protein could be estimated from a short-term nitrogen balance experiment, using rats or other suitable experimental animals. Obvious faults in the method were soon recognized. In 1924 Mitchell ('24) published a critical study of the nitrogen balance technic and proposed modifications which were claimed to constitute adequate improvements. Since then Chick and Roscoe ('30), Boas Fixsen and Jackson ('32), and others, have described further modifications.

Space will not permit a thorough discussion of the method, but it is important that the student have presented at least a brief outline of it. As described by Mitchell ('26), "The experiments on rats involve direct determinations of the amounts of nitrogen in the feces and in the urine, and indirect determinations of the fractions of the

fecal nitrogen and of the urinary nitrogen that are of dietary origin. The biological value of the protein is taken as the percentage of absorbed nitrogen (nitrogen intake minus fecal nitrogen of dietary origin) that is not eliminated in the urine."

As stated further by Mitchell, "Obviously the uncertainty of the method is related to the factoring of the fecal and urinary nitrogen into food and body components. Since no direct method is available, indirect methods must be relied upon. There is reason to believe that such estimations may be based on the values for the daily excretion of urinary and fecal nitrogen on adequate amounts of a nitrogen-free ration. The 'metabolic nitrogen' of the feces on a protein-containing diet . . . is related to the amount of food consumed and may be measured by the total excretion of fecal nitrogen on a nitrogen-free diet. The latter may be used with the most confidence when the 'roughage' content of the nitrogen-free diet approximates that of the protein-containing diet. The 'endogenous nitrogen' of the tissues is related to the body weight of the animal, and may be measured by the total urinary nitrogen on an otherwise adequate nitrogen-free diet. Both of these values are, of course, to be determined only after a period of preliminary feeding of sufficient length to insure equilibrium between the animal and its food.

"The method may be illustrated by the following analysis of an actual metabolism study on a rat receiving a ration containing approximately 4 per cent of a mixture of proteins derived from corn and tankage. The rat consumed an average of 56.9 mgm. of nitrogen per day in a 7-day collection period. On this diet, the daily excretion of fecal nitrogen was 27.6 mgm., but of this nitrogen it is estimated from the fecal nitrogen excreted on a nitrogen-free diet, that 21.7 mgm. were derived from the body, leaving only 5.9 mgm. contained in the indigestible food protein. Hence, the rat was receiving daily $56.9 - 5.9 = 51.0$ mgm. of absorbed nitrogen. During this period, the excretion of urinary nitrogen was 48.6 mgm. daily, of which 37.7 mgm. were the result of the catabolism of the body's own tissues (estimated from the excretion of urinary nitrogen on a nitrogen-free diet). Hence, only $48.6 - 37.7 = 10.9$ mgm. of the absorbed dietary nitrogen were wasted in metabolism, and $51.0 - 10.9 = 40.1$ mgm. were retained in the body. The biological value of the protein, therefore, is equal to $100 \times (40.1 \div 51.0) = 79$."

Mitchell's formula which expresses the nutritional (biological) value of a protein is as follows:

$$100 \times \frac{\text{Body Nitrogen Spared}}{\text{Food Nitrogen Absorbed}}$$

It should be stated further that the basal diet used in this method contains sufficient carbohydrate and fat to satisfy the energy requirements; thus tending to prevent the catabolism of body protein for energy. The diet must be complete as respects mineral elements and vitamins, these being supplied as the pure substances or the most potent concentrates obtainable, so that the only inadequacy will be with respect to protein. However, as indicated in Tables I, II, and III (see appendix), on the nutritive value of proteins, it will be observed that practically all determinations made by the nitrogen balance method, as well as others, have not satisfied this criterion. In most cases inadequate amounts of vitamin B-complex were provided or else the amount of nitrogen introduced with the vitamin supplement was sufficiently large as to probably supplement or augment the test protein.

Nevertheless, this method does furnish useful information regarding the nutritive value of proteins. It should be recognized, however, that there are some valid and significant objections to its use. One of these relates directly to technical problems of determining nitrogen balance under such conditions. Obviously feces and urine must not only be collected quantitatively, but they must be accurately separated. Also, even slight contamination of the excreta with the diet will vitiate the results. Moreover, the so-called endogenous nitrogen output must be determined for each experiment since it varies not only from animal to animal, but in the same animal at different times.

In order to ascertain the endogenous nitrogen output characteristic of the test animal it is necessary to keep the animal on a nitrogen-free diet to bring it into a condition where the endogenous type prevails. This preliminary period must not be prolonged to a point which causes loss of appetite.

The estimation of fecal nitrogen of endogenous origin presents practical difficulties. A part consists of undigested protein, or protein which has been fixed in the intestine as bacterial protein and which escapes absorption; and a part consists of unabsorbed protein excreted into the intestine with the digestive juices, which is lost in the feces. If the diet contains much indigestible material, which increases the water content, the latter fraction is increased. Obviously this loss should be kept as low as possible. In general, the endogenous fecal nitrogen has been found to be proportional to the food intake.

Since the nutritional value of proteins, as generally determined by the nitrogen balance method, are expressions of the utilization of absorbed protein, it should be evident that a protein might be of very poor quality, owing to low digestibility, yet rate a relatively high

nutritional value. This is shown in the data of Seegers and Mattill ('35), who compared the nutritional value of dried liver with that of heated dried liver, using the nitrogen balance method. With their basal diet, containing 7.2 per cent of whole dried liver, young rats weighing 40 gm. grew to an average of 165 gm. in 45 days. The same diet with heated liver induced rats of similar initial weight to increase to an average of 110 gm. in 50 days. In contrast to these large differences in the growth-promoting value of the two proteins, it was found that the nutritional value of whole dried liver, was 57 (average of eight experiments) and that of heated liver was 51 (average of eight experiments). It is apparent that the ability of the animals to utilize the proteins from these two sources for growth is only approximately expressed by the nutritional value of the protein as determined by the nitrogen balance method.

Growth and Weight Maintenance Methods.—In order to determine the value of a protein in the promotion of growth the most natural method of making that determination is the actual measurement of growth occurring with a given intake of protein. The problems of magnitude, in this procedure, are (a) food consumption, (b) length of experimental period, and (c) level of test protein in the diet. Their significance will appear in the discussion to follow.

The extensive and important studies of Osborne and Mendel were based entirely on some form of growth performance with a given intake of protein. Osborne, Mendel and Ferry's ('19) method relates the growth obtained to the intake of protein, and expresses the nutritional value (X) of the protein by the ratio

$$X = \frac{\text{Gain in Weight}}{\text{Intake of Protein}}$$

As pointed out by Boas Fixsen ('34-35, l.c.), "Since, before any protein can be used for growth, the maintenance requirement must be satisfied, the value of X will rise as the protein intake is increased until an optimum is reached at which the intake of protein is sufficient to allow the maximum rate of growth. Thereafter, with further increase in protein intake, the value of X declines, the surplus protein being used for energy requirements." Osborne, Mendel and Ferry attempted to determine the level at which a given protein should be fed in the diet in order to obtain the maximum value for X. Most workers, however, have fed the test protein at an arbitrarily fixed level. This is usually from 5 to 12 per cent. If fed at higher levels appreciable and increasing amounts are used to satisfy

energy needs and if fed at lower levels appreciable and increasing amounts are used to satisfy maintenance needs.

In this method, as in the nitrogen balance procedure, strict attention must be given to the provision of adequate energy, as carbohydrate and fat; and adequate vitamins and minerals without the introduction of appreciable protein or other nitrogenous material.

It seems unnecessary to place a low estimate on the results of this type of experimental comparison of proteins. They yield results which do compare proteins in an intelligible and practical way and we should never be able to deduce these values from the nitrogen balance procedures.

McCollum and Shukers (McCollum and Simmonds, '29) have modified this general procedure by determining the actual protein ($N \times 6.25$) retention which is possible when a given protein is consumed at an arbitrary level of intake. Three groups of young rats are selected which are strictly comparable with respect to age, weight, and genetic composition. Group 1 rats are killed, the contents of the digestive tracts removed by washing, and the entire carcasses are hashed and analyzed for total nitrogen. The remaining two groups are fed for 28 and 56 days respectively, on screens to prevent access to feces, the food consumption being carefully determined. The total nitrogen contents of their carcasses is determined as in the case of group 1 rats. From these data, including the protein consumption, it is obviously possible to estimate the per cent of ingested protein which is used to form tissue protein ($N \times 6.25$). The per cent so retained represents the nutritional value of the food protein subjected to test.

The advantage of this method over that of growth procedures, such as those of Osborne and Mendel, is that the actual protein (nitrogen) retention is determined. This may be of some importance since it has been shown that the composition of tissues may vary considerably during growth owing to differences in dietary ingredients. With reference to the nitrogen balance methods it also has some advantages. The animals are under less stress as obtains in conditions imposed by the quantitative collection of excreta. Also the procedure requires less technical work. It expresses the nutritional value of protein in practical terms, i. e., the percentage utilization of ingested protein for growth rather than the percentage utilization of absorbed nitrogen for the sparing of body nitrogen after nitrogen starvation.

The following table provides a comparison of values obtained by the Osborne and Mendel growth method, the McCollum and Shukers nitrogen retention method, and the Mitchell nitrogen balance method:

COMPARISON OF THE NUTRITIONAL VALUE OF PROTEINS AS DETERMINED BY THREE DIFFERENT METHODS

SOURCE OF PROTEIN	PER CENT PROTEIN IN DIET (N × 6.25)	AVERAGE NUTRITIONAL VALUES		
		Osborne and Mendel	McCollum and Shukers	Mitchell
Whole wheat	8.25	1.18	25.0	82
Menhaden meal	8.7	1.25	21.1	72.5
Menhaden meal	12.1	1.11	22.0	67.3
Menhaden meal	14.4	0.98	20.9	60.5
Menhaden meal	17.8	1.30	24.7	68.6
Whole wheat 67.5, casein 10.0, and whole milk powder 10.0	17.4	1.67	34.3	71.9
Whole wheat 35.5, casein 5.0, and whole milk powder 10.0	10.4	1.86	37.6	85.8

Paired Feeding Method.—This method, devised by Mitchell and Beadles ('29-30), is generally regarded as a particularly suitable procedure in studies of the supplemental relationships between proteins and the amino acids in which they might be deficient. Of course it has other applications. At the beginning of an experiment young rats are paired with respect to weight and genetic capacity. If, for example, the problem is the determination of cystine deficiency in a protein one rat is given the basal diet containing the test protein and the food intake is carefully determined. The mate to this rat is given the same basal diet and test protein plus cystine, but the food intake is restricted to the amount ingested by the first rat. If, over a given period of time, the rat receiving added cystine weighs more than its mate it is concluded that the protein in question is deficient in cystine or that the cystine is unavailable in adequate amounts. The degree of deficiency is determined by the differences in weight of the two rats. Thus, numerical results are obtained and differences in palatability of test proteins are controlled. In order to make the data statistically valid eight or more pairs of rats are used in each assay.

Boas Fixsen ('34-35, l.c.) has called attention to the limitations of the paired feeding method for comparison of the utilization of proteins. The principal question is whether an animal which is not allowed to satisfy its appetite is strictly comparable, for the purpose of the paired-feeding method, with one which eats all it wants.

McCollum Long-term Studies of Protein Foods.—McCollum and his associates ('21) attempted to compare the relative values of pro-

teins from different sources by feeding a series of groups of rats on comparable basal diets containing equal proportions of different proteins. The results were judged on the basis of growth, fertility, lactation, infant mortality, and longevity. Boas Fixsen ('34-35, l.c.) has criticized these studies. She states: "Where so many factors are involved, the assessment of results such as these must be largely a matter of individual judgment, some proteins giving better results for one function, others for another. It seems unjustifiable, also, to assume that all the dietary essentials are yet recognized, particularly those whose absence would be felt only after long periods of deprivation. Since no records were made of food intake, it is impossible to ascertain whether the intake of the rats receiving the different diets was comparable."

Her criticism seems to justify the too common belief that there is unique value in knowing the nutritional values of the proteins of different foods apart from their palatability, and hence the consumption of food by animals fed different natural products, and also that the point of singular interest is the determination of the nutritional values of proteins for growth. It is, of course, a matter of individual interest whether it is desirable to know the subsequent physiological history of animals fed a comparable basal diet, but supplying proteins from different sources, as shown by their growth, fertility, capacity to secrete milk, and longevity. The experiments of the type we are considering do show the comparative values of different foods, expressed in terms of their protein, as respects their effects on the life histories of the animals. They do not enable one to express the nutritional values of these proteins numerically, and were never claimed to have this merit.

Biological Availability of Protein in Foodstuffs.—Before ingested protein can be absorbed and utilized by the body it must be hydrolyzed (digested) into its constituent amino acids, or possibly simple peptides. In the case of certain foodstuffs the protein is completely hydrolyzed, under physiologic conditions, but others are only partially reduced to the amino acid stage. The protein of eggs is a good example of the former and those of most legumes represent one class of the latter. This, however, does not constitute the only criterion of nutritional quality, as subsequent discussion in this chapter will reveal.

Soy beans are useful examples of protein apparently containing all of the amino acids essential for good health. Yet, upon biological analysis, they have consistently shown low nutritive values. But, as demonstrated by Hayward, Steenbock and Bohstedt ('36a), the mere application of heat, sufficient to produce a temperature of 140 to 150° C. for 2½ minutes, practically doubles the nutritive value. A sim-

ilar improvement in nutritive value was demonstrated by supplementation of this protein with 0.3% l-cystine. This suggests that cystine or its equivalent may exist in the raw soy bean in a form which is not available to the animal and that the appropriate application of heat is sufficient to render the cystine available. Apparently analogous situations exist with respect to several other protein foods, particularly in meats which contain relatively large amounts of connective tissue.

Another factor is the effect of roughage on protein utilization (Funnell, et al., '36). Numerous studies of this subject have been made, but opinion seems to differ concerning the significance that should be attached to it. Adolph and Wu ('34), for example, claim ". . . a slight tendency toward a lowered degree of protein digestibility only when the fiber was ingested in an abnormally large amount." They suggest that lowered values for protein digestibility on a given diet result only when the food material passes through the alimentary tract with abnormal rapidity. In view of the present evidence this appears to be a reasonable conclusion.

A third factor which is probably of greatest practical importance is that of heat, particularly dry heat. This will be considered subsequently along with data on the effects of ultraviolet irradiation and deamination.

Space here is too limited to permit much consideration of the effect upon digestion of carbohydrate and protein mixtures. But brief comment seems necessary since there is a surprisingly large class of lay people who seem to be of the opinion that mixtures of protein and carbohydrate interfere with gastric digestion. A large amount of evidence may be cited in refutation of this faddist's belief. Two studies of particular value are those of Rehfuss ('34) and those of Shay, Gershon-Cohen and Fels ('36), who found no evidence whatsoever of incompatability between carbohydrate and protein digestion.

Nutritional Value of Common Proteins.—It has been emphasized that the nutritive value of protein is ultimately dependent upon the amino acid content. Moreover, consideration has been given to the relationship between nutritive value and such factors as protein digestibility and the level of protein in the dietary. These variables are considered in estimating the nutritive value of proteins.

In practical dietetics neither animals nor man are ever restricted to a single protein as a source of the necessary amino acids. Hence, data upon the nutritive value of individual proteins are actually of less utilitarian value than those of the supplementary relationships between such dietary essentials. But it is advantageous to consider first, as a basis for subsequent attention, the data on individual proteins. These are summarized in tables I and II (Appendix). How-

ever, one should be particularly critical in the use of this and subsequent tables of proteins in nutrition. One must realize that there is no general agreement concerning methods of estimating the nutritive value of proteins. Consequently it has been necessary to employ an arbitrary policy in preparing such tables. At best the numerical data constitute only approximations of the actual nutritional value of the proteins which they represent. Certainly one is not able to make deductions from them concerning the relative value of proteins in such general functions as growth, maintenance, reproduction, and lactation. It must be assumed, until more refined analytical distinctions can be made, that the value of a protein in any one of these functions is approximately similar with respect to all of them.

In general, as revealed by table II (Appendix), proteins of animal origin have a higher nutritive value than those of any other class. This is understandable, particularly in the case of milk and eggs, since these proteins must provide all the amino acids required by the young mammal or developing chick.

Whole eggs, which constitute a mixture of proteins, have a higher nutritive value than any other source of protein known. However, egg white, separated from the yolk, gives a rather low value, as shown by the table.

Considerable difference of opinion has existed concerning the status of casein and lactalbumin, the principal proteins of milk. It appears that lactalbumin constitutes a better source of essential amino acids than casein alone. Undoubtedly the method of isolating these proteins influences in considerable measure their nutritive value. This probably accounts for some of the early conclusions, 10 to 15 years ago, that lactalbumin is an inferior protein. Dry heat is known to lower the nutritive value of proteins if it is applied even for short intervals of time. It appears that the limiting factor in casein is cystine (Mitchell, '23-24a), although that amino acid is not essential if an adequate amount of methionine is present. Hence it is a common practice to fortify casein with 0.05 to 0.10 per cent of cystine in studies of rats if it is desired to feed this protein at a low level.

It is generally found that the proteins of liver, kidney, and heart have higher nutritive values than those of skeletal muscle meats. As pointed out by Mitchell, Beadles and Kruger ('27), the nutritional value of meat is inversely dependent, in considerable measure, upon its content of connective tissue. Hence some of the cheaper cuts of meat, which contain large amounts of connective tissue, are distinctly less valuable as sources of proteins. However, there is some supplementary relation between connective tissue and the protein of muscle. It appears that the nutritive values of sweetbreads, tripe, beef cheek meat, and ox lips are definitely lower than those of skeletal muscles.

Hoagland and Snider ('36) claim that there appears to be scarcely any difference between veal, ox, and hog brains, ox and hog tongues, ox spleens, dried milk, and skeletal muscles of ox, hog, and sheep. Mitchell claims, however, that he consistently obtains a higher nutritive value for milk proteins than for meat proteins.

A consideration of proteins derived from animal tissues would not be adequate unless some attention were given to gelatin. This protein, derived from the collagen present in white fibrous tissue, has been the subject of controversy, regarding its nutritional significance, since the early part of the nineteenth century. It is indeed notoriously deficient in certain indispensable amino acids and, consequently, will not even support life when it constitutes the only source of amino acids in the diet. However, it appears to be well tolerated and may be of value as an appetizer. But, as concluded by the Council on Foods of The American Medical Association ('36), it is not established that gelatin aids in the digestion of milk or the treatment of myopathies. For the latter purpose gelatin has been in some favor owing to its high content of glycine, the amino acid alleged to be of benefit in myopathies.

On the basis of dietary studies made several years ago by the U. S. Department of Agriculture it appears that white flour (wheat) contributes almost 30 per cent of the protein in the average American dietary. There is no reason to assume that the value has changed materially since that time. Owing to the prominent place of wheat, oat, and corn products in the dietary of both man and agricultural animals these and certain other cereals should be given some attention here.

As is indicated by the tables, all cereal proteins have lower nutritional values than those, in general, of meat and animal products. For example, according to Mitchell and Carman's data ('26), the nutritional values of white flour, whole egg, egg albumin, milk, veal, and beef are respectively, 52, 94, 83, 85, 62, and 69. Boas Fixsen et al. ('34) claim that there is scarcely any difference between the nutritive values of whole wheat and whole corn but Mitchell's ('23-24b) data indicate slight superiority of oats over corn. Mattill ('30), using the nitrogen balance method, obtained results indicating approximately the same differences between wheat and oats. The values for wheat endosperm and whole wheat were essentially alike, but those of precooked oats were definitely higher. In the interpretation of these data, obtained by means of the nitrogen balance procedure, it should be remembered that what was determined was the percentage of absorbed protein used in anabolism. The amount wasted through failure of digestion or absorption was ignored in expressing the numerical ratings.

The legumes, in general, contain rather high percentages of protein and in some parts of the world they are the chief source of dietary proteins. In this country, soy bean protein is of considerable interest as an animal food, but, as indicated previously, the nutritive value is relatively low unless it is cooked or supplemented with cystine, procedures which are obviously impractical. Data, such as are available on peas, peanuts, and other legumes may be found in table I.

Tubers contain significant amounts of protein but neither white nor sweet potatoes are particularly valuable as sources of all the essential amino acids.

Yeasts, molds, and other microorganisms probably could be relied upon to furnish protein, as well as other dietary essentials, if present sources should become limited. It has been demonstrated that rats can grow on diets containing yeast as the sole source of protein. Skinner ('34) believes that all the essential amino acids are synthesized by *P. flavo-glaucum,* but that cystine is a limiting factor when rats are restricted to this mold as the sole source of protein. A more extensive study of the problem by Gorcica, Peterson and Steenbock ('35) indicates that *Aspergillus sydowi* is of quite poor quality when fed as the only source of protein. However, whole wheat or corn gluten, fed at a low level, was able to confer a marked supplementary effect upon the mold. Undoubtedly the further study of microorganisms, as sources of dietary essentials, will yield interesting and valuable results.

Supplementary Relationships Between Proteins and Amino Acids.—For reasons presented in Chapter V, practical dietetics and animal husbandry are dependent, as respects protein nutrition, upon the reinforcement of essential amino acid inadequacies in available proteins with other available proteins which contain such amino acids in relative abundance, but which, perhaps, are inadequate in those possessed in relative excess by the first. Thus an understanding of supplementation is simple indeed.

As a rule animal proteins constitute good supplements for cereals and vegetable proteins. Evidence of this fact was made available by the extensive early studies of Osborne and Mendel and those of McCollum and Simmonds. Most recent contributions have been made by Mitchell et al., and others.

Instructive data upon the supplementary relationships between beef and vegetable proteins, for example, are furnished by Hoagland and Snider ('27) (Table III). When one part of beef protein was mixed with two parts of vegetable protein, the rations containing the following vegetable products had approximately the same value for inducing growth, viz., wheat, bolted wheat flour, corn meal, and oat

meal. Each of these mixtures also had approximately the same value for inducing growth as a ration containing the same percentage of beef protein alone.

According to Mitchell and Carman's ('26, l.c.) nitrogen balance data, milk, and white flour mixed (1 to 2) gave a value of 71, whereas the computed value, based on the nutritional value of each reckoned separately, was only 62. Moreover, white flour and beef (2 to 1) gave a value of 73, whereas the computed value was about 64. Thus in each instance a definite supplementary effect is indicated.

In certain countries, particularly the Orient, it would be of great hygienic and economic importance if some combination of vegetable proteins could be found which supplement each other to the extent of providing an amino acid mixture as satisfactory as that furnished by combinations of animal and vegetable proteins. According to Adolph and Cheng ('35) a flour made from a mixture of corn, millet, and soy beans, has a nutritional value considerably higher than that of any of the three components alone. Moreover, this mixed cereal flour is said to occupy a prominent place in the Chinese dietary. However, it appears improbable that any combination of vegetable proteins can be found which has the marked supplementary relationships as exists between animal and vegetable proteins. Vegetable proteins tend, almost invariably, to be somewhat deficient in lysine. And, as emphasized by Mitchell and Smuts ('32), tryptophane tends to occur in inadequate amounts, particularly in corn. In general, beef and soy bean proteins are deficient in cystine, wheat protein in lysine, corn protein in lysine and tryptophane, and oat protein in lysine.

Protein Requirements.—A perennial problem is the question of protein requirements for growth, maintenance in adult life, pregnancy, lactation, various conditions of disease, live stock production, milk and egg production, and even other departments of nutrition. Nevertheless, there is considerable basis for belief that good health can be maintained although the protein intake, provided it is of satisfactory quality, varies considerably from the level which is optimum for the body.

In the last century deductions of Voit, Atwater, and others, on the basis of statistical studies of human dietaries, placed the adult daily requirement for protein at about 110 to 120 gm. On the basis of present evidence, obtained by experimental procedures, this value is probably somewhat near the average normal requirement. Thus in this particular case the results, secured by unphysiological methods of calculation, appear to be approximately correct.

At the beginning of this century Chittenden conceived the idea that a low protein dietary will best meet the nutritive requirements of the body. He emphasized the desirability of relieving the body of the

task of degrading more protein than is necessary for the replacement of nitrogenous compounds used in metabolism and tissue repair. The principal deduction was that protein intake should be reduced to as low a level as possible, and still keep the body in nitrogen equilibrium. In the evaluation of this thesis numerous adults were observed over a period of 9 months during which the protein intake was reduced to about half the accepted requirements. Since they appeared to be in excellent condition at the end of the period of observation it was concluded that such a dietary regimen appeared to be suitable for maintenance of well-being in the adult for an indefinite period. Since that time numerous advocates of low-protein regimens have made themselves heard but convincing proof of such a policy, for general application, has not been demonstrated.

Of the numerous experimental data of McCollum and associates, none support this view. They all point to the conclusion that a generous protein ingestion which just suffices to induce maximal growth in the young serves to maintain vigor in adults for the longest possible period. Since these studies were made twenty years ago, before knowledge of dietary essentials was as advanced as it is now, it is believed that further study of the question should be made, using as criteria, longevity and general health throughout adulthood. However, there is no reason to believe that further research, in the light of present knowledge, would indicate a reversion to the thesis of Chittenden and others who advocated a low-protein regimen. The vigorous arguments of Christiansen ('34), Süsskind ('34), and others, against the low-dietary protein thesis of Hindhede ('34) and his supporters, provide further evidence that, in general, the safest policy is to maintain liberal amounts of protein in the diet.

As subsequent discussion will show, several types of disease are referable to inadequate protein intake. Even persons with certain types of nephritis appear to be benefited by the maintenance of the protein intake at a fairly liberal level (McCann, '31). As shown by Peters ('32), tuberculosis, diabetes, and some gastrointestinal diseases may be treated by the restoration of serum protein to a normal level through an increase of protein in the diet.

Space does not permit a consideration of the protein needs of various age groups and such special cases as pregnancy and lactation. The discussion of these subjects is more appropriate in special books and monographs written for that purpose. In addition to brief comments it suffices to mention that the protein requirements of children have been studied by numerous workers including Wang, Hawks and Hays ('28), McQuarrie ('29), Parsons ('30), and Daniels et al. ('35). Daniels et al. conclude that children of preschool age should receive approximately 3.2 gm. of protein per kilogram in diets furnishing at

least 50 per cent of the protein from animal sources. There seems to be no very authoritative opinions regarding the protein needs of adolescence. Undoubtedly the great variability in growth rate, as well as other factors, during that period precludes the application of more than very general standards.

Slonaker's Observations on the Effects of Different Amounts of Protein in the Diet.—As indicated above, it is of interest to determine the effects upon various life functions of different amounts of protein in the diet. The studies of Slonaker ('31), although unfortunately faulty in some important aspects, are sufficiently instructive to warrant some discussion. The five diets used in this study had the following composition, as calculated from Slonaker's data:

Components	Per Cent				
	I	II	III	IV	V
Cornstarch	48.31	44.44	40.48	36.49	32.36
Whole ground wheat	19.32	17.78	16.19	14.59	12.95
Whole ground yellow corn	9.67	8.89	8.13	7.30	6.41
Skimmed milk powder	3.87	3.56	3.24	2.92	2.59
Alfalfa leaf flour	3.87	3.56	3.24	2.92	2.59
Commercial casein	1.93	1.78	1.62	1.46	1.31
Meat scrap	0.96	8.89	17.00	25.18	33.66
Wheat germ	2.90	2.67	2.43	2.19	1.94
Unsalted butter	4.83	4.44	4.05	3.65	3.24
Yeast	1.93	1.78	1.62	1.46	1.31
Sodium chloride	0.96	0.89	0.81	0.73	0.64
Calcium carbonate	1.45	1.33	1.21	1.09	0.97

In addition to the above table it is helpful to estimate the calcium and phosphorus content of the different diets. Although it is impossible to make more than a very rough approximation, the errors should be relatively constant. The table which follows was calculated on the assumption that the calcium and phosphorus content of Slonaker's dietary components approximated the values given in standard tables on the composition of foods:

Diet I		Diet II		Diet III		Diet IV		Diet V	
Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.6414	0.2013	0.5917	0.2349	0.5459	0.2664	0.4977	0.3003	0.4534	0.3342

According to Slonaker's calculations, the protein content of these diets was: 10.3; 14.2; 18.2; 22.2; and 26.3 per cent respectively for diets I to V inclusive. Some of the results of feeding these diets to rats throughout life, were as follows: The average life span from longest to shortest was in the order of Groups II, III, I, IV, and V. During the first 120 days the average per cent increase in body weight from greatest to least was in the order of Groups V, IV, III, II, and I. Group V averaged 306 per cent and I averaged 112 per cent. The order of maximal weight was Group III, 307; II, 294; I, 288; V, 253; and IV, 247 gm. The average number of litters and the average number of young born, from greatest to least, was in the order of Groups II, I, III, IV, and V. The size of the litters was in the order of I, II, III, IV, and V.

On the basis of his numerous observations and calculations, Slonaker concluded "that when best growth is considered the amount of protein should be slightly in excess of 14 per cent." But when the method of preparing the diets is considered it is apparent that instead of one variable, namely protein, there are at least eleven variables, i. e., all of the various components of the diet! This was caused by the unusual method used to construct the diets. Diet I was used as the basal ration. Diet II was made by adding 900 gm. of meat scrap to 10,350 gm. of diet I. Two thousand grams of meat scrap were added to 10,350 gm. of diet II to make diet III, and so on. Thus this procedure caused the inclusion of many variables, as is indicated by the above table on the percentage composition of the diets. For example, in diet I the amount of yeast is 1.93 per cent but in diet V it is only 1.31 per cent. Also, the amount of calcium carbonate in diet I is 1.45 per cent and it is only 0.97 per cent in diet V. Finally, the calcium: phosphorus ratio of diet I is much higher than that of diet V. Since the content of vitamin D is almost nil it may be supposed that the effect of diet I on calcium and phosphorus metabolism is much different from that of diet V. Diets II, III, and IV occupy intermediate positions in these relationships. Since all of the variables undeniably influence rate of growth, reproduction, longevity, etc., it is obvious that the results of these studies are practically meaningless owing to the multiplicity of uncontrolled variables.

Moreover, meat scrap is not a pure protein. At best it represents a concentrated source of digestible protein, and at worst, material which consists in great measure of connective tissue and cartilage. It contains large and significant amounts of phosphorus as well as other minerals, especially potassium.

These experiments, although practically impossible of interpretation, are somewhat typical of nearly all investigations concerning the effects of various levels of protein in the diet. The general fault is

that nearly all so-called proteins, as used in dietary studies, are at best crude materials containing significant amounts of substances which are not protein. For example, the most commonly used protein, casein, contains almost one per cent of phosphorus. It is easy to imagine, therefore, that the results obtained with casein at a high level, perhaps 65 per cent of the diet, as compared with this material at 15 per cent of the diet, are not open to interpretation only on the basis of a difference of protein level in the diet; the differences in phosphorus must also be reckoned if an accurate evaluation is to be made. In the past there has been, perhaps, some excuse for failure to consider some of these things in studies or proteins, owing to the lack of information concerning the "importance of little things," but in the future investigators must pay stricter attention to the planning of their experiments if they are to obtain significant information.

The Effect of Heat upon the Nutritional Value of Proteins.— Most proteins are profoundly changed in their physical properties by being heated to their coagulation temperatures. The evidence available points to the nature of this change as one of polymerization. Attention has been called to the changes in the molecular weights of proteins at different points along the pH range (Chapter V). Chick and Martin ('10) have shown that water must be present when denaturation occurs. Also Wu and Wu ('25) offer evidence that some hydrolytic splitting of the molecule occurs. They have discussed the views of different investigators concerning the nature of denaturation, which is a term loosely used to designate the change of proteins from a soluble to an insoluble form by heat, light, pressure, acids, alkalies, certain organic solvents, salts of heavy metals, dyes, etc. We are here interested only in the effect of heat treatment, which is widely applied in the preparation of foods.

Many foods are subjected to more or less dry heat treatment, as in popping corn, roasting peanuts, baking crackers, and the preparation of flaked or puffed cereals. Frying foods or deep frying in fat may result in superheating of a part of the protein of a food. Pressure cookers, which are widely used, superheat the proteins. We should, of course, carefully distinguish between heating with superheated steam, involving the exclusion of air, which would cause hydrolytic cleavage of proteins, and dry heat treatment, where oxidation might occur.

Morgan ('30) conducted growth studies with young rats which showed that the protein of cereals was not well utilized after dry heating or toasting at approximately 200° F. for 45 minutes. Toasted whole wheat showed 15 per cent less absorbability than raw wheat protein. The addition of 5 per cent of unheated casein very nearly made up the discrepancy between the toasted and the raw diets,

showing that the heat treatment affected the protein rather than another constituent of the diet.

When young rats were fed diets containing raw and toasted (150° F. for 30 minutes) wheat gluten as a source of protein at 9, 12, 15, 18, 21, and 24 per cent levels, in an otherwise adequate diet, the growth in 56 days, per gram of protein eaten, was found to be 1.29 to 1.40 for raw gluten at levels up to 18 per cent, and to fall to 1.15 and 0.98 at 21 and 24 per cent respectively. The corresponding figures for the toasted gluten were 0.94 to 1.09, and 1.12 and 0.86. The maximum growth rate on both raw and toasted diets occurred at the 18 per cent level.

The nutritional values of raw, water-cooked, and toasted whole wheat protein, as determined by the nitrogen balance method of Mitchell, were found to be 64, 67, and 52 respectively, and 67, 75, and 69 when supplemented by 5 per cent of unheated casein. In a similar comparison of the values of raw and toasted wheat gluten at an approximately 12 per cent level the figures obtained were 66 and 54 respectively. Large and mature rats, when fed raw and toasted wheat gluten at 6 per cent level, gave nutritional values of 83 and 64, and when fed at a level of 8 per cent protein the values were 66 and 53 respectively.

The digestibility of the toasted proteins was but little different from that of the unheated, particularly in older animals, and the unexplainable loss of nitrogen occurred chiefly in the urine, indicating that the change produced by the heat treatment was probably in assortment or availability of the amino acids absorbed. Cooking with water had scarcely any effect on the nutritional values of proteins, and caramel formation during toasting was found to account for only a small part of the injury.

Chick and her associates ('35) compared the nutritional values of several proteins. They were the proteins of whole wheat, white flour, wheat germ, maize endosperm, whole milk, lactalbumin, heated lactalbumin (at 120° C. for 72 hours), casein, heated casein (at 112-125° C. for 72 hours), and heated casein purified by reprecipitation and extraction with dilute alcohol. These were fed at levels varying from 3 to 10 per cent of the diet. The value of casein was not lowered by heat treatment at 112 to 125° for 72 hours or by further purification. Heating it to 150° for 66 hours decreased its value from 64 to 44 (5 per cent of protein in the diet), and reduced the digestibility from 93 to 73 per cent. The heat-treated lactalbumin (120° for 72 hours) was scarcely reduced in nutritional value. However, its digestibility was reduced from 95 to 69 per cent.

Seegers and Mattill ('35) observed essentially the same rate of growth in rats fed adequate diets deriving almost all of their protein

(15 per cent) from beef heart, kidney, round, and liver. Pork liver was equal to beef liver. When these tissues were continuously extracted during 60 hours with 95 per cent alcohol there was but little reduction of nutritional value of the protein except in the case of liver, the proteins of which were greatly altered by prolonged contact with hot alcohol. When liver was extracted 130 hours it did not support growth or life. It was shown to have but slight digestibility. Liver heated at 100° C. for 14 days no longer supported growth although its digestibility and nutritional value were not lowered appreciably. Above 100° C. there was progressive lowering of its digestibility. Heart and round were resistant to change in nutritive value at 120° C., and kidney was still digestible after heating at 130° C. At higher temperatures these tissues also became refractory to digestive enzymes. It is pointed out that these changes in digestibility and in nutritive value do not occur to a significant degree in domestic cookery, but are of great importance in the preparation of purified proteins for nutrition studies.

Morgan and Kern ('34), however, found the value for maintenance of raw beef muscle protein at 7 per cent level to be greater for rats than that of the same meat cooked in three different ways. The value for raw beef, by the Mitchell method, is 67, for that boiled at ordinary pressure (internal temperature 85° C.), 60; boiled for 7 minutes at 15 lbs. pressure (internal temperature 85° C.), 60; and boiled for 1 hour at 15 lbs. pressure, 56. A heat injury appears to occur, which increases with length of exposure and intensity of heating.

That the depreciation of protein on heating is not solely one of physical change which interferes with digestion, follows from the fact that the observed nutritional values fall with heat treatment, and also from the findings of Greaves and Morgan ('34), who supplemented heat-treated casein with 0.2 per cent of lysine, histidine, tyrosine, cystine, and tryptophane respectively. Cystine addition increased the rate of growth with both raw and heated casein, uniformly by about 15 per cent above the unsupplemented values, but the difference between raw and heated protein still remained. It seems probable that cystine is not the damaged part of the molecule. Both lysine and histidine supplements increased the value of the heated casein but did not affect that of the raw, as illustrated by their figures.

	Gain per Gram of Protein Eaten
Unsupplemented heated casein	1.76 grams
Lysine supplemented heated casein	1.89 "
Histidine supplemented heated casein	1.96 "
Lysine supplemented raw casein	2.00 "
Histidine supplemented raw casein	3.14 "
Unsupplemented raw casein	2.10 "

Tyrosine and tryptophane additions did not increase the value of heated casein. It is concluded that heat treatment of casein in these experiments caused change in the lysine and histidine units of the protein which destroyed their nutritive value.

Block, Jones and Gersdorff ('34) were stimulated by the results of Greaves and Morgan to determine the lysine in casein which had been heated to 150° C. for 65 minutes. Their results show that this treatment does not materially affect the content of this amino acid. Their yield of lysine in unheated casein was 6.5 per cent, and that in two samples of heated casein was 5.5 and 6.6 per cent respectively. The results of Greaves and Morgan are, therefore, not to be explained on the basis of existing data.

Hayward, Steenbock and Bohstedt ('36b) observed effects quite contrary to the above described deterioration of nutritive value of proteins from heat treatment, in their studies of soy bean proteins. They cite several investigations which show that ground raw soy beans when fed with the necessary supplements, as the sole or principal source of protein, do not support growth. Normal growth resulted when the beans were cooked. Osborne and Mendel ('17) and Vestal and Shrewsbury ('32) reported that cooking soy beans increased the digestibility of their protein by about 4 per cent. They both stated that the improved nutritive value of these beans caused by heating appeared to be due to an increase in food consumption and nitrogen absorption.

Johns and Finks ('20) found that phaseolin, an isolated protein from the navy bean, produced markedly better growth when cooked than when raw. A similar result was observed with the isolated proteins of the velvet bean (Finks and Johns, '21). Both of these proteins were better digested *in vitro* after cooking than when raw. The results of Hayward et al., obtained by means of the Osborne, Mendel and Ferry method, are summarized as follows:

Soy Beans	*Growth per Gram of Protein Eaten*
Raw	0.93 (32% of diet)
Heated in electric oven at 135° C. 1.5 hr.	0.48 (18% of diet)
Heated in sealed bomb at 125° C. 1.5 hr.	0.93 (18% of diet)
Autoclaved at 125° C. 1.5 hr.	1.22 (18% of diet)
Raw, oil extracted with ether at 25° C.	0.31 (18% of diet)

By the balance sheet method they found the average digestion coefficients of raw and heated soy beans in six experiments to be 85 and 87 per cent respectively, and the average nutritional values 41 and 51 respectively. Commercial soy bean meals from which the oil had been expressed at temperatures of 112 to 130, and 140 to 150° C. during 2.5 minutes, or hydraulic meals cooked at 105 and 121° C. for

15 minutes, as in the commercial solvent method of oil extraction, was an effective method of heat treatment. Efficient heat treatment changes the meal from light brown to brown color, and the color may be used as an index to quality of protein in these meals, but only in the product of the expeller and hydraulic processes.

The food intake during the first few days of all rats in these experiments was similar when raw or heated meal was fed. This suggests that the poor growth on raw bean meal, and low temperature meal, was due to some deficiency in the proteins rather than to a lack of palatability. When casein was added as a supplement to raw soy beans, normal growth resulted. This is evidence that the deficiency lies in the protein fraction. Heating raw soy beans to the higher temperatures reached in commercial processing increased the digestibility by about 3 per cent, and the nutritional value by about 12 per cent.

Effect of Ultraviolet Irradiation on the Nutritive Value of Proteins.—Koch and Koch ('32) state that irradiation of casein and of egg white, in the dry state, slightly reduces their digestibility by pepsin and trypsin *in vitro*. Metabolism experiments on young rats, on the other hand, demonstrate a slightly higher growth rate when the protein is irradiated. The nitrogen retention on their low protein ration was slightly less for the irradiated than on the non-irradiated protein. Hence the increase in growth is due to some factor other than improved utilization of protein. Ample vitamin D was provided in both experiments, so the stimulation of growth is attributable to some other effect which is unknown at present. Scarcely anything more is known about this relationship. It should be further investigated.

The Nutritional Effect of Deaminized Protein.—When a protein is treated with nitrous acid deamination of its free amino groups occurs. This results in extensive mutilation of the protein as shown by Van Slyke and Birchard ('13-14), who demonstrated that casein loses half its lysine when deaminized in this manner. But Wiley and Lewis ('30) claim that in deaminized casein all the lysine is destroyed, half the histidine is lost, tyrosine is partially destroyed, and arginine and tryptophane are unchanged. According to White ('33) cystine reduction does not occur with deamination.

It appears that in addition to the very poor nutritional value, a striking effect of deaminized casein is the anemia which results from its inclusion in the diet of rats even at a level as low as 5 to 10 per cent. This was first demonstrated by Hogan and Ritchie ('34). Gelatin and gliadin together were an adequate source of protein, but when deaminized casein was added to the mixture the animals became anemic, failed to grow, and died. Although milk, egg yolk, wheat

germ oil, ventriculin, muscle, liver, stomach, laboratory-prepared lactalbumin, autoclaved casein, and autoclaved yeast, are ineffective in preventing the anemia, commercial preparations of lactalbumin, untreated casein, and dried yeast are protective against the anemia (Hogan, Guerrant and Ritchie, '36).

The evidence suggests that deaminized casein contains something which causes anemia. Some elucidation of the question was furnished by Smith and Stohlman ('36), who showed that the deaminized casein produced in the rat a characteristic macrocytic megaloblastic anemia with many Howell-Jollie bodies. They affirm that supplementation of the deaminized casein with good quality protein does not prevent the pathological process, though it reduces the severity of the anemia. That the anemia is an intoxication, rather than a deficiency disease, is indicated by Smith and Stohlman's observation that the anemia is reproduced by the intraperitoneal injection of a hydrolysate fraction of deaminized casein. Whether other proteins are similarly affected by deaminization is not known.

High Protein Diets and Renal Damage.—The question whether kidney injury may result from the ingestion of large amounts of protein has been studied by various investigators. Newburgh ('19) observed that rabbit's kidneys were injured by a high protein dietary. Squier and Newburgh ('21) reported that human kidneys were damaged by diets containing a high content of beef protein. Newburgh and Clarkson ('23) fed rabbits 26.8 and 36.2 per cent of protein and found kidney lesions. In the belief that the rabbit, being an herbivorous animal, and accustomed to succulent vegetable foods of low protein content, might be less tolerant of a high protein diet than the Omnivora or Carnivora, Polvogt, McCollum and Simmonds ('23) conducted comparable experiments with rats, using diets containing 31 to 41.3 per cent of protein. They observed hyalinization in the glomeruli, and hyaline material in the convoluted tubules, with adhesions between glomerulus and capsule in the Malpighian bodies. There was also some infiltration and congestion surrounding the capsules.

Then followed several investigations by others, who failed to confirm these results. Osborne and Mendel ('24-25), Osborne, Mendel, Park and Winternitz ('25), and Miller ('25), reported marked hypertrophy of the kidneys, but no abnormalities in histological structure in rats fed excessive amounts of protein. Evans and Risley ('25) confirmed the early observations, but Anderson ('26), Jackson and Riggs ('26), Smith and Moise ('27), and MacKay, MacKay and Addis ('24) reported hypertrophy without lesions.

It is possible that these conflicting researches might be explained, at least in so far as hypertrophy is concerned, on the basis of an

increased requirement for thiamin when high levels of protein are fed. Such a relationship was suggested by Drummond, Crowden and Hill ('22); by Reader and Drummond ('24-25); Hartwell ('24); and Urquhart, MacLean and Smith ('26). The last named investigators did not find kidney lesions when rabbits receiving 60 per cent of protein were given green food every day. But upon withdrawal of this supplement renal injury developed as evidenced by albumin and casts in the urine. Hassan and Drummond ('27) noted such a relationship, and Hartwell ('28) observed that the protective substance was not destroyed by autoclaving. Her findings suggest that it is not thiamin which prevents the renal injury. Since the rabbit synthesizes ascorbic acid, the effect of green food is not attributable to deficiency of this factor. Cox, Smythe and Fishback ('29), using rats, observed definite tubular lesions when 0.3, 0.6, and 0.9 per cent of cystine was added to the diet. The lesions were called "acute toxic nephrosis." Cox et al. found that the kidney injury did not occur when cystine was fed and the diet was supplemented by the daily addition of 300 mg. of the Osborne and Wakeman yeast concentrate (B complex).

Longwell, Hill and Lewis ('32) studied this problem, and concluded that vitamin B complex in the diet prevents hypertrophy of the kidneys of the rat under the influence of much cystine or excess of casein. Cystine fed at 0.3 per cent of the diet causes no greater degree of renal hypertrophy than the absence of yeast extract from an otherwise normal diet. Feeding 0.6 per cent of cystine, however, causes hypertrophy which may be prevented by the administration of the vitamin B complex. Similar results are observed when high casein feeding is the cause of renal hypertrophy. Graded doses of the B complex from yeast causes a progressively decreasing response of the kidneys to the administration of cystine, up to a certain point, above which increase does not have a further beneficial effect. According to these findings cystine is not directly nephrotoxic to rats, but it does, in the absence of some water-soluble vitamin, cause renal hypertrophy. However, one must conclude that the relation of dietary protein level to kidney lesions remains a mystery.

Tissue Injury Caused by Amino Acid Imbalance.—The indications that excessive protein ingestion causes renal injury, particularly when certain proteins are used, suggest that amino acid imbalance might be involved. The work of Newburgh and Curtis ('28), especially, indicates this relationship. For instance, a diet containing 75 per cent of dried liver produced a granular kidney in less than one year, but the same amount of casein, fed 16 months, caused only moderate tubular injury. It is not improbable, of course, that other factors associated with the proteins were etiological factors in renal damage. For example, both liver and casein contain large amounts of phos-

phorus which, if fed in excess, injures the kidneys and other organs. However, amino acids alone are harmful when ingested in large amounts. Newburgh and Marsh ('25) observed definite nephrotoxicity in rabbits fed arginine, aspartic acid, histidine, lysine, tyrosine, tryptophane, and cystine. Also, Lewis ('25) found cystine to be nephrotoxic.

Striking evidence of the relationship of amino acid imbalance to renal and hepatic injury is furnished by the studies of Sullivan, Hess and Sebrell ('31) and Lillie ('32), in addition to that of some other workers. For example, a basal diet containing approximately the maintenance level of protein, casein, at 4 per cent, was supplemented with various amounts of pure amino acids. When fed at the 5 per cent level, tyrosine was decidedly toxic, more so than cystine at that level. Glutathione at a 25 per cent level, equivalent to 10 per cent of cystine, was less toxic than 10 per cent of cystine. Also, 10 per cent of tyrosine and 5 per cent of cystine was less toxic than 10 per cent of tyrosine alone. The injury caused by tyrosine was strikingly different from that caused by cystine.

These data suggest that the injuries related to proteins and amino acids are less severe if the amino acids are ingested as protein, or if the amino acids are in certain proportions. They raise the question whether certain proteins which yield some amino acids in very large or small amounts might be injurious if they constitute the principal source of amino acids in the diet. They also increase interest in the problem of the proper range of protein level in the diet and the effects of excessive protein intake.

Edema Caused by Protein Deficiency.—Edema has been often observed in persons suffering from beriberi. In the dry form of this disease there is usually emaciation; in the wet form edema complicates thiamin deficiency. A history of epidemics of edema associated with beriberi is given by Hirsch (1885). Under conditions of war, of prisons and of famine, edema has frequently occurred in epidemic incidence. It has been variously spoken of as wet beriberi, war dropsy, hunger swelling, etc. The subsidence of edema in human subjects upon improvement of the diet has been many times observed, but the nature of the etiological factor concerned remained quite unknown until after edema was produced experimentally in animals by restriction of the protein intake. Studies of this type opened the way to important observations on edema in man associated with protein deprivation, but there are some points the nature of which is still obscure.

Denton and Kohman ('18), while studying the dietary properties of carrots, observed that some of their animals, when restricted to carrots alone, or carrots with certain nutrient supplements, developed edema. Later Maver ('19) and Kohman ('20) found that edema could be produced in rats by restricting them to carrots, cornstarch, lard, and

salts, for periods of 8 to 12 weeks. They made various additions to this diet, and found that only by increasing its protein content could the edema be alleviated. Frisch, Mendel and Peters ('29) repeated and extended these studies. They employed diets of the following composition:

	Wet Diet	*Dry Diet*
Carrots	4500 gm.	550 gm.
Starch	360 gm.	360 gm.
Lard	60 gm.	60 gm.
Salt mixture (McCollum and Davis, J. Biol. Chem., *21*, 615, 1915)	35.6 gm.	35.6 gm.
Proteins	0.7 per cent	6.3 per cent
Fats	1.6 per cent	7.0 per cent
Carbohydrates	15.5 per cent	68.8 per cent
Salts	1.4 per cent	6.3 per cent
Water	80.0 per cent	13.0 per cent

Rats developed edema on both the wet and the dry diet, as Kohman had observed. The inclusion of 5 per cent of casein in the wet diet prevented the development of edema. There was general anasarca, varying in degree. There was practically no gross fat visible and the muscles were small and pale. Some of the animals died of lung infections. Serum proteins showed a marked decrease in almost every case after two to three months, the reduction amounting to about 40 per cent. That reduction of serum protein was the important factor is supported by the work of Leiter ('28) who centrifuged off the plasma of dogs, mixed the cells with normal saline, and reintroduced them into the same animals. The serum proteins were reduced and edema developed. Darrow, Hopper and Cary ('32) confirmed these observations with human infants who were being fed flour or rice and water because of digestive disturbances, and who developed edema. Butter, salt or sugar failed to relieve the condition, but the infants improved rapidly when protein was added to the food, or when blood transfusions were given. Frisch, Mendel and Peters pointed out that serum protein reduction is only partly responsible for the production of edema. "Here, as in other conditions, they determine a tendency toward water retention which is influenced by other factors. The fluid intake is very important, for if it is reduced in any way . . . no edema develops, even if the serum proteins are actually quite low."

Cutting and Cutter ('34-35) placed 26 normal human subjects for two days on a diet containing only 500 calories per day, and no protein. Fluids were not restricted. Curiously, instead of falling, the total serum protein concentration rose during the test. The concentration of blood and of serum protein was attributed principally to low fluid intake. Two days of protein deprivation is an insufficient time to make any significant change in the character of the blood proteins,

but the concentration is definitely lowered. This observation is in harmony with that of Torbert ('35), who showed that starved rats lose a small percentage of protein of the plasma in 4 to 6 days and remain at a constant reduced level until death. The lost protein is principally albumin.

Weech, Snelling and Goettsch ('33) studied the edema produced in dogs by restriction of the protein in the diet. When edema is considered as a degree of interstitial fluid accumulation great enough to permit detection by finger palpation, a fairly close correlation is found between this symptom and the level of plasma albumin. With concentrations less than 1 gm. per 100 cc. of plasma edema is always present; with concentrations above 2 gm. per 100 cc. it is nearly always absent. The subcutaneous edema fluid from dogs with nutritional edema contains in most instances between 0.10 and 0.35 gm. of protein per 100 cc. Plasma specific gravity follows the fluctuations in total plasma protein and does not exhibit a correlation with edema. Reference has already been made to the studies of Block (Chapter V) which show that the albumin of blood plasma is not a distinct protein, but is variable in amount and composition depending upon the method of preparation.

Shelburne ('34) has produced edema in dogs by prolonged restriction to a low protein diet. He found the critical level of plasma proteins for the production of edema to be 4 gm. of total protein, and 2 gm. of plasma albumin per 100 cc. He observed fatty changes in the renal tubules in all of his edematous animals, and offers evidence that these changes were not caused by the accompanying anemia which invariably complicates such experiments.

Youmans and his associates ('32) have recorded the clinical findings of a group of patients whose principal complaint was mild edema, apparently of nutritional origin. The diet of 12 of these was studied before the beginning of treatment, and in 11 cases it was found that the total caloric intake was below the basal requirement. The average protein ingested ranged from 20 to 52 gm. daily, and was below the minimum requirement in all but four cases. The minimum is variously estimated to be from 0.5 to 0.7 gm. per kg. of weight. He refers the edema to chronic dietary deficiency, particularly to shortage of protein.

Lin and his associates ('32) studied two patients with nutritional edema. Edema was reproduced or accentuated twice by placing the patients on a protein-free diet, with accompanying significant changes in the plasma proteins. A vegetable diet fed at a level of 1 gm. of protein per kg. of weight, and 7 per cent of total caloric intake, was not sufficient to establish a positive nitrogen balance, and to increase the plasma protein to the point of disappearance of edema. Two grams

of vegetable protein sufficed for this purpose. This corresponded to 14 per cent of the caloric intake. Animal protein at a level of 7 per cent of the caloric intake was effective in restoring the plasma protein and in relieving the edema. The digestibility and nutritional value of their animal protein determined by the Mitchell method was much superior to the vegetable protein.

The subject of dietary protein in relation to edema is still in need of further investigation. Bloomfield ('33) has called in question the experimental work of Frisch, Mendel and Peters, by pointing out that the repeated withdrawal of blood for analysis may have played a part in the lowering of plasma protein. In rats kept on a low protein diet during 21 weeks he found no significant decrease in serum protein concentration aside from an initial drop. His data are, in general, supported by those of Weech, Goettsch and Reeves ('35), who include in their paper a good discussion of dietary protein and hypoproteinemia.

Dietary Protein and Plasma Protein Regeneration.—Several researches have provided data concerning the value of the proteins from different sources for plasma protein regeneration. McNaught and his associates ('36) have studied this problem, using dogs whose plasma proteins were kept at a low level, i.e., about 4 per cent, by means of repeated plasmapheresis and a low-protein diet. The basis of evaluating the proteins was the increased plasmapheresis needed to maintain the standard level of plasma protein when different sources of protein were added to the diet. They expressed the power of the food protein to yield plasma protein by the number of grams of food protein required to form 1 gram of plasma protein.

They reported that the protein of potato, bran, rice, and rice polishings have a higher value than animal proteins such as liver, brain, or spleen. Those of the soy bean were especially effective. Most plant proteins favored the production of globulin, but those of soy beans resembled the animal proteins in favoring the formation of albumin. The potency ratios, or grams of food protein required for regeneration of one gram of blood proteins, as determined by McNaught et al., are shown in the table on page 148.

McNaught and associates found that infection and intoxication disturb the plasma protein production of standardized dogs and may reduce the output of plasma proteins to very low levels in spite of considerable protein intake. There may be a very sharp drop in the protein level during the first day of intoxication.

Cartland and Koch ('28) state that diets deficient in proteins, vitamins, or minerals delayed regeneration of blood in rats made anemic by bleeding. Koessler and his associates ('26) claim that blood regeneration cannot take place in vitamin A deficiency, and that the

Source of Protein in Food	*Potency Ratios*
Beef serum	2.6
Wheat protein (60 per cent)	4.2
Bran	4.3
Potato-bran, basal ration	5.5, 4.8, 4.1, 2.7, 4.2, 4.4, 4.5*
Rice polishings	5.3
Gizzard	5.3
Kidney basal ration	5.3, 5.3*
Rice basal ration	5.4
Lactalbumin	5.5
Skeletal muscle	5.7
Egg white	5.8
Irish potato	6.1
Liver	6.4, 6.5, 6.8*
Soy bean	4.6, 7.1*
Liver residue	7.4
Beef heart	8.0
Liver extract	8.6
Casein	10.0
Spleen	10.2
Brain	11.8
Stomach	13.6
Salmon	15.2
Pancreas	19.0
Kidney	20.8

* Values obtained in different experiments.

rate of regeneration is a function of the amount of vitamin A available. Whipple and his associates ('28), as a result of numerous studies, conclude that many tissues and fruits, especially apricots, when fed to dogs stimulate hemoglobin regeneration, and that there exists some unknown substance which aids in the utilization of proteins in the synthesis of hemoglobin. Saunders ('26) found that when young rats were restricted to wheat gluten as the sole source of protein, they became anemic, whereas when given 10 to 18 per cent of casein a high concentration of hemoglobin was produced. They were able, on the casein diet, to regenerate their blood volume completely every 8 to 15 days. Feeding red corpuscles does not appear to stimulate the rate of hemoglobin regeneration (Cartland and Koch). These investigators observed, contrary to Koessler et al., that rats do not become anemic when fed diets deficient in vitamins A, B, or E.

Madden and coworkers ('37) suggest that plasma can contribute protein readily to body tissues or body stores, but only in small amounts and with difficulty can the body tissues contribute protein to form blood plasma as, for example, in fasting. A sterile abscess (turpentine) induces a marked reduction in plasma protein regeneration, even when the animals are consuming an ample diet. Large quantities of live yeast upset digestion and interfere with plasma formation, whereas autoclaved yeast is well utilized, and has a high potency ratio (4.4).

The studies which have been reported here clearly show that blood

protein regeneration is easily upset by faulty nutrition, and especially by a low protein intake. There appear to be great differences in the value of proteins for this function. The biological values of food proteins for this purpose are apparently quite different from those for growth of the tissues. The significance of this consideration is recognized and emphasized by Melnick, Cowgill and Burack ('36), whose recent studies of diet in the regeneration of plasma protein are based on well-planned technical procedures.

The Relation of Low-Protein Diets to the Etiology of Ulcer.— Clements ('34) states that natives of the Island of Manus, New Zealand, who eat a well-balanced diet rich in protein and vitamins are immune to tropical ulcer. Other natives who subsist upon a less well-balanced diet in which the ratio of carbohydrate to protein is high, and the supply of vitamins is poor, are susceptible. The highest incidence was found among natives existing upon a diet composed largely of sago, rich in carbohydrate, and deficient in vitamins. He postulates that imperfect diet induces changes in the skin epithelium, making it a suitable medium for the growth of fusiform bacilli and spirochaetes, which, upon entering a wound, rapidly convert it into an ulcer. The incidence, which ranged up to 15 per cent of the population of certain villages, fell when the diet was supplemented with fish or taro. There was complete absence of ulcers in fishing villages where the diet contained 60 to 70 per cent of protein. Clements suggests that deficiency of vitamins A and B-complex may play a role as well as protein deficiency.

Hoelzel and DaCosta ('31) studied the effect of the major vitamin deficiencies, of high and low carbohydrate, of fat, of protein, and of various amounts and kinds of roughage in the diet, as possible etiological factors in ulcers of the forestomach. They conclude that ulceration can be produced in the forestomach by simple protein restriction. An exclusive diet of bran was eaten by the rats in enormous amounts but no ulcers developed although the animals starved to death. Hence, malnutrition or inanition does not cause ulcers. The effects of bran were attributed in part to its acid-binding power, and in part to the diluting effect of its bulk. These investigators believe that the 61 per cent incidence of gastric ulcer in rats observed by Pappenheimer and Larimore ('24), when the animals were restricted to a diet containing 95 per cent patent flour, was due to protein restriction rather than to ingestion of hair as suggested by Pappenheimer. The suggestion is offered that ulcers may occur as a result of lack of acid-binding power of a diet poor in protein.

The indicated relationship between low-protein intake and gastric ulcers is further supported by the observations of Sharpless ('37) that rats on a purified diet invariably developed squamous epithelium

hyperplasia of the forestomach when the only protein was casein and yeast at 4 and 5 per cent levels respectively. Neither vitamins A nor B-complex seemed to be involved and the type of carbohydrate used was not significant. Neither cysteine hydrochloride nor glutathione prevented the lesion but cystine at a 0.2 per cent level conferred complete protection from injury. When the content of casein was trebled lesions never occurred.

In view of the present slight evidence one is at a loss to explain the mechanism of this relationship. Methionine and perhaps other amino acids should be studied with respect to their ability to prevent the stomach injury. Certainly the general question of dietary protein in relation to gastric ulcers should be further investigated.

Toxicity of Egg White.—Boas ('27) reported a novel syndrome in rats which were fed Chinese dried egg white. She characterized the condition as "eczematous dermatitis," accompanied by alopecia, blepharitis, spasticity, and in some cases by edema of the feet. Occasionally there were skin hemorrhages. Attention was called to the similarity of some of the conditions to those seen in pellagra. Salmon and Goodman ('34), using a similar basal diet containing raw egg white, noted fur-like or woolly hair, alopecia, exfoliating dermatitis, hyperemia, skin hemorrhages, blepharitis, stomatitis, salivation, variable edema, erythema of the feet, symptoms of nervous disturbance, hypochlorhydria, and some anemia. Both fresh and dried egg white caused the condition when fed in the raw form. Diets containing no more than 18 per cent of protein from this source cause the syndrome. They state that the skin lesions were more severe on low fat diets than on diets containing 18 per cent of butter fat or hydrogenated cottonseed oil or linseed oil.

Salmon and Goodman found that when 18 per cent of raw egg white was fed the symptoms were prevented by the inclusion of brewer's yeast, dried liver, the extracted residue of yeast or liver, but not by extracted casein, extract or hydrolyzed residue of brewer's yeast, white corn, or baker's yeast. Liver extract was more effective than yeast extract, but was only partially preventive. Well-developed cases were cured by brewer's yeast, dried liver, milk, or extracted residue of brewer's yeast, or dilute hydrochloric acid. Coagulation of fresh egg white by heat and extraction of the coagulum with 51 per cent alcohol rendered it innocuous. The concentrated extract of egg white had a slightly harmful effect but failed to produce the severe symptoms caused by raw egg white. Salmon and Goodman interpret their data to indicate the presence in raw egg white of a positive harmful factor which is antagonized by the protective substances rather than the existence of a deficiency.

Parsons and Lease ('34) have made extensive studies on this syn-

drome. They point out that while the symptoms are strikingly similar to those due to "vitamin G" deficiency, the provision of a great abundance of this factor does not prevent the egg white injury. No studies have been made with nicotinic acid, riboflavin, or vitamin B_6 individually. They have shown that the injury is not due to amino acid deficiency since 30 per cent of dried beef heart or 60 per cent of dried milk in the diets containing raw egg white failed to effect a cure of the dermatitis. Cooked pork kidney was the most effective substance studied for preventing the egg white injury. One-sixth of the weight of egg white fed, in the form of pork kidney, sufficed for protection. Cooked beef liver and kidney and cooked pork liver are good sources of the protective substance. One to three times the weight of egg white, as dried yeast, dried egg yolk, wheat embryo, or dried milk must be added to effect a cure. Spleen, heart, ovary, adrenal, blood, or hemoglobin, have little or no protective property. The activity resides in the residue after the preparation of Eli Lilly & Company liver extract No. 343, but not in the extract. Prolonged heating at 100° C is destructive, especially if the material is dry. Boiling with hydrochloric acid of 5 per cent or more concentration for 1 hour decreases the potency appreciably. They found that low hemoglobin is not a feature of the syndrome due to feeding egg white. They conclude that the injury is not due to destruction by egg white of some dietary factor in the ration.

Balls and Swenson ('34) conclude that egg white injury may be the result of its anti-tryptic activity. The proteinase inhibitor is found in the thin or watery fraction of egg white, whereas the thick white is definitely proteolytic. They separated these two fractions and described a procedure for concentrating the trypsin-inhibiting substance in the thin white, one gram of active material being obtained from 20 gm. of thin egg white. Parsons ('36) has employed such concentrates in further studies, and found that these showed no corresponding increase in the capacity of egg white rations to produce the nutritional disorder, but on the other hand, permitted the healing of the characteristic lesions when the diets incorporating them held a much higher concentration of anti-trypsin than did the physiologically injurious diets containing the egg white or extracted residues. She concludes, therefore, that the egg white syndrome is not attributable to the anti-tryptic content of this substance. The problem is still unsolved.

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CHAPTER

VII

Calcium, Phosphorus, and Magnesium

SINCE calcium and phosphorus constitute the principal mineral components of bone it must have been assumed several centuries ago that these elements are essential for the maintenance of life. Parey, at least as early as 1649, recognized that calcium plays an important part in bodily functions. However it is indeed amazing that so little attention should have been given to the nutritional significance of magnesium until the present decade. A tremendous volume of literature exists concerning calcium and phosphorus. But this, in addition to the relatively numerous contributions on magnesium, fails to furnish satisfactory answers to some significant problems relating to the nutritional roles of these elements. It appears that we are now in possession of sufficient knowledge to outline the general facts on which an intelligent use of calcium, phosphorus, and magnesium is possible in the maintenance of health and the prevention of diseases related to these elements. A relatively brief discussion of these subjects will be the purpose of this chapter.

Content of Calcium, Phosphorus, and Magnesium in the Body.— The calcium, phosphorus, and magnesium content of the body varies over a considerable range depending upon various factors. According to Bessey and associates ('35) the skeleton of normally developed adult rats contains approximately 99.3 per cent of the total calcium in the body. In normal rapidly growing rats and in adult rats on a calcium-low diet a somewhat smaller percentage of the total bodily calcium is found in the skeleton. Even under such conditions, Bessey et al. state that the value is of the order of 99 per cent. The skeletal structures contain on an average about 70 to 80 per cent of the total bodily phosphorus. But owing to considerable variations in the ratio of skeletal tissues to soft tissues this value is by no means constant.

About 70 per cent of the total bodily magnesium also is in the skeleton.

With respect to the absolute values for these elements there are marked differences. The average adult human body may contain 1400 to 2000 gm. of calcium, or about 2 per cent of the total body weight. The phosphorus content appears to be even more variable. Approximate figures for the absolute values are 450 to 700 gm. Scarcely anything is known concerning the total magnesium content.

According to Cox and Imboden ('36a) rats at birth contain relatively small amounts of calcium in comparison to infants at birth. The following table taken from their paper illustrates these differences and the marked increase in these elements during early growth in the rat.

	Weight	Per Cent Ash	Total Weight		Per Kilo Body Wt.		Per Cent Ash		Ca : P Ratio in Ash
			Ca	*P*	*Ca*	*P*	*Ca*	*P*	
	gm.		*gm.*	*gm.*	*gm.*	*gm.*			
Rat pup . . .	5.37	1.754	0.0144	0.015	2.68	2.79	15.3	16.0	0.96
Human fetus, full term . .	3000	3.33	24.21	13.32	8.07	4.44	24.2	13.3	1.82
Rat, 21 days old * . . .	44.7	2.738	0.313	0.236	7.01	5.27	25.7	19.3	1.33

* Average of 420 stock animals, 21 days old.

Recalculation of data by Greenberg and Tufts ('36), in terms of this table, shows that normal rats at birth contain about 0.0012 gm. of magnesium and at 21 days the value is approximately 0.0151 gm. These results indicate that gestation in rats causes relatively less need for the minerals in question as compared with the need in human gestation.

The forms in which these elements exist in the body are of interest. Since practically all of the calcium is in the bones and teeth its principal compounds occur there. Chemical analyses and X-ray diffraction patterns, as well as some other types of evidence, suggest that it exists in forms such as CaF_2 $[Ca_3(PO_4)_2]_3$ (fluorapatite), $CaCl_2$ $[Ca_3(PO_4)_2]_3$, $Ca(OH)_2$ $[Ca_3(PO_4)_2]_3$, $CaCO_3$ $[Ca_3(PO_4)_2]_3$ (podolite, and possibly as the mixed apatite), and francolite $CaFCaCO_3$ $[Ca_3(PO_4)_2]_3 \cdot H_2O$. Although no completely satisfactory solution of the problem has been found it is rather certain that bone salts are members of the apatite series.

Studies in this field must eventually clarify the relation of mag-

nesium to calcium in bone. It is the general tendency to dismiss this element from serious consideration as though it were of no consequence. However the ash of normal adult bone contains approximately 0.6 to 0.8 per cent of magnesium. There is scarcely any information on the chemical forms of magnesium in the body. About 20 to 30 per cent of the plasma magnesium is non-diffusible. It would be of much interest if compounds of this element should be found in animal tissues comparable in any way to that of chlorophyll in plants which contains magnesium as an essential component of the pigment.

The compounds of phosphorus in the body are far more numerous than those of any other mineral element. Some of the known compounds are inorganic phosphate, organic pyrophosphates which include cocarboxylase (thiamin pyrophosphate), hexose diphosphates, hexosemonophosphates, glycerophosphates, creatine phosphate, adenylic acid (adenine pentose phosphate), inosinic acid, lecithin, kephalin, sphingomyelin, nucleoproteins, phosphoproteins, and apatite of bones and teeth. Much of the present data on the distribution of phosphorus in soft tissues is in terms of such non-specific variables as acid-insoluble, acid-soluble, inorganic, lipid ester and total phosphorus (Sherman and Quinn, '26; Cole and Koch, '31). This practice, although superficial, is useful in revealing some aspects of phosphorus metabolism. For instance, in typical clinical rickets the serum inorganic phosphorus decreases markedly, but scarcely any change occurs in lipid phosphorus.

Effects of Calcium Deficiency.—Studies on the bodily effects of calcium deficiency are necessary to an understanding of the nutritional significance of this element. One of the first significant experimental investigations of calcium deficiency was made by Aron and Sebauer ('08) whose work is distinctive because they were the first to demonstrate the advantages of combining chemical and histological procedures in the elucidation of a nutritional problem. In this instance the problem was rickets but unfortunately no fundamental facts or points of view were established. Young dogs were given a diet of horse meat, maize, and beef fat. After about 2 weeks there was a temporary flow of bloody feces. Lacrimation occurred and after several more days the feces were foul-smelling and the appetite failed. Shortly thereafter tonic-clonic convulsions intervened. Autopsy showed rarification of the bones and a generalized inflammation of the intestinal mucosa.

These observations are of considerable interest in relation to the recent report of Martin ('37), who noted that young dogs fed a purified diet containing extremely small amounts of calcium showed similar degrees of irritability. In this case vitamin D was provided. In addition Martin claimed that total serum calcium was not appreci-

ably decreased. But in view of the resultant tetany it would seem that the content of ionizable calcium must have been somewhat reduced. That variable was not determined. Striking effects were the conditions of extreme tactile hypersensitiveness, the halisteresis and diffuse areas of hemorrhage. The latter were observed throughout the body including the viscera, muscles, and bones. Whether it is related to a failure in ascorbic acid synthesis or metabolism in the dog is not known but the appearance of the hemorrhages and the extreme hypersensitiveness are suggestive of the scorbutic state. This suggests that studies should be made of the possible relationship between calcium deficiency and ascorbic acid synthesis.

Rats appear to be more capable of maintaining stability of the nervous system in conditions of calcium deficiency. But if vitamin D is absent the musculature becomes tense. However there is no tetany (Shelling and Asher, '32; Templin and Steenbock, '33). Templin and Steenbock found that adult female rats maintained themselves in a surprisingly good condition during a period of 8 months during which time the ration contained no vitamin D and only 0.058 per cent of calcium. However, loss of weight and evidence of premature senility were observed. The femurs suffered a 10 per cent reduction in ash whereas the loss was only 6.5 per cent when vitamin D was furnished. This, incidentally, is indicative of the value of vitamin D in adulthood.

Without discussing the question at length it suffices to itemize some of the effects of calcium deficiency on young animals as follows:

1. Decreased rate or cessation of growth.
2. Probable slight reduction in blood calcium.
3. Changes in intestinal flora.
4. Intestinal inflammation which may or may not be alternative with intestinal atony and inflammation.
5. Hyperplasia of parathyroid glands.
6. Hyperirritability and perhaps tetany.
7. Resorption of bone salts and probably rickets.
8. Negative calcium balance.
9. Premature death.

In adult animals sterility occurs and lactation may be reduced or stopped, depending upon the severity of the deficiency. The relation of calcium to lactation is not well clarified but deficiency of this nutrient as the etiological factor in milk fever is supported by considerable evidence.

Calcium Deficiency and Milk Fever: Milk fever occurs among live stock in various parts of the world. A principal symptom is tetanic convulsions. The disease occurs soon after parturition and the onset of lactation, usually in heavy milk producers. A method of treatment widely practiced in former years was inflation of the udder with air.

Temporary respite was afforded by this treatment, apparently owing to the arrest of milk production. Studies have shown that the disease is characterized by a reduction in blood calcium. Since various factors, including calcium and vitamin D-poor diets, and pregnancy, tend to produce a condition of calcium deficiency in the parturient animal, it is not surprising that the sudden demands of heavy milk production should exceed the animal's ability to maintain an adequate level of blood calcium. Proof of the role of calcium in this disease is the dramatic effectiveness of calcium gluconate, calcium chloride, or some other suitable calcium salt in alleviating the condition. Greig ('31) has discussed the subject.

Effects of Phosphorus Deficiency.—Although compounds of phosphorus participate in numerous important functions of the body it is a significant fact that the literature does not yet contain any reports of experimental studies on the effects of diets very low in phosphorus but adequate in other respects. In 1918 Osborne and Mendel restricted rats to a relatively satisfactory diet somewhat deficient in phosphorus but unfortunately their only observations were those regarding growth. Since that time there have been numerous investigations of phosphorus in relation to rickets, but the principal emphasis has been on the calcium: phosphorus ratio. A high ratio was secured by the addition of a calcium salt, usually calcium carbonate, to a basal diet containing considerable amounts of phosphorus. Such diets produce extreme degrees of rickets in suitable animals but they are not a satisfactory means of studying the effects of simple phosphorus deficiency.

A difficult problem is the preparation of a suitable diet deficient in phosphorus. Almost all sources of protein contain relatively large amounts of phosphorus, and the provision of vitamin B components without significant amounts of phosphorus has been even more trying. Heretofore a diet containing 0.13 per cent of phosphorus was regarded as approaching the lower limits of practicability in studies of phosphorus deficiency (Forbes, '37). Day and McCollum ('38) have devised a diet containing approximately only 0.015 per cent of phosphorus but adequate for growth and reproduction when supplemented with phosphorus. It consists of: edestin 16.0, gelatin 4.0, sucrose 62.95, salts (P-free) 4.0, choline hydrochloride in sucrose (1:9) 1.0, cystine in sucrose (1:19) 1.0, Crisco 10.0, carotene in corn oil (3:1000) 0.3, viosterol 15 drops per kilo, and vitamin B complex 0.75. The latter is prepared from wheat germ and yeast by precipitation of phosphates with calcium hydroxide and alcohol. The procedure is too tedious to recount here. Weaned rats restricted to this diet grow slowly for 2-4 weeks, then decline and die after 7 to 9 weeks. The effects of the deficiency are therefore acute and are scarcely without parallel in the

rapidity of death from mineral deficiencies except in the case of magnesium deprivation.

Young rats restricted to the above diet maintain a fairly good appetite throughout the first half of the survival period. Marked loss of bodily calcium occurs but the metabolic balance data indicate that some of the phosphorus taken from the bones, with calcium, is transferred to the soft tissues and actually used to promote their growth. The animals gradually become inactive and use their legs as little as possible. This is understandable since X-ray examination shows a progressive rarification which becomes so acute that as death approaches only a faint shadow is formed on the plates, as shown by Follis, Day, and McCollum ('38). Spontaneous fractures occur and the lungs and heart become compressed by collapse of the ribs. There appears to be no marked increase in water consumption as is said to occur in phosphorus-deficient animals.

Phosphorus Starvation in Farm Animals: Phosphorus deficiency in farm animals is reported from many parts of the world (Theiler and Green, '32). It has been observed in Minnesota, Michigan, Pennsylvania, Ohio, New York, Virginia, West Virginia, Alabama, Mississippi, South Carolina, New Zealand, Australia, Hawaii, South Africa, and various parts of Europe. The deficiency is generally attributable to phosphorus-poor herbage, particularly grass and hay, grown on low-phosphorus soils. In several regions the soil depletion has been caused by continuous removal of phosphorus in the form of animals which were pastured on the land and in others by the continued marketing of hay and grain. Under natural conditions animals and herbage of a given region tend to remain there. The animals die on the land and the fertility is not lost. Mineral deficiencies of pasture lands in Australia and elsewhere are now attracting much attention because the soil has been so depleted by long export of animals as to make the present animals unthrifty and their production unprofitable.

The studies of Eccles, Palmer and associates ('32, '35) have shown that phosphorus deficiency in farm animals is much more widespread in the United States than was previously supposed. Mineral balance experiments with dry and lactating animals showed that a copious milk flow may greatly augment the loss of phosphorus from the animal's body. It was found that the level of inorganic phosphorus in blood serum is a useful index to the severity of the disease. The bones become greatly reduced in ash constituents. They have a lower phosphorus and a higher calcium carbonate content than normal bones. Supplementation of the diet with suitable sources of phosphorus, e. g., sodium phosphate, calcium phosphate, etc., causes an immediate stimulation of the appetite, more efficient utilization of feed, and a return to good health.

An indirect result of phosphorus deficiency in cattle is a disease called *Lamsiekte* (lame-sickness) by South African stock raisers who have suffered enormous losses from its ravages. The disease is localized and it occurs sporadically. As shown by Theiler in 1919 the disease is an intoxication caused by the eating of carcass debris infected with a toxigenic saprophyte, *Parabotulinus bovis*. Since cattle suffering from acute degrees of phosphorus deficiency greedily eat all bones and other debris they do not discriminate against carrion even though it is reeking with the botulinus saprophyte. In 1922 Theiler and associates discovered that the craving for bones by such cattle was due to phosphorus starvation. On the provision of any assimilable form of phosphorus such as bone meal, bran, sodium or calcium phosphate, the perverted appetite, pica, for bones and other debris soon disappeared.

Horses and sheep do not develop the habit of bone chewing although in an unthrifty condition from aphosphorosis. Likewise rats restricted to an extremely phosphorus-deficient diet exhibit no symptoms of pica. Also there is no significant degree of polydipsia in rats. This symptom is said to occur in phosphorus-deficient pigs (Aubel, Hughes and Lienhardt, '36).

Effects of Magnesium Deficiency.—Although it has been known for many decades that magnesium occurs in considerable amounts in all tissues of the body it was not until 1926 that experimental evidence was adduced which indicated the indispensability of this element. Leroy ('26) showed that young mice restricted to a ration containing about 10 parts of magnesium per million failed to grow and died in about 30 days. The nature of the deficiency was not studied. McCollum and associates ('31-'35) first described the spectacular syndrome of magnesium deficiency in rats and dogs.

Young rats 35 to 45 gm. in weight show marked vasodilatation 3 to 5 days after being restricted to a diet containing about 2 parts of magnesium per million. The condition becomes more pronounced until the 11th and 14th day when it begins to subside. During this period the animals become progressively apprehensive and show alarm at noises or shadows. By the time vasodilatation has begun to decrease hypersensitivity may be sufficiently advanced to cause convulsions from disturbances such as the rustling of paper or the hissing of air. The onset of a convulsion is striking. The animal races rapidly in a wide circle until it falls on its side. Tonic-clonic convulsions follows. The skin blanches and the eyes protrude. About 86 per cent of the animals die in the first attack. A small per cent will survive several attacks. Those which survive as long as 10 weeks develop an extraordinary fibrous hypertrophy of gum tissue covered by a thin layer of oral epithelium. The hypertrophied tissue almost

EFFECT OF MAGNESIUM DEPRIVATION ON THE MINERAL CONTENT OF RAT BONES

INITIAL AGE	FINAL AGE	DAYS ON DIET	MEAN ABSOLUTE VALUES PER ANIMAL FOR FEMORA, TIBIAE AND FIBULAE									
			Dried Bones		Ash		Ca		Mg		P	
			Control	Mg def.	Control	Mg def.	Control	Mg def.	Control	Mg def.	Control	Mg def.
days	*days*		*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
25	25	0	180.5	180.5	79.4	79.4	17.7	17.7	0.5	0.51	17.4	17.4
25	30	5	344.9	285.4	114.1	145.8	35.8	51.1	0.9	0.64	23.7	27.4
25	35	10	256.8	308.1	113.2	156.4	36.1	50.2	0.8	0.66	21.8	32.5
25	40	15	392.6	381.9	184.2	195.9	52.4	66.2	1.5	0.65	40.1	40.7
25	45	20	334.5	375.1	158.8	199.2	52.4	67.7	1.8	0.78	28.9	33.3
25	50	25	362.0	500.5	173.9	276.3	52.5	98.4	1.8	0.86	30.0	47.2
25	55	30*	513.9	591.0	254.8	313.5	87.4	107.8	2.4	0.75	44.6	54.2

* Animals sacrificed in first stage of induced convulsions.

completely buries the molars. At first this was thought to be due to resorption of alveolar bone forming the tooth sockets, but later studies showed that bone resorption does not occur.

Additional effects of magnesium deprivation are cardiac arrhythmia, increased sensitivity to electrical stimuli, marked decrease in serum magnesium, moderate decrease in serum calcium and a large increase in serum cholesterol esters. The ultrafilterable fraction of serum magnesium is lowered in correspondence with the decrease in total magnesium. Consequently the percentage of diffusible magnesium remains essentially unchanged. Likewise there is no appreciable change in the percentage of diffusible calcium. Calcification of bones is increased but the total magnesium content of the skeleton is scarcely able to increase at all. This is shown in the table (page 162) adapted from the paper by Orent, Kruse and McCollum ('34).

Several workers, including Cramer ('32), Brookfield ('34), Watchorn and McCance ('37), and Greenberg and associates ('38), have found marked changes in the kidneys. There is not complete unanimity of opinion concerning the exact pathological effects but it appears that there is calcification in the cortico-medullary zone, in the pyramids, and, at a later stage, in the cortex.

The following table prepared from data published by Tufts and Greenberg ('38) indicates the effect of magnesium deprivation on the magnesium and calcium content of rat viscera. (The results are expressed in mg. of element per 100 gm. of wet tissue.)

CONTENT OF RAT VISCERA

(The results are expressed in mg. of element per 100 gm. of wet tissue.)

TISSUE	CONTROL ANIMALS				MG-DEFICIENT ANIMALS			
	Mg		Ca		Mg		Ca	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Heart . . .	19.3–28.6	22.4	3.6– 7.9	6.4	20.5–26.5	23.1	6.1– 14.7	9.3
Muscle . .	24.6–35.1	29.6	4.1– 7.9	5.7	15.2–33.5	26.5	7.5– 15.2	10.1
Kidney . .	16.0–28.0	21.5	6.9–15.4	8.5	15.6–46.7	25.2	14.2– 232	106.7

From the table it appears that the magnesium content of the soft tissue is not greatly altered by magnesium deficiency. However the calcium content is greatly increased, reaching 15-fold the average value of normal kidneys. The diet contained two or more times as much magnesium as the diet used by Orent and associates.

State of Calcium and Magnesium in Foodstuffs and Their Biological Availability.—It is understandable that the state of minerals in foodstuffs determines in considerable measure their absorption and utilization in the body. The studies of Schmidt and associates (Schmidt and Greenberg, '35) indicate that much of the calcium and magnesium, except magnesium bound in chlorophyll, forms complex ions with protein in plant tissues. In plant juices these elements appear to exist as salts of carbonic, oxalic, citric, malic, tartaric, and other such weak acids as occur in plants. They also occur in combination with inositol (hexahydroxyhexahydrobenzene) as the calcium-magnesium salt of inositol phosphoric acid. This compound is widely distributed in the plant kingdom, especially in the pericarp of seeds (Anderson, '20), and is generally called phytin.

Calcium and magnesium in milk probably form complex compounds with proteins but a large percentage may be regarded as existing in the form of inorganic phosphates.

The reaction (pH) of the gastrointestinal tract is one of the important factors in the absorption and utilization of calcium and magnesium as well as phosphorus. Unless the food is made distinctly acid in the stomach, absorption and utilization are deficient. This is indicated by numerous types of evidence. Among the more recent studies are those of Bussabarger, Freeman and Ivy ('38), who have found that gastrectomy in dogs is followed by marked demineralization and consequent osteoporosis. Presumably the lack of sufficient acid in the initial steps of digestion and absorption was responsible for the marked withdrawal of bone minerals.

Factors which tend to keep calcium in solution promote absorption. Normally the acidified gastric contents upon passing into the upper intestine keep the reaction somewhat acid in that portion of the alimentary tract. It is believed that this favorable circumstance causes most of the calcium absorption to take place there since the lower part of the intestinal tract tends to be alkaline and precipitation of calcium occurs (Courtney, Tisdall and Brown, '28). As shown by Zucker and Matzner ('23-24), and others, the feces are alkaline in untreated ricketic rats. Administration of vitamin D causes the return of an acid reaction in the feces simultaneously with clinical improvement. The relation of vitamin D to the maintenance of proper reaction (pH) in the intestinal tract is not understood. A critical study of this relationship might be profitable. It is at least interesting that estrogenic substances, closely related to vitamin D compounds in a chemical sense, are capable of quickly producing an acid reaction in the vagina, which is a normal condition in sexually mature individuals.

In some cases of disturbance in fat metabolism, fatty acids form insoluble soaps with calcium, thus causing the latter to be excreted.

But as shown by Boyd, Crum and Lyman ('32) the presence in the diet of moderate amounts of fat seems to have a beneficial influence on the absorption of calcium and phosphorus by maintaining a favorable acidity of the intestinal contents. The absorption of calcium soaps was studied with rats on a fat-low diet. When the calcium intake was 20 to 32.5 mg. per rat per day the utilization values were calcium stearate 45 per cent, calcium palmitate 65 per cent, and calcium oleate 91 per cent.

The opinion has prevailed for many years that calcium of certain vegetables is very poorly utilized and that in general the biological availability of plant calcium is less than that of milk calcium. It required some time to elucidate the basis for this difference since vitamin D and some other factors were not controlled in many of the studies. However the best evidence indicates that oxalic acid is the chief factor. Suggestive of this is the correlation of oxalate content of plant foods with the unavailability of calcium. Fincke and Sherman ('35) found that calcium of kale was nearly as well utilized as the calcium of milk, but calcium of spinach was utilized very poorly if at all. That these findings correspond with the oxalate content of kale and spinach is indicated by the table of the oxalate content of common foods prepared by Kohman ('34). Mackenzie and McCollum ('37) fed potassium oxalate to rats and determined the relationship between oxalate ingestion and calcium retention. At a level of 1.7 per cent potassium oxalate hindered bone growth and calcium retention when the diet lacked vitamin D but contained 0.35 per cent calcium and 0.35 per cent phosphorus. With higher levels of calcium ingestion the oxalate was not deleterious. In view of the evidence it may be concluded that the liberal consumption of foods containing large amounts of oxalate might cause an actual calcium deficit unless the diet contains generous amounts of calcium.

Another consideration has been the relative availability of calcium (and phosphorus) from the various kinds of calcium phosphates. Cox and Imboden ('36b) determined the effect of mono-, di-, and tricalcium phosphates on reproductive ability in rats when the salts were fed at the high level of 2.45 per cent. At this level the primary salt was definitely unsuitable and the tertiary form appeared to be slightly superior to the secondary phosphate. Rottensten and Maynard ('34) compared the assimilation of phosphorus from dicalcium phosphate, C.P., tricalcium phosphate, C.P., bone dicalcium phosphate, and cooked bone meal. The data seemed to indicate a slight superiority of a secondary phosphate over a tertiary product but it was concluded that no really significant differences exist between the four phosphates tested. Presumably the assimilation of calcium from these products is similar to that of phosphorus. The data of Higgins and Sheard

('33), who worked with chicks, tend to support the above conclusions that scarcely any differences in the assimilation exist between secondary and tertiary phosphates.

The above discussion has applied almost exclusively to calcium. This is due to the almost total lack of critical studies on magnesium, the other element of interest here, and the existence of numerous data on calcium, which is of much practical importance since deficiencies of it are more likely to occur.

State of Phosphorus in Foodstuffs and its Biological Availability. —The ubiquity of phosphorus compounds differing greatly in chemical and physiological properties merits considerable attention. Apparently all compounds of phosphorus in biological material exist as derivatives of orthophosphoric or pyrophosphoric acids. Some type compounds of the first are inorganic salts, especially calcium phosphates of bones and teeth, phospholipids, phosphoprotein, phytin, nucleoprotein or its derivatives, hexosephosphates, glycerophosphates and even flavin phosphoric acid. Pyrophosphoric acid participates in the intermediary metabolism of carbohydrate as adenylic acid pyrophosphate. It has been identified also as a component of cocarboxylase (thiamin pyrophosphate).

It became a question of considerable interest at the beginning of this century whether the body requires any particular types of phosphorus compounds to provide for its needs of the various phosphorus complexes. These early studies are reviewed by Forbes and Keith ('14). It was even believed for some time that beriberi was due to a deficiency of some organic phosphorus complex removed in the polishing of rice. Since it is not unlikely that thiamin (vitamin B_1) does occur in cereals as a complex with pyrophosphoric acid, that early idea based on totally inadequate proof, may have been partly correct.

An early experiment by McCollum, Halpin and Drescher ('12) conclusively proved that phospholipids in general can be readily synthesized by the hen since young hens confined to a diet practically free from that class of substances produced many eggs containing much more of the complex lipids than the bodies of the birds could possibly furnish. Investigations of other workers have indicated that phosphoproteins, nucleoproteins, and various other esters of phosphoric acid can be synthesized by the body. Hence it is generally concluded that inorganic sources of orthophosphates can adequately serve the requirements of all species irrespective of age and degree of development. In general this is probably true but there is, as yet, no adequate experimental proof that it is invariably the case. Future studies may show that certain types of organic complexes are utilized more efficiently than inorganic compounds of phosphorus. With respect to the latter, Shelling ('32) studied the utilization of pyrophosphate,

metaphosphate, and hypophosphite in parathyroidectomized rats and found that pyrophosphate is utilized while the latter two types are inert.

The nutritional significance of phosphoproteins has not received the critical attention this subject deserves. It does not seem probable that casein, for example, quantitatively the most important protein in the diet of all suckling animals, should contain approximately 0.8 per cent phosphorus in ester combination unless the particular complex is of nutritional importance and not merely a fortuitous circumstance. As pointed out by Rimington and Kay ('26): "Either this group (organic phosphorus complex of casein) (a) has some definite part to play in the economy of the suckling, or (b) is there because of certain synthetic necessities of the mammary gland, or (c) is present adventitiously in the milk, and could be replaced equally well by inorganic phosphoric acid as far as the needs either of the mother or of the growing suckling are concerned." Schmidt and Greenberg (l.c.), in their extensive review, as well as Rimington and Kay (l.c.), regard the ester-complex as a wise provision of nature, since they believe the low rate of hydrolytic cleavage (assumed) in the intestinal tract permits most of the calcium to be absorbed before much of the phosphate is released. It is assumed that this is a means of preventing the formation of calcium phosphate in the gut which they think is incapable of being absorbed efficiently. Whether these opinions are valid is not known since the subject has not been adequately investigated.

It is of interest to consider some of the present evidence concerning the mode of combination of phosphorus in casein. Rimington ('27) reported that tryptic digestion of casein quickly releases most of the phosphorus as a complex which is relatively resistant to further tryptic hydrolysis. The complex was called phosphopeptone. Hydroxyglutamic acid, hydroxyaminobutyric acid, and serine were isolated after subjecting the material to acid hydrolysis. Later Lipmann ('33) reported that serine is the sole phosphoric acid bearing component of the casein molecule. This peptide, isolated by mild acid hydrolysis, was named serine phosphoric acid. Levene and associates have made various chemical studies of the phosphoric esters of hydroxyamino acids and in the opinion of Levene and Schormüller ('34) the dipeptide phosphoric acid complex of phosphoproteins probably has special physiological significance. Since critical nutritional studies have not been reported, no definite conclusions can be drawn. The subject requires careful investigation.

Another subject about which more definite information is at hand is the availability of phosphorus in cereals, particularly phytin phosphorus. Harris and Bunker ('35) found that the proportion of the

total phosphorus in the form of phytin is variable and may range from 30 to 70 per cent in different samples of a single cereal such as corn. The phytin phosphorus is poorly utilized. As shown by McCance and Widdowson ('35), in studies on humans, 20 to 60 per cent of ingested phytin was excreted unchanged. They believed that such utilization as did occur was probably the result of bacterial action in the intestines. Also it has been shown by Bruce and Callow ('34) that the heretofore alleged ricketogenic effect of cereals is due to unavailability of phytin phosphorus. When oatmeal, for example, was subjected to mild acid treatment the so-called anticalcifying tendency was decidedly reduced. Presumably the slight acid hydrolysis rendered more of the phosphorus available to the body. These observations were confirmed and amplified by Lowe and Steenbock ('36).

In view of these data there is no further basis for Mellanby's postulate that cereals contain an anticalcifying substance, designated as a "toxamin" (American Medical Association, '37). Moreover there is no unique advantage to be secured by the irradiation of cereals in order to destroy the alleged anticalcifying effect of such foods. The principal deduction to be drawn from these investigations is that the chemical value of the phosphorus content of a ration is not necessarily synonymous with its biological value as respects the available phosphorus.

Numerous types of evidence demonstrate the ability of the body to synthesize nucleoprotein, hexose phosphates, and glycerophosphates, using inorganic phosphates and the appropriate organic substances as starting material. In the earlier investigations of rickets considerable attention was given to the nutritional role of hexose- and glycerophosphates but none of the data suggested significant differences between the efficiency of their utilization as compared with that of inorganic phosphates. However, it has not been conclusively determined whether phosphorus combined in such forms is utilized with any greater efficiency than phosphorus in inorganic forms.

Cations which Interfere with Phosphorus Utilization.—There are various cations which form insoluble compounds with phosphates under conditions which exist in the intestinal tract. Some of these are iron, aluminum, beryllium, and manganese. Waltner ('27) showed that rickets could be produced in rats by the addition of soluble iron salts to a non-ricketogenic diet. This was confirmed by Brock and Diamond ('34). Severe phosphorus depletion was produced in young chicks by Deobald and Elvehjem ('35) who treated their normal ration with soluble iron and aluminum salts equivalent to 0.5 and 0.75 per cent of the theoretical amount required to combine with the dietary phosphorus as $FePO_4$ and $AlPO_4$. Normal bone ash values of 50 to 60 per cent were reduced to 25 per cent. The latter investigators warned

that the clinical usage of very high doses of iron in the treatment of anemia might seriously interfere with phosphorus utilization.

A few studies have been made on the effect of beryllium in the production of rickets (Guyatt, Kay and Branion, '33). When a normal diet is supplemented with 0.5 per cent or more beryllium carbonate severe phosphorus deficiency occurs. This can be prevented by means of a soluble phosphate administered in sufficient quantity to combine with most of the beryllium. Beryllium phosphate appears to have no deleterious effect.

In view of these data it is safe to generalize that all cations capable of forming insoluble phosphate compounds in the intestinal tract will interfere with phosphorus utilization, depending on their concentration in the diet. Reciprocally, excess of phosphorus in the diet will interfere with the utilization of these cations. The effect on calcium will be discussed in relation to the calcium: phosphorus ratio and iron will be considered in the chapter on iron and copper. Whether the utilization of copper and manganese may be seriously affected by a relative excess of phosphorus remains to be investigated.

Acid-Base Balance of the Diet and Utilization of Calcium and Phosphorus.—Various data have been published concerning the relationship between acid-base balance and the metabolism of calcium and phosphorus. *A priori* it would seem that a preponderance of acids would augment the excretion of urinary calcium and that an excess of alkali would increase the excretion of urinary phosphorus. This actually occurs. But owing to the solubility relationships between calcium and phosphorus, to be discussed in the section on Ca: P ratios, the abnormal excretion of one effects an abnormal excretion of the other. However, calcium and phosphorus do not appear to play an important role in the regulation of acid-base equilibrium. Goss and Schmidt ('30) studied the effect of various acid-base levels on the calcium and phosphorus metabolism of pregnant and lactating rats. The basal diets, containing 0.74 to 0.82 per cent of calcium and 0.50 to 0.55 per cent of phosphorus, were derived from whole wheat, wheat germ, alfalfa leaf powder, whole milk powder, casein, butter fat, and added salts. Within the range of acidity employed, no significant difference was found between the diets except that the second litters from rats on diets alkalinized with sodium bicarbonate were not nourished by the mothers owing to failure of lactation. Litters continued on the diets of their mothers, either alkalinized to the extent of 440 cc. of 0.1 N NaOH per 100 gm. of food, or acidified to the extent of 206 cc. of 0.1 N HCl per 100 gm. of food, stored both calcium and phosphorus in the normal manner. These observations support those of Aub and coworkers who reported that calcium excretion is only slightly affected by acid or alkali. And it should be noted that the

diets of Goss and Schmidt contained rather generous amounts of calcium and phosphorus. It may be concluded that the dietary acid-base balance, although important in some respects, is relatively of slight significance in the utilization of calcium and phosphorus.

Vitamin D in the Assimilation of Calcium and Phosphorus.— The physiological action of vitamin D will be discussed more extensively elsewhere (see chapters on vitamin D) but it is necessary to outline here, even at the risk of repetition, some of the data which bear upon the subject. Calcium and phosphorus assimilation is promoted by vitamin D but the mechanism involved in this relationship is but poorly understood. As pointed out by Bills ('35) this ". . . is understandable when one contemplates the permutations and combinations which are possible in a system comprising several forms of calcium and phosphorus in the blood, a reserve and depository of these elements in bone, a fluctuating intake and output of them in the diet and excretions, and a dumping place for them in the soft tissues—all under the influence of many factors besides the parathyroid glands and the several forms of vitamin D."

Since one of the most apparent effects of vitamin D deficiency is an increased loss of calcium and phosphorus in the feces, associated with an elevation of fecal pH, it was early assumed that vitamin D has a local action in the gut. But there is practically no experimental support for this view. For example, it is reported that aqueous solutions of vitamin D favorably influence the extent and density of ossification in ricketic bone fragments immersed in a solution containing calcium (Venar and Todd, '36). Also, the increase in urinary calcium following deprivation of vitamin D is indicative of a systemic rather than a local action.

Another suggested mechanism is that vitamin D promotes calcium and phosphorus utilization through its action on the parathyroid glands (Taylor, Weld and Sykes, '33). There is considerable lack of conclusive proof for this relationship although various experimental procedures have been employed in these studies. It appears significant that young dogs deprived of vitamin D but given satisfactory amounts of calcium and phosphorus showed scarcely any change in blood calcium when parathyroid hormone was administered. Control dogs receiving vitamin D did respond to the hormone (Morgan and Garrison, '33).

The reports of Nicolaysen ('37) are valuable since they indicate the nature of critical investigations that are needed. The object of his studies has been to determine how vitamin D decreases the fecal and urinary output of calcium and phosphorus. As he points out, all experiments up to the present have been performed with considerable amounts of calcium and phosphorus in the diet. Hence there is no

definite evidence concerning relationships between vitamin D deficiency and absorption or excretion of calcium and phosphorus since the fecal output constitutes both endogenous and exogenous products. The increased output may be due partly to decreased absorption of ingested calcium and phosphorus, partly to decreased secondary absorption of the endogenous moiety secreted into the gastrointestinal tract with digestive fluids, and partly to increased secretion into the gastrointestinal tract. Moreover, it is possible that a deficiency of vitamin D is marked by more or less specific differences in the effect on calcium and phosphorus. Obviously a critical evaluation of calcium- and phosphorus assimilation, as affected by vitamin D, is dependent upon clear-cut information concerning these questions. Unfortunately Nicolaysen's data are not very helpful since his basal calcium- and phosphorus-low diet was not only grossly deficient with respect to several dietary essentials, but it also contained enough egg albumin to produce some toxicity. One of the basal diets, for example, contained agar 4, NaCl 1, egg albumin 20, and sucrose 75. In rats fed this diet the percentage of absorbed calcium, given as the gluconate or carbonate, was considerably higher when vitamin D was fed. The absorption of phosphorus, given as NaH_2PO_4 or disodium glycerophosphate, was complete whether or not vitamin D was administered. This was interpreted by Nicolaysen as evidence that the action of vitamin D in the gut is confined to a direct action on the absorption of calcium. He concluded also that the decreased utilization of phosphorus in vitamin D deficiency, with diets containing calcium, is due to a precipitation by the increased amount of calcium in the intestinal tract. These conclusions are without convincing proof owing to the grossly inadequate diet, but as stated above, the work is significant because it suggests the type of data needed to aid in clarifying the role of vitamin D in calcium and phosphorus utilization.

Parathyroid Glands in the Assimilation of Calcium and Phosphorus.—Lack of space makes it impossible to adequately discuss this subject. Excellent reviews have been written by Thomson and Collip ('32) and by Shelling ('35). It suffices to state only a few of the important facts and opinions. Much experimental evidence suggests that the parathyroids are interrelated with vitamin D in the metabolism of calcium and phosphorus, but without duplication of function. There is no doubt also that a considerable degree of interrelationship exists between the parathyroids and other glands of internal secretion, particularly the thyroid. However, there is very little definite knowledge of the relationships involved.

It is fully established that hyperplasia occurs in the parathyroids when animals are maintained on calcium deficient diets, or in those lacking vitamin D. It is perhaps not without significance that Wilder

and Howell ('36) noted a higher incidence of parathyroid enlargement in the northern part of the United States as compared with other sections where the sunshine contains appreciable amounts of ultraviolet light. It is suggested, therefore, that deficiency of vitamin D, either directly or indirectly through disturbances in calcium and phosphorus metabolism, causes parathyroid hyperplasia. A few investigators have described an enlargement of the parathyroids in chickens deprived of vitamin D.

An outstanding effect of parathyroidectomy is the marked decrease in serum calcium and the small increase in serum inorganic phosphorus. Decalcification of bones occurs. The important work of McLean and associates ('35) has shown that the parathyroids function in the maintenance of the calcium ion concentration at a physiological level. In parathyroidectomized animals the ionized calcium may be reduced one-third. Conversely in conditions of extreme hyperparathyroidism, induced by parathormone injections, the calcium ion concentration is almost doubled and the inorganic phosphorus is markedly raised (Shelling, Asher and Jackson, '33). These blood changes occur at the expense of the bones. And, in turn, the utilization of calcium and phosphorus is affected, possibly to a certain extent through an interference with renal function.

It is possible to regard the parathyroids as non-essential for life, since it appears that both animals and man have been maintained in an apparently fair state of health for indefinite periods by careful regulation of the dietary calcium, phosphorus, and vitamin D. However, their important role in calcium and phosphorus utilization cannot be denied. As pointed out in the discussion of vitamin D in the utilization of calcium and phosphorus, the discovery of means for the preparation of satisfactory experimental diets in which calcium and phosphorus can be varied practically at will should make it possible to clarify somewhat the role of the parathyroids in calcium and phosphorus metabolism. The basal diet developed by Day and McCollum in the study of acute phosphorus deficiency should be of use for the above purpose. By means of such a diet information could be secured on the relationships between the parathyroids and endogenous and exogenous excretion, as well as of absorption of calcium and phosphorus. Moreover such a diet would permit the regulation at will of magnesium and vitamin D.

Significance of Calcium: Phosphorus Ratio.—It was not recognized until about 1921 that the ratio of calcium to phosphorus in the diet is of much importance in the promotion of bone growth and maintenance of good health. Previous to that time most of the emphasis was placed on calcium. Since most of the bone salts consist of some form of calcium phosphate it is remarkable that it took such a long

time for the importance of phosphorus to become realized. Early in 1921 Sherman and Pappenheimer published an important paper demonstrating that rickets can be produced in rats by a diet containing only 0.08 per cent phosphorus and about 0.6 per cent calcium. Rickets was prevented by replacement of some of the calcium, fed as calcium lactate, with potassium phosphate. Shortly thereafter McCollum, Shipley, Park and coworkers reported essentially the same results. Thus these two papers marked a distinct advance in the subject of mineral nutrition since they indicated not only the necessity of adequate phosphorus in rickets prevention but the importance of the dietary calcium: phosphorus ratio.

Upon consideration of the physico-chemical relationships between calcium and phosphorus it is understandable that a relative imbalance in the dietary content of these nutrients should render impossible their proper utilization. Practically all of the calcium in the body is deposited in the skeleton as some form of calcium phosphate, the solubility of which at the reaction (pH) of body tissues and fluids is very sparing. Since the bodily fluids, containing dissolved and ionized calcium and phosphorus, are contiguous with the solid calcium phosphate of bone, it follows, on the basis of mass action relationships, that a reversible equilibrium must exist between the two phases. Moreover, there is a continuous interchange of bodily fluid components, including calcium and phosphorus, with the gastrointestinal contents. Also there is a continuous although variable excretion of these nutrients in the urine. It is therefore apparent that if the dietary calcium-phosphorus ratio is very abnormal, as respects the ratio in the body, both elements will be excreted and the bodily calcium and phosphorus will tend to be withdrawn. An indication of this effect is afforded by the work of Shohl and Wolbach ('36). The following table prepared from their data is sufficient to illustrate the point:

Composition of Diet			Bone Ash	Blood Serum	
Ca: P	Ca	P		Ca	P
	per cent	*per cent*	*per cent*	*mg. per cent*	*mg. per cent*
1	.25	.25	46	6.0	11.4
1	.50	.50	48	7.6	10.0
1: 8	.25	2.00	40	6.3	15.2
1: 4	.50	2.00	42		11.2

It will be noted that a low ratio, in spite of the adequacy of calcium in absolute amounts, causes poor mineralization of the bones. An

abnormally high ratio, not shown here, causes even more deleterious effects. This is shown by the studies of Kletzien, Templin, Steenbock and Thomas ('32) who found that adult rats lost calcium and phosphorus from their skeletons when kept on the Steenbock and Black ricketogenic diet which contains about 1.25 per cent calcium and about 0.25 per cent phosphorus. This loss was reduced but not prevented by the addition of vitamin D.

This introduces the question of optimum calcium: phosphorus ratios. As pointed out by Sherman ('37), an optimum ratio probably should be defined in terms of the particular nutritive requirements since these may be variable. A suggestion of this is furnished by the studies of Stearns ('31) who considers that a retention ratio of calcium: phosphorus between 5:1 and 2:1 is normal for an infant, but for older children the ratio is somewhat lower, about 1:1, owing to the relatively greater amount of phosphorus required for soft tissue growth. Indirectly this suggests the optimum dietary ratios of calcium and phosphorus for infants and children provided these nutrients are furnished in a utilizable state.

Cox and Imboden ('36), using a purified diet, concluded that a calcium: phosphorus ratio of 1.0, at a calcium level of 0.49 per cent, is the ideal mineral level and ratio for successful gestation and lactation in rats. They found also that phosphorus in excess was better tolerated than calcium in excess. The experimental basis of their work was sound but attention must be called to the fact that no source of vitamin D was provided.

Possibly discussions of rickets have tended to stress calcium: phosphorus ratios to the extent that the necessity of maintaining adequate absolute levels of these minerals in the diet has been minimized. Rickets does occur in rats when the calcium and phosphorus content of the diet is quite low, but the ratio is approximately normal. Shohl and Wolbach (l.c.) observed rickets when the ratio was 1:1 but unfortunately the experimental diet was composed largely of cereals. Hence the exact amount of available phosphorus was not known.

If there are different optimum ratios for various stages and functions of life it is not probable that the differences vary significantly, in a practical sense, from the generally accepted range of 1 to 2. In view of the recent studies which show that phosphorus in cereals is only partially available, it is necessary to be cautious in accepting data and conclusions based on the use of cereal diets. Unfortunately most of the present information is of that sort. The high-cereal rickets-producing diets of McCollum and of Steenbock and Black served an extremely useful purpose when the fundamental aspects of rickets were being established but it appears that the peak of their usefulness

in research has passed. Profitable work of the future must be based on the use of purified diets.

Role of Magnesium in the Metabolism of Calcium and Phosphorus.—The evidence is predominant that large amounts of magnesium in the diet is a disturbing factor especially in the utilization of calcium. The view that there is a physiological antagonism between calcium and magnesium originated with Loew during the latter part of the last century in studies of plant nutrition. In 1905 Malcolm fed magnesium chloride to dogs and noted a small increase in calcium excretion. Eight years later Hart and Steenbock found that magnesium chloride and magnesium sulfate fed to swine caused an increased excretion of calcium in the urine but not in the feces. This observation was made really significant by the further observation that soluble phosphates fed with the magnesium caused a decrease in the loss of urinary calcium with scarcely any increase in the fecal calcium. Further studies by Palmer, Eckles and Schutte ('28) with cattle subsisting on phosphorus-low diets confirmed the favorable effect of phosphates in the sparing of calcium when the diet contains large amounts of magnesium.

Cunningham ('33), working with rats, observed a reduction in bone calcium and an increase in magnesium when magnesium was fed in the form of sulfate or carbonate. This did not occur when magnesium phosphate was fed. Moreover, Buckner and associates ('32) found that magnesium, as the carbonate, disturbed the mineral metabolism of chickens. The basal ration consisted of yellow corn 80, wheat middlings 20, NaCl 1, and cod liver oil 2, with skim milk ad libitum. Different groups of 30 day-old chicks received supplements of $Ca_3(PO_4)_2$; $CaCO_3$; and $MgCO_3$ in various proportions. Feeding of $MgCO_3$ decreased the percentage of Ca and increased the percentage of P and Mg in the leg bones.

Studies on rats by Elmslie and Steenbock ('29) contradicted the relationship between calcium, phosphorus, and magnesium, as indicated here. The explanation of these divergent findings is not apparent, but there can be scarcely any doubt that magnesium does interfere with calcium metabolism, especially in conditions of inadequate phosphorus intake. There is no conclusive explanation of this relationship, based on experimental evidence, but it appears possible that the excess magnesium is excreted, in part at least, as phosphate, thus rendering some of the calcium unassimilable and resulting in its excretion.

Indirect evidence that magnesium affects calcium utilization is afforded by the numerous observations on physiological antagonism between these minerals, referred to above. Classic experiments on this subject are those of Meltzer and Auer (1908) who found that deep anesthesia could be induced by the injection of magnesium salts

and that the effect could be prevented by means of calcium salts. This relationship has been thoroughly established by numerous experiments.

The recent studies of magnesium deficiency in rats and dogs also bear on the subject. Day, Kruse and McCollum ('35) found that dogs restricted to a diet deficient in both magnesium and calcium lived longer than similar animals deprived only of magnesium. Moreover, most of the animals failed to exhibit symptoms of hyperirritability, a principal feature of the magnesium deficiency syndrome. Also, Tufts and Greenberg (l.c.) have shown that a high content of calcium in the diet (1.16%) increases the severity of magnesium deficiency and raises the amount necessary to meet the minimal magnesium requirements.

Calcium, Magnesium, and Phosphorus in Tetany.—A discussion of calcium, magnesium, and phosporus in nutrition is not adequate without giving some consideration to tetany. Hess ('29) has written an admirable account of this condition in which he states that tetany ". . . is merely a symptom-complex resulting from and giving evidence of, an increased irritability of the nervous system and may be incited by a variety of factors." In general the various forms of tetany are characterized by one or the other of two conditions, namely, a reduction in blood calcium or a lack of change in blood calcium. Nearly all cases of tetany seen clinically are of the low-calcium type but, as shown by Kruse, Orent and McCollum, magnesium deficiency is characterized by tetany without apparent involvement of calcium or phosphorus. On the basis of numerous studies of irritability it has become established that the proper irritability of a tissue or organism depends upon the ionic balance of all cations and anions in the circulating fluids. At present calcium and magnesium may be regarded as the most important in this respect.

According to the significant studies of McLean and others, 50 to 65 per cent of the total serum calcium is ionized, the remainder being combined with protein. About 65 to 80 per cent of the total serum magnesium and apparently all of the inorganic phosphorus are ionized.

In absolute terms normal adult human blood serum contains about 10 mg. of calcium, approximately 2.0 to 3.0 mg. of magnesium, and about 3.5 to 5 mg. of inorganic phosphorus per 100 cc. In growing children the content of inorganic phosphorus is about 4.5 to 6.5 mg. per 100 cc. but there is no appreciable difference with respect to calcium and magnesium. All of the unionized calcium appears to be bound to protein and presumably this is the state of unionized magnesium. As shown by McLean and Hastings ('35a) the total calcium, total protein, calcium ions, and bound calcium are all interrelated in one equilibrium, which as a first approximation can be represented

by a simple mass-law equation yielding the ionization constant of calcium proteinate. But as they have further pointed out (McLean and Hastings, '35b), this equilibrium is affected by such variables as pH, temperature, albumin to globulin ratio (for want of a better term to express differences in the quality of serum protein), and magnesium. Since hyperirritability and tetany intervene when the concentration of ionized calcium is even slightly reduced from the physiological range, about 4.25 to 5.25 mg. per 100 cc. of serum, it is apparent that a well-regulated mechanism must exist to maintain constancy as respects this factor. As implied previously, the parathyroid glands play the most prominent role in this function. Parathyroidectomy causes a reduction of ionized calcium and parathormone increases the level above normal.

But the regulatory action of the parathyroids is dependent upon the calcium and phosphorus content of the diet, as well as the vitamin D intake. When the vitamin D intake is inadequate calcium-low diets always cause a decrease in total serum calcium, and presumably a diminution in the ionized moiety since hyperirritability intervenes. The same effect is caused by adding considerable amounts of phosphorus to a diet containing calcium. Presumably this is explainable on the basis of calcium:phosphorus ratios, already discussed. The roles of acid-base equilibria, serum protein, and other factors are of less practical significance and are afield from the present problem.

The role of magnesium in tetany has not been clarified although there is scarcely any doubt that a deficiency of this element causes a unique tetanic syndrome. As reported by Hoobler, Kruse and McCollum ('37) the diffusible (ionized) calcium of magnesium-deficient dogs, although reduced, did not reach the critical tetanic range. The reduction in diffusible magnesium was marked. Hence it was concluded that ". . . the tetanic syndrome of magnesium deficiency was closely associated with the extremely low concentration of magnesium ions in the blood."

It is significant that Hirschfelder ('34) observed low serum magnesium values (0.9 to 1.37 mg. per cent) in all of a series of 10 patients manifesting muscular twitchings or convulsions. The cases were diagnosed as follows: 1 parathyroid tetany, 2 epilepsy, 1 cerebral injury, the others acute or chronic nephritis. Three of the latter were given magnesium sulfate orally. This was followed by a rise of serum magnesium to 3.3-5.8 mg. per cent and a disappearance of the nervous symptoms.

"Grass Tetany" in Cattle: Although Hirschfelder's observations stand practically alone as respects indications of hyperirritability from magnesium deficiency in humans, there is a form of tetany in cattle known in Holland as "grass tetany," which is sufficiently extensive

to constitute an economic problem. In some parts of Holland lactating cows taken from stalls where they have been on dry feed and placed on spring pasture, following calving, have characteristic tetanic seizures which may terminate in death. Following Orent, Kruse, and McCollum's description of tetany in dogs and rats deprived of magnesium, Sjollema and coworkers ('32) noted the similarity between that syndrome and the grass tetany with which they were familiar. Analysis of blood from cows in this condition showed the following in terms of mg. per 100 cc. of serum: calcium 6.65, inorganic phosphorus 4.33, and magnesium 1.66. The phosphorus was approximately normal but the values for calcium and magnesium were only about two-thirds of normal. In his early studies Sjollema found that intravenous injections of calcium chloride solution caused an immediate curative effect. In later studies he reported the ineffectiveness of calcium gluconate, which is so effective in milk fever, and emphasized the importance of restoring a normal level of blood magnesium in order to cure or prevent the disease. Apparently there is a deficiency of both calcium and magnesium in this syndrome, produced by inadequate diet and the heavy drain on reserves occasioned by heavy milk flow. However, a deficiency of magnesium appears to predominate. As shown by Day, Kruse and McCollum (l.c.) tetany is not likely to occur in dogs deprived of both calcium and magnesium. Apparently the normal calcium: magnesium ratio of the blood must be altered before hyperirritability can occur.

Further indication that magnesium tetany may occur under certain dietary conditions is provided by the interesting observations of Duncan and associates ('35). Calves reared on a diet of whole milk supplemented with iron, copper, and manganese had normal serum calcium and inorganic phosphorus values but the level of magnesium was considerably reduced. A tetanic syndrome was observed which resembled that occurring in rats and dogs deprived of magnesium. Addition of magnesium salts to the diet prevented or cured the syndrome. This suggests that the cow's milk either contained insufficient amounts of magnesium to promote normal growth or, under the conditions of the experiment, magnesium was poorly utilized. Day and Orent-Keiles ('38) studied young rats restricted to a whole cows' milk diet supplemented with iron, copper, and manganese. They were unable to note hyperirritability or abnormal values in serum magnesium, calcium, and inorganic phosphorus.

Calcium, Magnesium, and Phosphorus in Ossification.—Mechanisms of ossification are only vaguely understood. Two basic concepts of the process are: (a) the idea of supersaturation of blood serum with calcium and phosphate ions and a precipitation of these as bone salts in cartilage when local conditions are favorable; (b) the view

that bone salts are formed through the action of an enzyme (phosphatase) in cartilage which hydrolyzes organic esters of phosphoric acid producing thereby a local concentration of inorganic phosphate ions and resulting in the precipitation of calcium phosphate of the composition characteristic of bone.

Important objections can be raised against the supersaturation theory. One defect is the lack of evidence that the mineral structure of bone bears any resemblance to the critical ion product postulated for supersaturated blood serum. The theory was advanced by Holt, LaMer and Chown ('25) who were led to believe on the basis of their experiments, that blood serum is normally supersaturated with tertiary calcium phosphate to the extent of more than 200 per cent, for on shaking serum with solid calcium phosphate there is deposition of the salt, while the content of calcium and phosphate decreases in equivalent amounts. No such deposition occurs when serum alone is shaken without the addition of the solid phase. They suggested that the serum bathing the bone matrix readily gives up its surplus, causing calcification. As pointed out by Stewart and Percival ('28), Holt and associates underestimated the complexity of the ionic equilibrium and ignored the fact that about 50 per cent of the blood calcium is in combination with protein.

The view that organic esters of phosphoric acid play the leading role in bone formation merits consideration since it is the most plausible of the present theories. This concept was advanced by Robison ('32) while studying the hydrolysis of hexosemonophosphate by various enzymes. As he states, "For these experiments the readily soluble calcium and barium salts were used, and when hydrolysis occurred the liberated inorganic phosphate was precipitated as the insoluble calcium or barium phosphate on the sides of the vessel." This observation suggested to Robison the possibility that such a mechanism might be concerned with the deposition of calcium salts in bone. Investigation showed that bone does contain a very active enzyme which can hydrolyze not only hexosephosphates but also other phosphoric acid esters. Robison's theory is made more plausible by the finding that the enzyme is absent from cartilage in which ossification is not taking place. It was shown that calcification occurs in slices of young bone, whether from normal or ricketic rats, when these are placed in a solution of calcium hexosephosphate at the correct pH.

Shipley and coworkers ('24, '26) found that no calcification occurs when the cartilage and bone of ricketic rats are incubated in the serum of ricketic rats. On the other hand they found that calcification does occur when a piece of the cartilage and bone from the same ricketic animal is incubated in the serum of normal rats. Since the inorganic phosphate value of the ricketic serum was only 2.5 to 3 mg. per

100 cc., as contrasted with the normal value of 8 mg., it was suggested that *in vitro* calcification is somewhat similar to that *in vivo*. They further studied the calcification of bone and cartilage slices immersed in sterile solutions of inorganic salts containing calcium and inorganic phosphorus in concentrations similar to those in plasma. It is significant that the presence of only 1 or 2 per cent of egg albumin inhibited calcification, although the serum contained 10 mg. of calcium and 4 mg. of inorganic phosphorus per 100 cc. In the absence of protein calcification readily occurred. This effect of protein was not explained but Shipley and associates concluded that in rickets failure of calcification is not caused by any primary inability of ricketic bone or cartilage to undergo calcification, but that it is attributable to an insufficient concentration of the necessary elements in the fluids bathing the bone tissues, which must exceed a certain minimum value.

Robison, however, pointed out that the above solutions, in the absence of protein, were highly supersaturated with respect to normal plasma. This is evident since not more than 5 or 5.5 mg. of calcium in normal plasma is ionized, the remainder being bound by protein.

The evidence in favor of the phosphatase theory is indeed impressive. For instance, Robison and associates have shown that *in vitro* calcification can be made to take place with relatively low levels of calcium and inorganic phosphorus if a phosphoric acid ester is added in very small amounts. As little as 0.5 mg. of phosphorus in this form is sufficient. Since the blood normally contains no more than 0.5 mg. per cent of phosphorus as phosphoric acid esters it has been suggested that phosphatase action cannot be at the basis of calcification, owing to the low concentration of its substrate. However, it would seem that an equilibrium might exist between inorganic phosphorus and the esterified forms entering into the calcification process. By means of such a mechanism the concentration of phosphoric acid esters could be maintained at a constant physiologic level and calcification could proceed at an unvarying rate. Experiments should be made to determine whether such an equilibrium occurs. On the whole, Robison's views seem to explain the process of calcification better than any other. The fact that phosphatase occurs in kidney, intestine, blood, and other tissues appears to be a disconcerting fact difficult to reconcile with Robison's theory. It is probable that there is more than one phosphatase, as discussed by Kay ('32), Robison ('36) and Linderstrøm-Lang ('37).

Mobility of Calcium, Magnesium, and Phosphorus in Bones and Teeth.—A great deal of attention has been given to the study of calcification as compared to the interest in mobility of calcium, magnesium, and phosphorus after they have entered the skeletal structures. It appears self-evident that an understanding on the mechanism of

"demineralization" of bone and teeth, if such occurs in the latter, and mobility of skeletal minerals are important.

As shown by Aub and associates (Bauer, Aub and Albright, '29), and other workers, much of the calcium and phosphorus deposited in bones may be regarded simply as a reserve supply to be used in time of need. Presumably some of the magnesium in bones may be regarded as fulfilling a similar function. This storage capacity of bones is a function of the trabeculae. The content of minerals in the cortex is not appreciably influenced except in the case of very unusual bodily demands.

Recently it has become possible to determine more precisely the movement of phosphorus in the body by means of radioactive phosphorus. It may be presumed that this provides some indirect means of estimating the mobility of calcium, since practically all of the calcium is combined with phosphorus. The radioactive isotope of phosphorus has a half-life value of 15 days, making it possible to utilize this substance as an "indicator" in metabolic investigations. The concentration of radioactive phosphorus can be readily estimated by means of a Geiger counter. Also, there is little reason to believe that the small amounts of material required for a metabolic experiment would have such unnatural effects as to invalidate the data.

Chiewitz and Hevesy ('35) fed rats a few milligrams of sodium phosphate containing radioactive phosphorus as indicator. By means of tissue analysis and mineral balance studies it was estimated that the average time a phosphorus atom spends in the body of a normal rat is about 2 months. They found that in the adult rat about 30 per cent of the phosphorus atoms deposited in the skeleton were removed in the course of 20 days. Since calcification, in the sense of bone growth, has practically ceased in mature rats it must be concluded that the deposited minerals are not "fixed" but continually in the process of mobilization, either from one part of the body to another, or excreted and replaced by the dietary source and the "moving" phosphorus in the body. Even the molar teeth, which do not grow in adult rats, took up some of the radioactive phosphorus. Hence it may be inferred that the mineral content of teeth is not completely fixed. This has important implications as respects relationships between the quality of diet and dental caries.

Pathological Calcification.—Owing to the relative insolubility of some calcium salts at the pH of bodily tissues and fluids, abnormal calcification is a problem of some importance from the viewpoint of nutrition, since dietary procedures can be used in its control. The question has been extensively discussed by Barr ('32). In general it may be stated that deposits of calcium salt tend to form in any dead or dying tissue and any nutritional factors which lead to devitalization of a tissue will favor calcification. A significant example is the formation

of renal calculi in conditions of vitamin A deficiency (Higgins, '35). The epithelial tissue of the urinary system undergoes marked changes (see chapters on vitamin A) and owing to the secondary invasion of microorganisms ammonia is formed by fermentation of urea. The induced local alkalinity causes the precipitation of calcium phosphate. These masses of insoluble material become large enough to obstruct tubules and ducts, thus causing serious damage.

As shown by Cox and Imboden ('38a, l.c.) and others, high calcium diets in the absence of vitamin D also cause the formation of renal concretions consisting of calcium phosphate. Another example is from the work of King (Chapter XVII) who showed that histological changes suggestive of arteriosclerosis occur in the blood vessels of guinea pigs whose reserves of ascorbic acid are low. The lesions are caused by poisons of bacterial origin since there is not sufficient ascorbic acid to inhibit bacterial action. It is probable that blood vessels injured by such agencies will calcify after some time. Conditions favorable to calcification may be brought about by various factors such as temporary interruption of circulation, and poisoning from mercury, iodine, iodoform, and other substances.

The Calcium Requirement of Man.—Since absorption and retention of calcium and phosphorus is dependent upon rate of growth, ionic relationships between these elements and others which form compounds of low solubility, the supply of vitamin D, and even the composition of the diet as respects other nutrients, it is not surprising that experimental data on the calcium and phosphorus requirement of infants, children, and adults, although available from numerous studies, do not lend themselves to simple presentation or unequivocal interpretation. Leitch ('37) has prepared an excellent review of the subject. As is true of other nutritional factors it is difficult to determine the best criteria on which judgment should be based concerning requirements for calcium. Certainly the maintenance of a positive calcium balance, even in adults, on a given dietary regimen is not entirely a valid basis for judgment. In this case, for example, it is possible that a slight but continuous positive balance is no more indicative of an optimal nutritional state, as respects calcium, than is true of positive nitrogen balances in determining the requirement for protein.

A great many mineral balance studies of humans and animals have been made as a means of estimating the calcium needs. Conclusions based on such procedures are subject to considerable error since they are usually too short to constitute an adequate sampling of the mineral exchange. Macy and associates ('36), whose contributions based on mineral balance procedures are well known, have a realistic attitude in this matter. They have observed in children marked fluctuations in the balance of various minerals and nitrogen, although the subjects were

in apparent good health. A short interval of sampling in such subjects, which are presumably typical, would give quite erroneous information. As emphasized by Leitch, the only adequate general criterion is the effect of various dietary regimens on the health of man observed over long periods of time.

The body of the newborn infant contains approximately 7 to 8 gm. of calcium per kilogram (birth weights averages 3 kg.). During the first 6 to 12 months the value is increased to about 12 to 15 gm. per kilogram. At 2 to 3 years of age it is 20 to 24 gm. per kilogram. The value rises steadily (on the basis of statistical analysis) until at 9 to 10 years of age the content is about 35 to 36 gm. per kilogram, after which the ratio of calcium to weight remains essentially constant up to at least 18 years of age. But it should be recognized that these data are based on a paucity of reliable information. On the basis of standard average growth records it may be estimated that the daily requirement of *retained calcium* at 6 months is about 0.3 gm. and rises as the child grows. Daniels and associates ('35) have concluded that normal children between 4 and 7 years of age require 45 to 50 mg. of calcium in the diet per kilogram of body weight. As shown by Hubbell and Koehne ('34) girls between 7 and 11 years of age retained about 5 mg. of calcium per day per kilogram of body weight. Stearns ('31, l.c.) believes that average daily retentions of calcium (and phosphorus) of less than 10 mg. per kg. of body weight are probably not optimal in very young children. Leitch has summarized his views regarding the daily minimum calcium requirements, assuming that 50 per cent of the amount ingested is absorbed. They are: from 6 months to 2 years, 0.8 gm.; from 2 to 9 years, 0.9 gm.; 1 gm. after 9 years and increasing to 2 gm. between 15 and 16 years. He assumes that thereafter the amount may be gradually decreased to the adult maintenance requirement. This value is estimated as 0.55 gm. per day.

Infants commonly absorb both calcium and phosphorus rather poorly, even from breast milk. Leitch concluded that it is impossible for the breast fed infant to maintain birth composition of the skeleton during the first 6 months since the average content of calcium in human milk is only 0.32 gm. per liter. In comparison, cow's milk contains about 1.20 gm. of calcium per liter. But when all factors are considered human milk may be regarded as superior to cow's milk. There is some reason to believe that normal infants at birth have a store of calcium in the bone trabeculae which is drawn upon during the first few months of postnatal life (Bauer, et al., '29, l.c.).

The classic studies of Sherman and Hawley ('22) are generally quoted concerning the calcium requirements of children. On the basis of balance studies they concluded that children from 3 to 13 years of age require an intake of 1 gm. of calcium per day if most of

this nutrient is furnished by milk. It requires about 1 quart of milk to furnish 1 gm. of calcium. As pointed out previously the calcium of some foods, especially certain vegetables, is poorly utilized because of their oxalate content.

Owing to the rapid skeletal growth which normally occurs in adolescence it must be assumed, for want of absolute evidence, that the calcium (and phosphorus) needs are particularly large. Todd's ('34) extensive roentgenological data indicates that skeletal rarification is a common occurrence in this period. Hence there may be considerable basis in Leitch's belief that 1 gm. of calcium per day is not optimal. But actually we have no real evidence in support of the tacit assumption that the skeletal structures of children and growing animals should be kept as fully saturated as diet can make them. Since it is definitely known that a large percentage of children do not receive enough calcium, this view should not be misinterpreted. As a general policy intelligent attempts should be made to increase the consumption of calcium-rich foods.

The unincumbered adult, according to Sherman's estimations, should have a minimum of 0.4 gm. of calcium per day. It is generally believed that this value should be increased by 50 per cent in order to assure an adequate intake. Hence the normal value may be regarded as about 0.68 gm. per day. That amount of calcium is furnished by a little over 1 pint of milk. Owing to the tendency to hypochlorhydria in adults past middle life it is probable that their calcium absorption is considerably diminished. Osteoporosis of varying degree is common in older people. This condition is probably brought on by long periods of insufficient calcium intake combined with failing calcium absorption. Since there is a lack of critical studies on calcium (and phosphorus) metabolism in persons who are past middle age, generalizations regarding the requirements cannot be made.

The requirements in pregnancy and lactation are obviously larger. According to studies by Coons and associates ('35) there is a high storage of calcium, as well as iron and nitrogen, in early human pregnancy which is in apparent excess of fetal needs if the dietary conditions are favorable for storage.

Coons and Hunscher have estimated that pregnant women store about 200 mg. of calcium daily from the 4th to the 9th month of pregnancy and 300 mg. daily during the last week of pregnancy. Macy and associates ('30, '31) have made extensive investigations. They found that women producing large amounts of milk were frequently in negative calcium balance although the diet, of high quality in every known respect, contained 3 to 4 gm. daily of calcium and phosphorus. Calcium and phosphorus in the diet of lactating women

were utilized more economically when cod liver oil and yeast were administered.

There are no satisfactory absolute figures for calcium requirement during pregnancy and lactation. On the basis of such evidence as we have it would seem that the diet should furnish 1.5-3.0 gm. of calcium per day during pregnancy and that this value should be increased, perhaps to 2.0-4.0 gm. during the lactation period. This amount is so large that it cannot be provided by any common dietary constituents except milk. Either tertiary or secondary calcium phosphates are satisfactory supplements to the diet of pregnant or lactating women. But it is advisable to rely upon milk and other suitable natural foods as much as possible to furnish calcium.

The Phosphorus Requirements of Man.—In general the utilization of phosphorus is influenced by the same factors discussed in the section on calcium. Also, the criteria upon which phosphorus needs are estimated differ in no significant way from those used in estimating calcium needs. Quantitatively, however, there are differences. Phosphorus is required for numerous purposes in addition to the formation of bones and teeth. Nearly all of the bodily calcium contained in the skeleton is combined with phosphorus. But large amounts of phosphorus occurs in the soft tissues unassociated with calcium. On this basis Stearns ('31, l.c.) has concluded that a Ca: P retention of less than 1.5: 1.0 is indicative of rapid muscle or tissue growth, and a retention ratio of approximately 2 suggests that nearly all of the phosphorus is being deposited in the bone. Perhaps owing to the likelihood of calcium deficiency in persons who do not regularly drink milk or use milk products which contain calcium, much more attention has been given to the estimation of calcium requirements than of phosphorus requirements. Many common foods contain considerable amounts of phosphorus; hence it is somewhat tacitly assumed that the phosphorus need tends to take care of itself. Moreover, unless calcium is ingested in considerable amounts as the carbonate or gluconate, for example, it is very unlikely that the phosphorus intake will ever lag behind that of calcium. Milk, the principal source of calcium, particularly of infants and children, contains almost 1 gm. of phosphorus per quart; the calcium: phosphorus ratio of milk is about 1.2: 1. The most desirable dietary Ca: P ratio, as discussed previously, is about 1: 1.

According to Daniels and associates the phosphorus needs of children 4 to 7 years of age is about 60 to 70 mg. per kilogram of bodyweight. Hence a child weighing 20 kilograms should receive 1.2 to 1.4 gm. of phosphorus per day. As a general rule it is regarded that the phosphorus requirements are about 25 per cent higher than those for calcium. Sherman calculates that a normal adult should receive

at least 0.88 gm. of phosphorus daily. The values for pregnant and lactating women may be regarded as about 25 per cent higher than those for calcium.

The likelihood of phosphorus (and calcium) deficiency in pregnancy, unless the diet contains very generous amounts of this nutrient is indicated by Cox and Imboden ('36c, l.c.) who compiled data from the studies of Toverud (1931). The table is as follows:

Mineral	Level of Intake	Average Intake	Number of Metabolism Periods	Average Month of Pregnancy	Negative Balance Per Cent
		gm.			
Calcium .	less than 1 gm.	0.83	17	7.6	41.2
Calcium .	more than 1 gm.	1.35	27	7.6	14.8
Phosphorus	less than 1 gm.	0.82	6	7.0	83.3
Phosphorus	more than 1 gm.	1.48	38	7.7	13.2

The Magnesium Requirements of Man.—Scarcely anything is known concerning the human requirements for magnesium. Similarly little is known about animal needs for this nutrient. It has been pointed out that calves restricted to a whole milk diet for long periods of time apparently suffer from lack of sufficient magnesium. Denis and Talbot ('21) noted low plasma magnesium values but normal calcium levels in certain children with various types of diseases. Hirschfelder's observations of low plasma magnesium values in some human cases of hyperirritability have been discussed. Thus there are indications that some degree of magnesium deficiency might occur in humans.

Daniels and Everson ('36) attempted to estimate the magnesium needs of children 4 to 7 years of age. Since the output of magnesium in urine tended to parallel the amount ingested, they adopted this relationship as a criterion of magnesium need. For example, low values for urinary magnesium, when coexistent with low retentions, were regarded as indicative of too low ingestions. High urinary magnesiums with high retentions following high ingestions were believed to indicate that the subject had been receiving previously less than the optimum amount. It was found that 75 per cent of the children studied were in the latter group. On this basis it was tentatively concluded that the diets of children should contain not less than 13 mg. of magnesium daily per kilogram of body weight.

Nothing is known concerning the magnesium requirements of unincumbered adults and pregnant or lactating women.

Factors Affecting the Content of Calcium, Magnesium, and Phosphorus in Foodstuffs.—The composition of soils and the vagaries of weather are important factors in determining the mineral composition of plants and vegetables. Since man, and most of the animals upon which he depends for foods, are directly affected by the amount of calcium, magnesium, and phosphorus in products of the soil, the present subject is one of obvious moment. It has been indicated that large areas of land contain such low amounts of phosphorus that animals indigenous to those areas are scarcely able to live. The data of Hart, Guilbert and Goss ('32) show that the phosphorus content of different grasses may vary from 0.03 to 0.68 per cent. Calcium shows similar wide variations, ranging from 0.10 to 2.90 per cent. Bishop's ('34) analysis of Alabama vegetables grown on different types of soil showed that the calcium and phosphorus content of a given vegetable varies widely, usually in opposite directions, so that extremes in the Ca: P ratio may result. Moreover, it seems probable that these unfavorable ratios result in excessive excretion of magnesium, thus tending to cause a magnesium deficiency. This view is supported by the following data supplied by Forbes and Keith (l.c.) showing the composition of certain American foodstuffs:

CALCIUM, MAGNESIUM, AND PHOSPHORUS PER 100 PARTS OF DRY SUBSTANCE

	Ca	Mg	P
	gm.	*gm.*	*gm.*
Soy beans	0.230	0.224	0.649
Linseed oilmeal	0.403	0.544	0.786
Peanuts	0.068	0.180	0.399
Corn (maize)	0.012	0.108	0.260
Oats	0.102	0.118	0.395
Milk (skimmed)	1.336	0.146	0.979
Bluegrass	0.336	0.240	0.242
Clover hay	1.236	0.292	0.183
Alfalfa hay	1.130	0.400	0.238
Millet hay	0.326	0.262	0.173

According to Sjollema the winter rations of cows which develop grass tetany include hay, a large per cent of maize and other cereal meals, and concentrates such as extracted soybean meal and peanut cake. It seems probable, therefore, that depletion of magnesium in grass tetany is due to deficient retention rather than to an insufficient supply of this element.

Also, on a given type of soil one strain of a given plant may have a considerably different content of a given nutrient than another plant

grown under similar conditions. This is illustrated by the studies of Greaves and Greaves ('33) working with wheat. Two varieties were grown on the same soil under similar conditions. The following table, which includes values for protein, potassium, iron, and sulfur, indicates the pertinent results:

PERCENTAGE COMPOSITION

VARIETY	PROTEIN	Ca	Mg	P	K	Fe	S
Kota	17.41	0.080	0.182	0.308	0.334	0.006	0.253
Turkey	14.42	0.055	0.159	0.153	0.276	0.006	0.202

Obviously one is not justified in assuming that the composition of a given ration, made up of natural foodstuffs, can be reliably estimated on the basis of classic food analysis tables. Proof of this is indicated by the fact that the skeletal development was superior in rats fed a diet containing 90 per cent of Kota wheat, as contrasted with those fed a similar basal ration containing 90 per cent of Turkey wheat.

Milk, eggs, and other edible animal products, in comparison to plants, appear to maintain a much greater constancy of composition, as respects calcium, magnesium, and phosphorus. Hart, Steenbock and Kline ('30) were unable to note any effect on the per cent of calcium and phosphorus in milk of cows fed irradiated yeast. Hart and associates have also shown that cod liver oil is similarly without effect. Even cows suffering from osteomalacia and showing a negative calcium balance, produced milk containing the same concentration of calcium and phosphorus as normal cows (Becker, Eckles and Palmer, '27). This remarkable capacity to maintain constancy of composition in spite of adverse dietary circumstances, is well illustrated in the fact that the calcium, magnesium, and phosphorus of eggs are scarcely affected as long as the hen is able to make hard egg shells. However, the production level of milk and eggs is greatly affected by dietary inadequacies.

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CHAPTER

VIII

Sodium, Potassium, and Chlorine

LOEB'S THEORY of physiologically balanced salt solutions has profoundly influenced scientific thought in the field of nutrition. From the fact that animal tissues respond so markedly to even slight changes in ion concentration in the fluids bathing them, many investigators have presumed that the proportions existing among the mineral elements in an animal's food are equally important to the maintenance of health and physiological efficiency.

Since nearly all natural foods contain considerable amounts of sodium, potassium, and chlorine, it has been possible for man and animals to subsist in many parts of the world, and in at least fairly good health, on the amounts of these elements supplied in their food. In the Odyssey reference is made to inlanders who use no salt in their food. Benjamin Rush (cited by Kellogg, '23) stated that "although the interior parts of our continent abound with salt springs, yet I cannot find that the Indians used salt in their diet till they were instructed to do so by the Europeans." The well-known fact that commerce in salt was one of the major enterprises in ancient time attests, however, that mankind generally has always desired salt, and where it was obtained with difficulty it has commanded high prices.

Carnivorous animals do not develop a craving for salt (NaCl) as do Herbivora, a fact which may reflect the dietary sufficiency of this element in the animals they eat. It may well be true that the liking of Carnivora for blood is due in part at least to its salty taste. Grazing animals crave salt, except in such areas as provide more than usual amounts of sodium chloride in the water, and such places are not rare.

Sodium, potassium, and chloride have been the subject of many investigations, particularly as relates to their ionic content in normal and pathological tissues to "total base" content of body fluids and

tissues, acidosis, acid-base equilibria, effects of the "level of intake," diuresis, edema, etc. These subjects are fully discussed in such works as Peters and Van Slyke's *Quantitative Clinical Chemistry,* in physiological chemistry texts by Bodansky, Hawk and Bergheim, and others, and will not be considered here. For discussion of the "ionic effects" or the effect of ions on irritability and contractility, reference may be made to any work on physiology. The discussion in this chapter will be limited to the recent experimental studies on the effects of specific starvation for each of these elements, and, to the role of sodium and potassium in the function of the adrenal cortical hormone.

Investigations dealing with the physiological role of sodium have been largely studies of the importance of salt, i. e., sodium chloride, rather than the element sodium. Few definite experiments dealing with the function of sodium alone have been made.

In 1873 Forster fed dogs on a low-sodium diet, extracting the food with water until very low in salts. Salt-free meat, starch, and lard were used as food. These dogs died more quickly than those which were completely starved. He explained this occurrence by the fact that urea is a diuretic and causes excretion of a larger volume of urine and probably more sodium; and the urea coming from the salt-free protein caused elimination of some sodium. He concluded that a dog will die if it does not get sodium. From our knowledge of nutrition today we can readily see that Forster had a multiple deficiency.

In 1874 Bunge repeated the same experiment on pigeons and obtained similar results. Forster considered it salt starvation, but Bunge thought it was due to a lack of alkali needed to neutralize phosphoric and sulphuric acids arising from the metabolism of proteins. Lunin, in 1881, found that mice died in 3 days if they were totally starved, but on a salt-free diet they lived on the average 23 days. These experiments indicated that we probably need sodium in our diet.

Dairy cows that do not have access to salt (NaCl) exhibit an abnormal appetite for it in 2 or 3 weeks' time, but the health of the animals is not affected generally until a much longer time has elapsed (Babcock, '05). The breakdown is most likely to occur at calving or immediately after, when the system is weakened and the flow of milk is large. In general, the cows giving the largest amount of milk are the first to show signs of distress. The breakdown in health due to lack of salt is marked by a loss of appetite, a generally haggard appearance, lusterless eyes, a rough coat, and a very rapid decline in both weight and yield of milk. When these events follow calving in a high-producing cow, the collapse may be sudden and death may rapidly ensue. If given before the animal is in a desperate condition, sodium chloride will effect a dramatically rapid recovery.

Cattle and sheep that have been deprived of salt (NaCl), on gaining access to a salt lick, may consume sufficient salt to cause toxic effects, i. e., 4 to 8 oz. in the case of sheep and 1.5 to 5 lbs. for cattle. In acute cases the animals show extreme thirst, depression excitement, abdominal pain, and death in collapse. In less acute cases, profuse watery diarrhea which may become hemorrhagic sets in. Pregnant animals may abort. On post-mortem examinations, gastrointestinal inflammation and injection of the cerebral membranes are generally found.

An excess of salt (NaCl) in the ration of chickens up to 8 per cent of the ration excites no apparent detrimental effect on their condition, nor after they become accustomed to such salty rations on their rate of growth (Mitchell, et al., '26). Baby chicks also exhibit a high tolerance for salt (Quigley and Waite, '32).

Distribution.—Sodium, potassium, and chlorine are widely distributed in nature. Potassium is especially abundant in both plant and animal tissues. It is the chief mineral constituent of muscle and of most other tissues, very little being present in the fluid portions of the organism. This element is especially utilized by newly formed and growing tissues. Practically all foods of vegetable or animal origin contain several times as much potassium as sodium. The greater part of the sodium in the ordinary daily diet is added as seasoning in the form of sodium chloride, while the potassium is furnished by the various organic and inorganic potassium salts of the animal and vegetable tissues employed in the diet.

In the blood of man, sodium predominates in the plasma and potassium in the red corpuscles. (Na-43 mg./100 cc. corpuscles; 335 mg./100 cc. serum. K about 425 mg./100 cc. corpuscles; 18-20 mg./100 cc. serum.) This is also true for the rat, rabbit, guinea pig, pig, and monkey. In the cat, dog, sheep, and cow, sodium and potassium concentrations in the red cells tend to approximate the concentrations of these ions in the serum. Sodium is the predominant base in lymph, edema fluid, cerebrospinal fluid, and other extracellular fluids. Cartilage is especially rich in this element. The whole human body has been estimated to contain 0.1 per cent sodium and 0.11 per cent potassium.

Vegetable foods are poor in chlorine but this element is found in all the tissues of the animal organism. The concentration of chloride in normal serum varies from about 362-376 mg. per 100 cc. It has generally been assumed that it all exists in the body in inorganic form. However, Hanke and Donovan ('27) stated that 15-20 per cent of the total chlorine of the body is in combination with lipoid substances. Cameron and Walton ('28) could not confirm these results,

Absorption and Excretion.—Sodium, potassium, and chlorine, under normal conditions, are easily absorbed from the intestinal tract and are readily excreted by the kidneys. Although little of an exact nature is known concerning the mechanism by which the excretory organs function in selecting the ratios of these elements for excretion, it is clear that they do so. Accordingly, there is maintained in the tissues and body fluids a fairly constant concentration.

Excessive ingestion of any salts leads to increased thirst and increased output of urine or sweat or both, which carry away the surplus. Ordinarily sweat contains 0.7 per cent sodium chloride. On an average man excretes 10 gm. sodium chloride in the urine and sweat daily which indicates that 10 gm. of the salt are consumed in the food, since normally the intake is just balanced by the output. During starvation the body retains the sodium chloride very tenaciously. In a metabolism study conducted at the Carnegie Nutrition Laboratory on a man who fasted for a month, it was found that the amount of sodium excreted progressively decreased from 2.5 gm. to 0.1 gm. on the tenth day. On administration of 10-15 gm. sodium chloride per day, it took 4 days for the excretion to rise above 0.1 gm. per day. The body evidently was retaining practically all of the salt to replenish the deficiency. Dill and others ('33) state that not more than 15 gm. of sodium chloride can be excreted daily in sweat. Heller ('32) found that with salt solutions for drinking water the upper tolerance limit was 1.5 to 1.7 per cent (NaCl) and animals died of thirst rather than drink such solutions. In many dry regions the water holes contain excessive amounts of sodium chloride and carbonate, and the forced drinking of such waters may adversely affect the health of animals. Mistaken views have arisen in this connection, as is illustrated by the belief of farmers in the Middle West that the so-called "alkali disease" was due to too much salts in the water. Later discoveries showed that the condition was selenium poisoning.

Gompel et al. ('36) found that rabbits consumed with reluctance food containing 1 per cent sodium chloride, and rejected it when the content was raised to 2.5 per cent. By gradually increasing the content of sodium chloride, rabbits could acquire tolerance and readily eat food containing 3.5 per cent (NaCl). When the concentration exceeded 0.4 per cent the water consumption increased in proportion to the salt concentration to such an extent that the final concentration of salt in the food was less than 1.3 per cent. There were some individual variations. Human subjects differ in their taste for salt with their food.

General Functions.—Although we know that sodium, potassium, and chlorine are indispensable in the diet, our knowledge of their

function and importance in metabolism is fragmentary. The functions of the minerals are so manifold and interrelated with so many other processes that the mechanism of their actions is still obscure. Minerals are part of every living cell. The chemical processes that characterize living matter take place in salt solutions. Osmotic pressure must be maintained within precise limits for the proper behavior of the cells in the mammalian organism. The maintenance of this osmotic pressure, as well as the maintenance of physiological neutrality, depends primarily on the total base content in both intra- and inter-cellular fluids.

The principal basic elements, calcium, magnesium, sodium, and potassium, influence many properties and functions of living cells including water content, osmotic pressure, permeability, irritability, contractility, secretion, excretion, and general metabolism. The irritability of the nervous system is dependent in a large measure upon the balance between the amount of these ions present in the tissues and body fluids. The maintenance of certain relationships among the individual bases appears to be necessary for proper muscle irritability.

Chlorine assists in the maintenance of physiological neutrality in the body fluids and plays a part in the distribution of water in the blood, the intercellular tissue spaces and serous cavities, and the body cells. Presumably the tissue cells in general swell and shrink as do the blood cells with changes in the salt content of the fluid bathing them and suffer if such changes are too great.

The Effects of Sodium Deficiency.—On the basis of their studies with diets containing very low concentrations of each of several of the essential inorganic elements, Osborne and Mendel ('18) concluded that "the law of the minimum" holds for all essential salts in the diet, and that failure of growth in the body as a whole results when the limiting factors are deficiencies of sodium, chlorine, potassium, magnesium, calcium, and phosphorus. They found that young rats grew normally on a ration containing but 0.035 per cent of potassium or sodium.

Miller ('26) secured satisfactory growth on a ration containing 0.07 per cent of sodium, and observed no deleterious effect of a ratio of K: Na 14: 1. He did not get normal growth when the sodium in the diet was reduced to 0.03 per cent, but growth was satisfactory when a sodium supplement was provided. Richards and associates ('24; '27) found that a diet composed of corn, oats, barley, and blood meal was improved as respects retention of nitrogen, calcium, and phosphorus, by supplementing it with sodium citrate or chloride. Similarly, Mitchell and Carman ('26) employed a ration for rats and chicks consisting largely of corn, and found it was below the optimum in both sodium and chlorine, and that its deficiency of sodium

was probably greater than of chlorine. Their diet contained 0.047 per cent of sodium and 0.041 per cent of chlorine. They found deficiency of these elements to limit the utilization of protein and energy for growth. The digestibility of protein on the unsupplemented ration was not impaired by deficiency of hydrochloric acid in the gastric juice, and the effects of the deficiency of chlorine seemed to be related more to the requirements of growth than to those of gastric digestion.

In 1928 St. John, using a somewhat better diet (containing 0.3% sodium) than the other investigators, demonstrated retarded growth and abnormal reproduction, and also noted that the eyes of his animals were affected, blindness resulting in many cases.

In 1934 Schoorl studied the influence of sodium depletion on growth and other physiological constants in rats and pigs. He also noted a decreased rate of growth. He states that fertility is not affected but reproduction is impossible on his diet because owing to the disturbed protein metabolism, milk secretion is impaired.

Sjollema ('35) observed that the growth of chicks was considerably retarded by a ration poor in sodium.

In paired feeding experiments, Kahlenberg, Black and Forbes ('37) employed a corn-supplemented ration and observed that sodium deficiency unfavorably affected appetite, growth, storage of energy, and synthesis of fat and protein. The proportion of energy stored as fat was larger with the sodium-supplemented than with the sodium-deficient diet. There was no impairment of digestion, and the water content of the bodies of their animals was normal. Heat loss was significantly higher in the sodium-deficient rats than in the supplemented ones. The diet used in this investigation, although containing but 0.03 per cent sodium, appear to be inadequate in some of the necessary dietary constituents, especially the vitamin B complex.

While the above-mentioned experiments have elucidated to some extent the importance of sodium, the diets used contained considerable amounts of this nutrient and almost without exception they were obviously deficient in some other dietary essentials. Nevertheless, these studies are important since they show, among other things, that a cereal diet is likely to provide too little sodium for maximum rate of development and utilization of food. They justify the addition of common salt to diets largely derived from cereals.

Orent-Keiles, Robinson and McCollum ('37) restricted young rats to a diet composed of acid-washed and alcohol-extracted casein, a methyl alcohol-gaseous HCl extract of yeast, sucrose, sweet butter fat, salt mixture, and viosterol. This diet contained 0.002 per cent of sodium, and was otherwise complete as was shown by its satisfactory character when supplemented with sodium salts. Ninety experimental and 50 control rats weighing 35 to 40 gm. were employed in the

investigation. In other experiments with the same diet crystalline thiamin, purified riboflavin, and B_6 in the form of Peter's eluate were used as the vitamin B-complex instead of the yeast extract. The results in this set of experiments were similar to the ones where the yeast extract was used. The animals on the sodium-low diet grew at more than half the normal rate for a few weeks, after which they began to lose weight. No striking symptoms were noted until the 6th to the 8th week. At this time the sodium-low animals began to show eye changes. Some exhibited bluish-gray corneae; in others the corneae were still normal at this time; some had perforated ulcers, and all showed a thin sanguinolent excretion covering both eyes.

During the next 2 weeks practically all the rats developed the following eye changes: thickening and bluish-gray corneae, sanguinolent secretion, corneal ulceration, perforation of the ulcers, hypopyon, marked bulbar and ciliary injection, edema of the lids, loss of hair of lids, loss of lashes, and abscess of the anterior segment of the eyes.

At the end of the 8th week of the experiment, 19 animals were selected for histological studies of the eyes. They were divided into groups A and B. Those in group A were killed; their eyes were removed and prepared for histological examination. Those in group B were continued on the sodium-low diet. These rats died in the 20th week of the experiment, all showing the symptoms described above. The eyes of these animals were also studied histologically.

Microscopic examination of the group A animals showed corneal lesions with extensive anterior synechiae, also posterior synechiae and beginning capsular cataract. Wandering cells were present in the posterior chamber. In others, the corneal epithelium was thickened in places and showed areas of ulceration. The entire thickness of the cornea was infiltrated with wandering cells of all types and there was apparently perforation, for the iris was adherent to the center of the cornea in this region. There were some round cells on the surface of the iris in the posterior chamber. The lacrymal glands appeared to be normal.

The histologic examination of the rats in group B, after 20 weeks on the sodium-low diet showed the following changes: in a number there was intense infiltration throughout the cornea which had evidently been associated with perforation of ulcers inasmuch as the iris was adherent to the back of the cornea over its whole extent. The corneal epithelium was composed of thin basal cells which were flattened. The superficial two-fifths were keratinized; in certain animals there was intense purulent keratitis with perforated ulcer and hemorrhage into the anterior-chamber. In places the corneal epithelium was greatly thickened. Basal cells were irregular in pattern and the superficial layer was keratinized. There was a thick plaque of hyaline mate-

rial over the ulcer which contained colonies of bacteria. The sections of the control rats' eyes were all normal.

These eyes differed from those of vitamin A deficient rats in that there was no diminution in secretions of tears. In A deficient rats the eyes are dry owing to keratinization of epithelium in the lacrymal glands. In the sodium-low animals there was at least normal tear production and in some instances it appeared to be excessive.

In the earlier literature on xerophthalmia in vitamin A deficient rats, several observers called attention to ulceration of the cornea and hypopyon in animals in this deficient state. Wolbach and Howe ('25), who made the most thorough study of the pathology of this state, assert that they "have not a single example of ulceration of the cornea or of hypopyon" in their series of animals. It is apparent, therefore, that the eye disturbances of the rat induced by deprivation of sodium differ in almost all details from those caused by vitamin A deficiency.

When killed, the sodium-deficient rats appeared almost free from fat deposits, the muscles were atrophied, the liver a darker reddish brown than normal, and with some mottling. The spleen was very dark and greatly reduced in size. The kidneys were abnormally dark and showed occasional mottling. The adrenal glands were orange rather than pink in color. The testes appeared atrophic. The bladder was usually filled with urine and the stomach and intestines were distended with gas. All animals showed lung infections.

The bones of the sodium-deficient rats appeared retarded with respect to growth and they were fragile. There was a deficiency of cartilage and osteoid tissue.

A study of the estrual behavior showed that sodium-deficient rats matured about the 66th day of life as contrasted with the 55th day in the controls. Daily vaginal smears demonstrated that the experimental animals exhibited an essentially normal ovulatory performance during an average period of 14 days. Thereafter a characteristic disturbance was observed in the depleted animals which resembled that of vitamin A deficiency. There was prolongation of the estrus desquamation stage in the vaginal epithelium; the smears showed almost exclusively large, flaky, cornified cells which normally characterize the actual period of estrus and ovulation. In these animals this occurred throughout the entire depletion period. In addition to cornified cells there were present small amounts of mucus; in some cases excessive numbers of leucocytes; and in others, a few cornified, nucleated epithelial cells. Additions of vitamin A in the forms of butter fat and carotene respectively were tested as supplements to that already present in the diet, but repair of the vaginal epithelium did not occur following the administration of large doses of this vitamin.

Twelve sodium-depleted female rats, separately caged, were placed

during each working day with a stock male, during a ten-week period. Postmortem examinations showed no signs of pregnancy in the case of 8 animals; 1 contained a single fetus, the other contained 5 fetuses all in one uterine horn. One fetus weighed 4.5 gm., another 2.2 gm., and the remaining 3 had a collective weight of 1.7 gm. The single fetus in the other rat weighed 3.6 gm.

Sperm motility tests by means of testicular smears, gross examination of testes at autopsy, and mating tests, were made on a series of 12 males. Motility of sperm remained normal until about the 96th day when in 5 animals there was a decrease in motility. The epididymides appeared to be somewhat atrophied, and there seemed to be a decrease in their fluid content. Although the testes were smaller than those of the controls they were normal in proportion to body weight, and their appearance was essentially normal.

Six other males were used for mating tests using vigorous stock females on which vaginal smears were made regularly to determine their regularity of ovulation. It was shown that these males remained fertile for at least 75 to 80 days on the sodium-low diet.

In a study of the metabolism of sodium-deficient animals Schoorl ('34, l.c.) carried on protein balance experiments and reported that the decreased growth of rats and pigs fed a sodium-low diet is the result of an increased nitrogen metabolism. He found that the greater part of nitrogen was excreted in the form of urea. His sodium-deficient animals did not exhibit any signs of uraemia in spite of the increased production of urea. He concludes from his findings that sodium was needed in the diet to enable the organism to utilize the resorbed protein for building up body protein.

Sjollema ('35, l.c.) showed that the excreta of sodium-deficient chicks were rich in nitrogen and contained but traces of sodium. However, the birds were in positive sodium balance throughout the duration of the experiment.

Metabolism balance studies on the sodium-low diet devised in this laboratory are in progress now, using the paired feeding technic. Analyses of the urine and feces of the sodium-deprived rats during the first 6 weeks of the experimental period (18-21 weeks) show progressive changes in the phosphorus and nitrogen retention. The sodium-deficient rats begin to show negative balances early in the experiment and by the 6th week on the diet all of the experimental animals are in negative nitrogen balance. Thus the increased protein metabolism explains the decreased rate of growth of the rats deprived of sodium. The sodium excretion of the animals on this sodium-deficient diet is at a very low level from the first week of the experiment, being 2 to 7 mg. in contrast to 86-124 mg. of the controls. Hence, the sodium-deficient animals are consistently in nega-

tive sodium balance. In case of phosphorus, during the first week a positive balance is exhibited. At the end of the second week it is still positive but to a lesser degree. From the third week on negative balances begin to appear and by the end of the 5th week all the sodium-deprived rats are in negative phosphorus balance. The calcium, magnesium, potassium, and chlorine metabolism is at this time still not disturbed. Similar analyses of the excreta of control animals, i. e., those receiving the sodium-low diet supplemented with sodium, show no abnormal changes in the metabolism of these constituents.

Effects of Potassium Deficiency.—In their physico-chemical behavior sodium and potassium are practically alike, so far as the reactions in which they are known to take part are concerned. Why they play such different roles in the organism we have not the slightest idea. Thus, although it has long been recognized that potassium is essential for animal life, the effect of a deficiency of this element on animals is at present unknown. Very little work has been done on the study of the symptomatology of potassium deficiency, undoubtedly due partially to the difficulties in preparing a diet free from or low enough in potassium to furnish this element in smaller amounts than the minimum requirements.

Osborne and Mendel ('18, l.c.) restricted rats to a diet containing 0.033 per cent of potassium and otherwise adequate. The growth was retarded somewhat more than when sodium was reduced to 0.035 per cent but the animals continued to gain slowly for over 300 days. Like sodium, potassium is held tenaciously when the supply is very limited.

Miller ('23) reported a very slow growth and an abnormal alertness as the two principal symptoms of rats fed a purified diet low in potassium. Leulier and Vanhems ('34) found that young rats fail to grow, and die in 12 to 24 days when fed a diet deficient in potassium.

The effects of deficiency of potassium in rats have been described by Schrader, Prickett and Salmon ('37). Their potassium-deficient diet consisted of an extracted grain mixture supplemented with purified casein, cod liver oil, and a salt mixture free from potassium. The grain mixture was composed of yellow cornmeal and wheat middlings and was extracted with 0.2 per cent acetic acid for 6 days and with distilled water for 1 day. In certain experiments the diet was supplemented with 10 mg. of a fuller's earth adsorbate, found by pigeon assay to contain 3 I. U. of thiamin. Their records indicate that this addition was without effect on weight changes. On this diet they observed that young rats made no growth and the average loss of weight during the average duration of life, which was 23 days, was 10 per cent. They describe the symptoms as early leth-

argy, with progressive increase of abdominal distention; pale skin with cyanosis; and pendulous sacs in the region of the thyroid gland and sternum. At necropsy severe ascites was usually present, and in a few cases there was hydrothorax and hydropericardium. Atony and transluscence characterized the intestine, and in the ileum there were thickened annular areas about Peyer's patches, showing congestion. Extensive pathological changes in other tissues were also described.

Unfortunately the diets of these different workers are obviously not adequate in the other dietary essentials aside from potassium. The diet of the last mentioned group was particularly unsatisfactory; therefore it cannot be accepted that the observations recorded by these investigators are actually due to potassium depletion of the animal organism.

Orent-Keiles and McCollum ('38) have observed the effects of deprivation of potassium in rats. Their results are so completely at variance with those of Schrader and associates (l.c.) that it is concluded that the latter did not produce in their animals a state of potassium starvation alone but a syndrome caused by deficiency of some dietary factors other than this element. Orent-Keiles employed a diet consisting of purified lactalbumin, wheat gluten, gelatin, salt mixture, sweet butter fat, dextrose, the vitamin B complex, viosterol and vitamin E concentrate.

With the exception of potassium, this diet was complete in all other respects as shown by the fact that in rats on the experimental diet plus potassium essentially normal growth occurred, ranging from initial weights of 35-40 gm. to 240-288 gm. at 8½ months, after which increment of growth became slower. These control rats have reproduced and successfully weaned several litters of young. Such effects as were observed in the experimental group are, therefore, to be attributed solely to potassium deprivation.

Young rats on the potassium-low diet grew very slowly but steadily and at 8½ months were still increasing 1 to 4 gm. per week. This result is comparable to but more pronounced than that of Osborne and Mendel ('18, l.c.) whose diet contained 0.033 per cent of potassium, or about 2.5 times the content of this element provided by the Orent-Keiles diet. In the potassium-low animals the time of sexual maturity and ovulation was retarded. The vaginal orifice was dry and the insertion of a thin glass rod was difficult even after the vagina had been open for some time. There was disturbance of the estrual rhythm. When mated with stock males no impregnations occurred. Control females produced and raised several litters of young. In the males, testicular smears showed that the reproductive system was affected. The motility of the sperm cells was reduced markedly and many spermatids were present. Later the smears showed dead

sperm and spermatids. Males, when mated with normal females, were found incapable of fertilizing them. In both sexes the fur was thin and somewhat rough. The animals were extremely alert and active, exhibiting an unrest which was strikingly apparent. They also manifested a form of pica which caused them to almost constantly search about the cage and lick various parts of the cage and its equipment; to lick each other, particularly their genital orifices, especially after urination.

The rate and rhythm of the heart beat in potassium-low rats was investigated by means of the electrocardiograph. The potassium-deficient rats show a slowing in the heart rate and irregularity in the rhythm.

Preliminary histological studies of rats deprived of potassium show that there is a characteristic scarring of the heart muscle, the kidneys increase in weight, and the bones are fragile and retarded in growth.

The probability of a deficiency of potassium in the human or animal diet under normal conditions is extremely remote, for the ubiquitous occurrence of this element in plant and animal tissues used as foodstuffs and the small amount of the mineral required, guarantee against a deficit of this base.

However, in the light of the observations recorded, the treatment of patients with Addison's disease by the present practice of restricting them to a diet low in potassium, especially over a prolonged period of time, may be fraught with some hazard. As yet no data are available on which to base judgment as to the minimum intake of this element by man which is consistent with safety, but caution may be advisable in planning low potassium diets to be used over long periods of time.

Effects of Chlorine and Sodium Chloride Deficiency.—Experiments dealing with the depletion of the organism of the chlorine ion alone are but few. Rosemann ('11) observed that when the diet is deficient in chlorides there is practically no depletion of the body content of chlorine (chlorine ions) since excretion decreases at once and practically ceases. Osborne and Mendel ('18, l.c.) fed to rats a diet containing but 0.035 per cent of chlorine. Starting at a body weight of 90 gm., one of the rats was but little retarded in growth until it reached a weight of 220 gm. Thereafter it continued to grow more slowly to a weight of nearly 300 gm. From then on it grew even more slowly. However, it was still able to respond with rapid growth when placed on a normal diet. They noted that it could not have been expected that on a diet so low in chlorine the rats should be able to continue to thrive so long or to attain so many times their original weight. This study showed that animals receiv-

ing but extremely small amounts of chlorine husband practically all of the quantity ingested.

Orent-Keiles, Robinson and McCollum ('37, l.c.) restricted rats to a diet practically free from chlorine and found that they were somewhat retarded in growth, but showed no other symptoms during a 90-day period. Animals on a diet deficient in both sodium and chlorine grew more slowly than did those deficient in chlorine only. After the third week rats deprived of sodium chloride became denuded of hair about the back and shoulders. They appeared very apprehensive. At the end of the first month the mouth, ears, nose, and forepaws were bloody and the urine was dark brown in color. Denudation increased as the experiment proceeded but was restricted to the anterio-dorsal parts of the body. The sodium chloride-depleted rats showed evidence of internal hemorrhages in the thymus and liver. The testes were small in proportion to the size of the animals but otherwise appeared normal. The adrenals were smaller than those of the controls. The bones were not fragile as were those of sodium-depleted rats.

The only circumstances under which normal human subjects are observed to suffer a deficiency of sodium chloride are where they are exposed to the excessively high temperatures and low humidity as in furnace rooms and mines. Men working under these conditions lose salt to an extent which causes "heat cramps" or "bends." This condition is prevented by drinking water containing sodium chloride. Excessive amounts of sodium chloride and sodium bicarbonate may also be lost through diarrhea.

The Role of Sodium and Potassium in Carbohydrate Metabolism.—Chaikelis ('34) showed experimentally that there is an inverse ratio between blood chlorides and blood sugar, and that this is not a compensatory mechanism concerned with the maintenance of normal osmotic relationship. He expressed the view that this change in blood chloride is associated with some phase of carbohydrate metabolism. Crabtree and Longwell ('36) have studied this problem further, and found that when young rats were fed the Osborne and Mendel ('18, l.c.), "chloride-free" diet, there was no effect on the liver glycogen stores. With the same diet fortified with 6.25 per cent of sodium chloride, and distilled water *ad libitum,* the glycogen content was about one-third higher than in the "chloride-free" livers. There was no significant difference in the muscle glycogen in the two groups.

These observations are of special interest in connection with the studies of McQuarrie and associates ('36) on the craving of young diabetic subjects for salt (NaCl) and the influence of excessive salt ingestion on carbohydrate metabolism. The daily ingestion of 1 to 2 gm. of sodium chloride per kilogram of body weight resulted

within 2 to 4 days in a gain of 4 to 5 per cent in weight and in increase in both systolic and diastolic blood pressure to new plateaus between 30 and 40 per cent above the control levels. These higher pressures were maintained so long as the salt was taken. In addition to these there was marked reduction in the degree of glycosuria. The effects were qualitatively the same in one mildly diabetic patient not receiving insulin, as in diabetic patients who received insulin at 6 hour intervals. Other conditions being unchanged, the fasting blood sugar remained at lower levels after a few days of a high sodium chloride intake. In severe diabetes, ketosis appeared to develop earlier after the withdrawal of insulin when the sodium chloride intake was low than during the periods of high intake.

Patients maintained on a constant protein intake estimated to maintain nitrogen balance were found to be in negative nitrogen balance during periods of low salt feeding, but retained nitrogen when the sodium chloride intake was high. Typical insulin reactions were at times observed during periods of high sodium chloride intake in patients receiving insulin in doses sufficient to prevent glycosuria during low-salt periods.

Sodium bicarbonate and sodium citrate, when given in amounts equivalent to the sodium values, in cases of sodium chloride administration, had similar though less marked effects than did sodium chloride. The maximum effects of sodium chloride on both blood pressure and carbohydrate metabolism were obtained only when the patients were given simplified diets low in potassium. Ordinary diets high in potassium either prevented or greatly lessened the effects of the sodium chloride. When potassium chloride was given in doses of 10 to 20 gm. daily along with the therapeutic diet low in sodium chloride, the blood pressure was lowered, and at times a significant increase in the glycosuria recurred. In these respects, therefore, there is shown a physiological antagonism between sodium and potassium, comparable with that which has long been known in relation to muscle irritability and contractility. In the studies of McQuarrie it appears that one part of potassium counteracts the effect of at least three equivalent parts of sodium. High sodium chloride intake, he found, depresses significantly the potassium content of blood serum. In view of these observations, it is thought that a low-sodium, high-potassium regime might prove to be beneficial in cases of spontaneous hypoglycemia.

Relation of Sodium and Potassium to Adrenal Function.—Of particular interest are the recent observations on the relation of the cortical hormone of the adrenals to sodium and potassium metabolism. The most striking feature of experimental and clinical adrenal insufficiency and one for which an explanation at this time is unavail-

able, is the metabolism of sodium and potassium. During the course of the syndrome the sodium of the blood is lowered and the potassium is increased. The condition may be corrected by the administration of sodium salts and cortin singly or in combination. However, it has been found difficult to maintain adrenalectomized animals, and patients who have Addison's disease, in an entirely normal condition and with a normal blood electrolyte pattern by supplying sodium salts alone. The increases in the serum potassium in these cases are not necessarily parallel to the degree of depletion of sodium and may at times be present even when other electrolytes of the blood have been returned to normal by the administration of sodium salts. Allers ('35) and Kendall ('35) were the first to show that adrenalectomized animals could be thrown into crises of adrenal insufficiency by the addition of potassium to their diet, even while adequate supplies of sodium salts were provided, and conversely, that a low intake of potassium in the diet was of great importance in maintaining such animals in optimal condition with normal blood electrolytes. Their studies have been confirmed and extended by Zwemer and Truszkowski ('36) who also have demonstrated the extreme sensitivity of adrenalectomized animals to potassium salts. These investigators have produced the symptoms of adrenal insufficiency in normal animals by raising the plasma potassium by means of repeated intraperitoneal injection of potassium chloride. Such studies seem to indicate that the role of cortical hormone depends essentially on its function in regulating the metabolism of sodium and potassium.

Loss of sodium in the adrenalectomized animal or in case of Addison's disease is accounted for by Vérzar and Laszt ('36) on the basis of inhibition of glucose absorption due to lack of cortical hormone. The sugar remains in the intestine, causing a transfer of water from the tissues by osmosis, and of sodium by diffusion. When sodium salts are included in the diet this diffusion is prevented.

Observations on patients with Addison's disease demonstrated that a low intake of potassium will protect the patient to a considerable extent against withdrawal of sodium salts. A high intake of sodium alone does not suffice to prevent a crisis of adrenal insufficiency. In the therapy of Addison's disease it is of greater importance to reduce the potassium in the diet than to administer high doses of sodium chloride and the best results are obtained if both of these measures are used. When such a regime is used, the patients require less cortin and sodium salt and are less subject to relapse.

The preparation of a palatable potassium-low diet (Wilder, et al., '36) requires the same careful planning as does that of a diet for a diabetic patient. The potassium values of foods, in percentage of the edible portion, that are available at present were compiled from

various sources and for this reason are not the most satisfactory, for there is a considerable variation in the potassium content of foodstuffs obtained from different regions due to the soil composition, fertilizer treatment, etc.

Relation of Potassium to Familial Paralysis.—The effect of potassium on certain types of paralysis is of interest. During the past year several reports have been recorded on the control of familial periodic paralysis with potassium salts. Familial periodic paralysis is a rare disease characterized by periodic abrupt attacks of flaccid paralysis, without sensory loss or psychic disturbances of any sort and from which recovery is complete. In about 80 per cent of the cases the disease is hereditary. It is transmitted by both sexes, but males are affected twice as frequently as females. The etiology of the disease is unknown. The attacks begin in most cases at puberty. The muscles of the limbs are chiefly affected, but in severe cases the abdominal and respiratory muscles are also attacked. The paralysis lasts from a few hours to a few days, or even longer. The attacks vary in frequency, occurring every few days or at intervals of months or years. Analyses of the blood during and between the paralytic attacks showed that the onset of paralysis was associated with a fall of serum potassium from the normal value of about 19 mg. to below 12 mg. per 100 cc. serum. The paralysis can be relieved in 15 minutes by the ingestion of potassium salts such as potassium chloride or potassium citrate (Mitchell and associates, '02; Aitkin, et al., '37; Herrington, '37; Gammon, '38).

Gammon (l.c.) observed no excessive elimination of potassium by the kidneys preceding the seizures. At the time of the attack the absorption of potassium from the gut was quite low. Administration of potassium salts prevented the development of seizures or prevented them from occurring as frequently or in as severe a form. This investigator concludes that such patients have an unusual need for potassium salts to maintain contractility of certain of their muscles.

Other bases involved in muscle metabolism such as sodium chloride, sodium bicarbonate, creatine hydrate, and prostigmin are ineffective.

These observations, suggesting a relationship of disturbed metabolism to this disease, are of interest for they promise to illuminate this hitherto obscure malady.

Relation of Potassium to Intestinal Stasis.—Robertson ('37) after observing that rats restricted to a diet low in mineral elements suffered intestinal stasis accompanied by dilatation and overloading of the large intestine with fecal matter, made a detailed investigation of the effect of inorganic salts on intestinal elimination. She found that stasis could be cured by supplying the missing elements. The

condition is relieved, or prevented, by supplying in the diet appropriate amounts of calcium and potassium. The contents of the large intestine is definitely more acid in stasis due to deficiency of these elements than in the controls receiving calcium and potassium. In a series of 19 children who were fed diets low in these elements, 14 showed constipation. Her studies suggest that the modern human diet, consisting to a large extent of refined cereal products and sugar, and providing far less total mineral elements than were contained in diets before the advent of modern methods of milling and of the manufacture of sugar, may be in some measure responsible for the prevalence of constipation.

Dietary Requirements of Sodium, Potassium, and Chlorine.— Very little information is available concerning the requirements of man and animals for potassium, sodium, chlorine, or sodium chloride.

From the growth and general physiological behavior of cattle subsisting upon rations of varying mineral content, South African investigators (Theiler, Green and DuToit, '27) have concluded that the requirements of growing cattle for sodium and chlorine are low, about 1.5 gm. daily of sodium and less than 5 gm. of chlorine. During lactation the sodium requirement of a cow (DuToit, et al., '34) is 11 gm. per day, and 15 gm. of chlorine daily suffices for the same animal at this time.

In the case of poultry 0.5 per cent sodium chloride in the diet appears to meet the requirements of either the growing chick, or the laying hen judged by growth, egg production, and mortality (Halpin, et al., '36).

It is of interest to note that the character and composition of the rations fed affect in large measure the sodium chloride consumption and requirements of fattening lambs (Evvard and associates, '26). Feeding beet molasses markedly decreased salt consumption, whereas alfalfa had the opposite effect. Also, the greater the proportion of roughage in the ration, the larger the sodium chloride consumption.

Man consumes 3-6 gm. of sodium in the ordinary daily diet, the greater part of which is supplied by the sodium chloride added as seasoning.

With regard to the potassium requirement, 25 grams of potassium daily seems to be more than adequate for calves through growth and gestation, while 32 gm. suffice during the period of lactation (DuToit and associates, '34, l.c.).

The ordinary diet of man furnishes 2-4 gm. of potassium which comes from the various animal and vegetable tissues used as food. It is thought that 0.06 gm. of potassium per kilo body weight suffices for the normal adult, but that the nursing child requires 0.07 gm. per kilo body weight for its growth. In early life the rapid growth

of the tissues requires potassium, and much of the potassium ingested in the food is retained. After the age of 12 years the retention gradually grows less and only that amount which may be regarded as more or less the normal requirement is held by the cells. However, exact data on the minimum physiological intake of potassium, as far as man is concerned, are not very definite.

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CHAPTER

IX

Iron, Copper, and Nutritional Anemias

IN ALL THE higher forms of life iron is an integral constituent of heme, also called hematin, the prosthetic group of various complex pigments required in the transfer of oxygen. The most commonly known of these substances is hemoglobin, but myohemoglobin and cytochrome are other important examples. It is estimated that the circulating hemoglobin of a normal adult man contains about 2-2.5 gm. of iron. Copper has not been demonstrated to occur in any specific combinations in the higher forms of life but in recent years it has been shown that the element is required to promote the utilization of iron in hemoglobin formation, probably as a catalyst. Lower organisms such as Mollusca and Arthropoda have a copper-containing respiratory pigment, hemocyanin, which acts as an oxygen carrier in a manner analogous to that of hemoglobin. Hemocyanin contains about 0.34-0.35 per cent copper. It is significant that this figure is practically identical with the value for iron in hemoglobin, about 0.34 per cent. Since these elements occupy integral roles in cellular respiration it is natural that nearly all reference to them in relation to nutrition concerns anemia and blood regeneration.

Anemia may be regarded as due to loss or abnormal destruction of blood, or because of deficient blood formation. Anemias of nutritional origin are in the category of deficient blood formation, owing to an inadequate intake of structural components or activators in hematopoiesis, or deficient utilization of such nutrients. There is, of course, no clear line of distinction between all the so-called nutritional anemias and those attributable to distinctly different causes such as acute blood loss, acute blood destruction, and injury to blood-forming organs by metastatic carcinoma, chemicals, infections, and physical agents.

It is intended in this chapter to review some of the pertinent facts and opinions concerning the relationships of dietary iron and copper to blood formation and anemia prevention, the factors affecting the utilization of these elements, their metabolism, and human and animal requirements for iron and copper. In addition, the relation of diet to the etiology and treatment of pernicious anemia will be briefly discussed.

Early Views Regarding the Role of Iron in Blood Formation.— Hirsch (1885) gives an interesting historical account of references in Ancient and Mediaeval writings to pallor or a "yellow tint" of the skin as symptoms of illness and conjectures that these terms signify chlorosis. According to Hirsch, Sennert in 1760, was the first to employ this term, meaning, in the modern sense, a form of anemia most common in young women and characterized by a marked reduction in hemoglobin and a slight diminution of red corpuscles. Also, Hirsch states, there can be no doubt that chlorosis has been among the subjects of medical observation at all times and that it is independent of climate, soil, and race. He records that the "general anemia" of the tropics is principally the consequence of deficient food, or a diet poor in animal substances, and that malaria contributes to it. The discourse is limited to chlorosis, general anemia of the tropics, and "mountain sickness."

As stated by Robscheit-Robbins ('29), in an excellent review of the literature on blood regeneration, iron has been regarded as an important factor in hematopoiesis since Menghini, in 1747, discovered this element in blood. But it appears that even ancient civilizations held iron-containing waters in high esteem. Robscheit-Robbins points out that the main interest of iron studies between 1850 and 1905 centered on absorption and elimination of the element. But those studies resulted in scarcely any worthwhile contributions to the role of iron in preventing anemia. Bunge (1889) is generally regarded as the originator of the present view that iron deficiency is the principal cause of anemia. Unfortunately he was led to believe that only the iron contained in foods (organic iron) could be utilized in blood formation. This view was shared for some time by Abderhalden (1900), Bunge's pupil. These two workers have the distinction of being the first to record that prolonged restriction of animals to milk diets causes anemia. Eventually Abderhalden, at the beginning of the century, found that inorganic sources of iron are effective in blood formation.

After the demonstration by many investigators that inorganic salts of iron could be absorbed and utilized for hemoglobin regeneration, the puzzling observation continued to be made that the administra-

tion of iron compounds failed, in many instances, to bring about relief from anemia. Eventually it was realized that there are various types of anemia having origin in different causes. As early as 1843 Andral (1843) first described the blood in "spontaneous" and "secondary" anemia. In the same decade Addison (1849) characterized pernicious anemia—and initiated the study of endocrinology. Simple hypochromic anemia was described by Faber in 1913. As distinguished from pernicious anemia, there are now recognized certain so-called secondary anemias. The most common cause of anemia is deficiency of iron for the construction of hemoglobin. This is due to the lack of sufficient available (utilizable) iron in the diet, or to conditions in the alimentary tract which are inimical to its absorption. Anemias which accompany hookworm infestation are due to chronic blood loss and the inability of the body to restore the loss, often due in part to inadequate intake of iron and other essential nutrients. The anemia of malaria is also due to blood destruction. Nephritis, rickets, scurvy, myxedema, malignancies, and several other conditions usually are accompanied by anemias. We will not attempt to classify them here. The endeavor to learn the relationships between these apparently different causes of anemia occasioned an enormous amount of research and the accumulation of many detailed descriptions of the blood picture in anemic individuals. The results of these various investigations are among the great achievements in experimental physiology.

The Discovery of the Indispensability of Copper in Hematopoiesis.—It has been shown by Bunge (l.c.) that milk is deficient in iron and that in the newborn young, with the exception of the guinea pig, there are stores of iron in the liver and spleen sufficient to tide them over the suckling period. The guinea pig is so far developed at birth that it is able to secure iron in the succulent vegetables which it begins to eat within a short time after it is born; hence it does not need a reserve supply of iron. Following Abderhalden's (l.c.) demonstration that inorganic salts of iron cause hemoglobin formation in animals restricted to a milk diet, the problem was not further investigated critically until Hart and his associates took it up in 1925 (Hart, Steenbock, Elvehjem, Waddell, and others, '25, '28).

In their first study they showed that rabbits suffered a marked reduction in hemoglobin and red cells when limited to whole milk supplemented with sodium citrate (added to soften curds formed in the stomach). Ferric oxide added to this diet did not correct the anemia. But ferric oxide together with fresh cabbage, or an alcoholic extract of desiccated cabbage, or of yellow corn meal, cured the anemia although these extracts were free from iron. Chlorophyll, free from iron, had a similar beneficial effect on hematopoiesis.

In later studies Hart and coworkers (1928) employed rats since these animals also developed anemia when restricted to the milk diet. The condition was not cured by the daily administration of 0.5 mg. of iron as pure chloride, sulfate, citrate, acetate, or phosphate. But the anemic condition was quickly alleviated when the same dosage of iron was administered in the form of the ash of dried beef liver, lettuce, yellow corn, or as acid extracts of these ashes. This inevitably led to the conclusion that their effectiveness was due to some inorganic element in addition to iron. The precipitate obtained by treatment of the ash solution with hydrogen sulfide was considerably more potent than the original material. Eventually it was demonstrated that the active substance is copper, and that without it iron cannot be utilized in hemoglobin formation.

Controversies Regarding the Significance of Copper.—Following this fundamental discovery various investigators became actively interested and a controversy soon ensued over whether copper can be replaced by certain other elements in hematopoiesis. Titus, Cave and Hughes ('28) asserted that manganese could replace copper in hematopoiesis but further work has failed to substantiate the claim (Krause, '31). Myers, Beard and coworkers ('29) were the most aggressive proponents of the view that iron alone was sufficient to induce hemoglobin regeneration under the experimental conditions employed by Hart and associates. They claimed, however, that the supplementation of milk diets with manganese, nickel, germanium, and arsenic, could, like copper, increase the rate of hematopoiesis. Mitchell and Miller ('29) also expressed the belief that a group of elements, rather than copper alone, could function in hemoglobin building. Some clarification of the problem was furnished by Keil and Nelson ('31) who found that pure ferric chloride alone promoted hematopoiesis when the milk employed was not carefully protected from metallic contamination. When the milk was collected in glass, ferric chloride did not cause improvement in the blood. After testing vanadium, titanium, manganese, nickel, arsenic, germanium, zinc, chromium, cobalt, tin, and mercury, they concluded that copper is the only element which must be provided along with iron to cure or prevent anemia produced by Abderhalden's procedure, the restriction of animals to whole milk diets. Others (Underhill, Orten and Lewis, '31; Cunningham, '31) have fully demonstrated that copper is the unique element necessary for iron utilization in hemoglobin production.

In retrospect it is not difficult to understand why the results of different investigators were in disagreement. As shown by Sheldon and Ramage ('32), copper was absent in only one out of five therapeutic preparations of iron examined by the spectrograph, and manganese was a constant impurity. Also Davies ('33) found the natural

copper content of milk to vary between 0.30 and 0.75 p.p.m. Obviously, therefore, it was difficult for investigators to be certain that their mineral supplements were pure and that the milk was comparable in copper content. The entire subject has been reviewed by Elvehjem ('35).

Cobalt Polycythemia.—Although cobalt apparently plays a significant physiological role in nutrition (Chapt. XI) particularly in hematopoiesis, there is no clear evidence regarding its function. The administration of only 0.5 mg. of this element as the chloride or sulfate produces a polycythemic state in young rats (Orten, Underhill, Mugrage, and Lewis, '32). The effect was first noted by Waltner and Waltner ('29). As shown by Orten ('36), cobalt administered to young or adult rats causes a reticulocytosis about the 4th day and a definite polycythemia by the end of 6 weeks. He believes that the polycythemic state is due to an increase in the rate of hemoglobin and red cell formation and not to diminished red cell destruction. This opinion is supported by the data of Barron and Barron ('36) on the basis of red cell respiration studies. The latter workers believe that cobalt depresses the respiration of immature cells in the bone marrow, thus causing them to be thrown into circulation. The result is an excess of circulating erythrocytes.

Goat's Milk Anemia.—The so-called "goat's milk anemia" has been studied by several investigators. Rominger and associates ('33), as well as others, claimed that it is not curable by iron and copper, or vitamin A and ascorbic acid. Since liver extract, but not gastric mucosa, was said to be effective they postulated that the alleged goat's milk anemia was due to a deficiency of Castle's "extrinsic" factor, a hypothetical substance necessary in the prevention of pernicious anemia. But at present there is little credence in the view that this form of anemia is essentially different from the simple type produced by restriction of animals to cow's milk diets (Kohler, Elvehjem and Hart, '35; Beard and Boggess, '35; Orten and Smith, '36). Since Kohler and associates have shown that the growth-promoting property of goat's milk is low, unless the lactating animals have a good diet, it is possible that the confusion in this matter had its origin in the use of milk from poorly fed goats. The subject has been reviewed by Orten and Smith.

Dietary Factors Other than Iron and Copper in Hematopoiesis.—The illuminating researches of Whipple and Robscheit-Robbins (Robscheit-Robbins, l.c.) showed that the character of the diet, as respects other factors than the iron content (exclusive of copper), is of extraordinary significance in determining the capacity of the body to regenerate hemoglobin. Their method was based principally on the removal of measured amounts of red blood cells from experimental

dogs and observation of the effect of specific foods on the regeneration of hemoglobin. The routine experiments included the determination of plasma volume, hemoglobin, and erythrocyte and leucocyte counts on blood withdrawn by venipuncture. The following table shows the influence of several foods on hemoglobin production:

HEMOGLOBIN PRODUCTION INFLUENCED BY DIET

Diet, Grams Daily	*Hemoglobin Production (Two-week Feeding Period) grams*
Bread, 400	3
Milk 450, Bread 400	3
Cream 100, Bread 400	10
Butter 100, Bread 350	15
Asparagus 200, Bread 300	9
Spinach 200, Bread 300	15
Raspberries 200, Bread 300	5
Raisins 200, Bread 300	25
Apricots 200, Bread 300	48
Eggs 150, Bread 300	45
Whole fish 250, Bread 300	13
Beef muscle 250, Bread 300	17
Pig muscle 250, Bread 300	30
Chicken gizzard 250, Bread 200	80
Kidney 250, Bread 300	70
Chicken liver 250, Bread 300	80
Beef liver 300, Bread 300	80
Beef liver 450	95

The precise basis for differences in the value of these foods has not been determined. Obviously it was not entirely attributable to differences in the iron content since raspberries have a higher iron value than apricots, yet the former were practically inert. The potency of liver and kidney was regarded as due to the presence in the parenchyma of a large amount of potential red cell and hemoglobin building material other than iron. These data attracted much attention and owing to the high potency of liver, Minot and Castle began to investigate the efficacy of this food in the treatment of pernicious anemia. That it is remarkably effective was soon learned. A discussion of the subject will be given subsequently.

It is of particular interest that Hogan and Ritchie ('34) have observed an anemia in rats caused by the ingestion of deaminized casein. The evidence suggests that casein contains a chemical group which overcomes the toxic action of deaminized casein and which occurs in very small amounts, if at all, in gelatin and gliadin (Chap. VI). As shown by Sebrell ('30), onions, either cooked or raw, when given in quantities of 15 gm. or over, per kilogram of body weight, produce a severe anemia in dogs. Gruhzit's ('31) data suggest that the effect is caused by certain disulfide compounds which occur in

onions. It has not been demonstrated that anemia is caused in humans by the ingestion of onions.

Several investigators have observed anemia in animals on diets apparently deficient in one or more components of the vitamin B-complex. Miller and Rhoads ('32-33) claimed that dogs on a vitamin "G" deficient diet developed marked anemia although the diet contained iron. This was not confirmed by Stucky and Brand ('32-33). P. L. Day, Langston and Shukers ('35) have reported that severe anemia and leukopenia, with ulceration of the gums, occur in monkeys restricted to a diet of casein, whole ground wheat, polished rice, cod liver oil, salt mixture, and oranges. Since the syndrome could be prevented by supplementation with dried brewer's yeast, the evidence suggests the involvement of some component of the vitamin B-complex in blood formation. Suzman and associates ('32), while studying the effect of vitamin A deficiency on the spinal cord, using dogs, employed a diet consisting of 76.8 per cent of rolled oats supplemented with sugar, lard, bone ash, a salt mixture, and calciferol (vitamin D). Anemia developed on this diet, irrespective of whether the animals were given vitamin A. Loss of weight, dermatitis, skin ulcers, and alteration in the concentration of blood lipids also occurred. The diet was deficient in some components of the vitamin B-complex. This was suggested as the cause of the anemia since iron was ineffective in relieving the condition. Various other evidences might be cited of the complexity of nutritional factors in blood formation.

Absorption of Iron.—Iron is almost entirely excreted by the intestine. This fact makes it difficult to study iron absorption quantitatively, since it is not now possible to differentiate between "ingoing" and "outgoing" iron. According to Witts ('36), iron is absorbed chiefly by the duodenum, but the stomach and the whole of the small intestine may take part. Schmidt ('28) depleted mice of iron by restricting them during a period of 3-4 generations to a diet of milk and rice. The iron content of the intestine could not be made to entirely disappear, but was reduced to a very low minimum. There were no detectable stores of the element in the tissues. He then fed iron compounds to these animals and made histological studies of the intestines. It was found that iron is absorbed by the tips of the villi of parts of the duodenum and appears in the underlying cells, apparently already combined with preformed cell granules. Absorption took place both through the portal circulation and the lymphatics, and iron was carried thence to the liver and the mesenteric lymph glands. The iron which promptly appeared in the liver was diffusely distributed, but at this time there was none demonstrable in the spleen. Siderosis was not evident in the spleen until the hemoglobin content of the blood had returned to approximately normal. From this it

appears that visible iron deposits in the spleen and liver represent degradation products of hemoglobin. Freshly absorbed iron, which is presumably stored in the liver, stains diffusely, or is present in colorless granules.

There is general agreement that iron, to be absorbable, must be soluble, ionizable, and ultrafilterable. Such forms are reduced iron salts soluble in acid solution, and the hydroxides of iron, which are readily changed to salts by acids. Hematin iron, and such complex ions as ferro- and ferricyanide, do not pass through the intestinal wall. Lintzel ('31) has shown that hematin iron is not liberated by even prolonged peptic digestion.

Availability of Iron.—It has been thoroughly demonstrated in various laboratories that inorganic salts of iron can be used as the sole source of iron for the cure of iron-deficiency anemia in rats, mice, chickens, and pigs (Elvehjem, '32). It is concluded therefore that the body's need for iron can be entirely satisfied by inorganic iron compounds. Since organic forms of iron, unless they are readily digested, tend to lack the properties required for absorption, it is evident that the inorganic forms are generally superior. Unfortunately there is no generally satisfactory method of determining the form in which iron exists in foodstuffs. However, the method devised by Hill ('30) has been of much use as a means of estimating chemically the approximate availability of iron in various materials. Hill's method is based on the use of $\alpha\alpha'$-dipyridyl, a reagent which differentiates between ionizable and non-ionizable iron. Since there seems to be a fair correspondence between the "biologically" available iron and the iron determined by Hill's reagent, the chemical method has been used in various investigations of iron in its relation to nutrition. Sherman, Elvehjem and Hart ('34a) employed the reagent in an investigation of the available iron in several foodstuffs. According to their findings the fraction of the total which is available in wheat, oats, and yeast is 47, 57, and 47 per cent respectively. Beef skeletal muscle has an availability of 50 per cent, while that of oysters, spinach, alfalfa, and blood is 25 per cent or less. These workers claim that hemoglobin regeneration tests of the above materials agreed with the values furnished by the dipyridyl reagent. But the general reliability of $\alpha\alpha'$-dipyridyl has been denied by a few workers. Rose and associates ('34) and Free and Bing ('36), employing biological assay methods, claim that wheat, for example, contains a much higher percentage of available iron than is indicated by means of this chemical procedure. As Borgen and Elvehjem ('37) have shown with further evidence, the dipyridyl method, in general, does give a good indication of the availability of iron in various substances. The content of available iron in foodstuffs will be given subsequently.

Hill's reagent is extremely valuable in investigations of biological problems relating to iron. The compound combines with ferrous but not ferric iron at pH 3.5 to 8.5, forming an intense red complex. Ferric iron can be determined after reduction, sodium hydrosulfite generally being employed. It has been shown by Lintzel ('31, l.c.) that ferric iron, but not ferrous iron, readily forms complexes with proteins and sugars. This is important since ferric salts would tend to combine with mucus and undigested protein in the digestive tract, thereby becoming unabsorbable. But in the alimentary tract the oxygen tension is low and there are present many easily oxidizable substances, a situation which would tend to reduce ferric to ferrous iron, in which form it would become ionizable and absorbable. This view is supported by the observation of Lintzel ('33) that anemia can be produced in rats by feeding them dipyridyl while on a diet which normally would maintain adequate hematopoiesis. If they had been able to absorb ferric iron, anemia should not have occurred. However, this does not suggest that ferrous iron should be taken in preference to the ferric form. As we have indicated, ferric iron is readily reduced to the ferrous state in the gastrointestinal tract.

Ferric iron evidently becomes available only when hydrochloric acid is present in the stomach since in achlorhydria anemia occurs. It appears that hydrochloric acid is important for the solution and reduction of food iron in preparation for its absorption. In achlorhydria there may be a failure to liberate iron from protein thus preventing absorption of the iron. Organic acids in the food influence the solubility of iron and the amount of iron in solution in the stomach is greater than can be accounted for on the basis of pH, owing to the presence of lactic acid.

The Ca:P Ratio in Iron Utilization.—That other factors affecting the solubility of iron in the gastrointestinal tract play important roles in the utilization of iron is made evident by the studies of Orten, Smith and Mendel ('36), Day and Stein ('38), and others. The former showed that when rats were fed a diet qualitatively complete but low in inorganic salts, there occurred an increase in the number of erythrocytes per unit volume of blood and a simultaneous decrease in the concentration of hemoglobin. The diet was deficient in calcium and to a lesser extent in sodium, potassium, magnesium, chloride, and iron. Since the diet contained 18 per cent of the phosphoprotein, casein, the phosphorus content was relatively high. Orten and associates were able to prevent the abnormal blood picture by the provision of calcium carbonate. Since small amounts of ferric chloride were only partially effective they postulated that there exists some subtle relationship between calcium and the utilization of iron in blood formation. Recognizing that excess of dietary iron causes a marked loss of phos-

phorus from the body as shown by Brock and Diamond ('34), Deobald and Elvehjem ('35), and others, Day and Stein believed that an excess of phosphorus would cause the loss of iron, thus accounting for the effects observed by Orten et al. They investigated the subject and found that calcium does not have a unique function in hematopoiesis. When the low-mineral ration was supplemented with calcium carbonate the results of Orten and associates were confirmed but the same amount of calcium, furnished as the diphosphate, was totally without effect. Moreover, supplementation of the diet with generous amounts of ferric chloride, so as to remove most of the excess phosphorus, as iron phosphate, produced a normal blood picture while the same amount of iron as ferric phosphate was without effect. The addition to the diet of beryllium carbonate, which produces an insoluble phosphate, likewise prevented abnormal hematopoiesis. Finally, by means of a basal ration adequate in all respects it was possible to produce mild anemia and polycythemia by adding an excess of phosphorus or by the reduction of calcium to a low level. It was concluded, therefore, that neither calcium deficiency nor an extremely low Ca: P ratio are incompatible with normal hematopoiesis, if the diet contains some factor capable of preventing phosphorus from combination with iron. They stated, however, that calcium is the only cation that is of practical value in this respect and that to assure efficient utilization of iron attention should be given to the provision of an adequate Ca: P ratio. Thus it appears that the mechanism whereby calcium "spares" iron has been demonstrated. Von Wendt (1905) and Sherman (1907) first announced this relationship and as shown clinically by Davidson and associates ('33), a normal blood picture can be maintained on a diet rich in calcium and moderate in iron, but anemia occurs on the same iron intake if the calcium is considerably reduced. It would seem that the clinical significance of this relationship should be further investigated.

Vitamins in Utilization of Iron.—Although, in general, there is no more similarity between vitamins as respects their physiological roles than there is between minerals, for example, it is important to note that anemia accompanies deficiencies of vitamin A and ascorbic acid. When the Ca: P ratio is unfavorable (low), as shown by Day and Stein (l.c.), vitamin D aids in reducing the degree of anemia.

It seems probable that the anemia frequently observed in marked degrees of vitamin A deficiency is related to the infectious states which accompany vitamin A depletion. As pointed out by Josephs ('36), in a review of anemia in infancy and childhood, infections cause marked interference with hematopoiesis.

That anemia occurs in scurvy is well known, but it has been attributed by some to undernutrition and hemorrhage. Mettier and

associates ('30) found in scorbutic patients (eight elderly men) that a diet rich in fruit, green vegetables, and fresh liver, all of which are rich in ascorbic acid, caused a prompt reticulocytosis and rapid regeneration of blood. Neither large doses of iron nor the substance potent in pernicious anemia appeared to accomplish these results. Mettier and Chew ('31) concluded that the cytological changes in the bone marrow of scorbutic guinea pigs were indicative of retardation in erythrocytosis. Pure ascorbic acid administration caused a marked hematopoietic response in a scorbutic man who was continued during the experiment on his deficient diet of bread, syrup, margarine, corned beef or smoked sausage, cheese, and tea made with sugar and canned milk (Dunlop and Scarborough, '35).

Metabolism of Iron.—From the available evidence, much of it of dubious significance, it is possible to construct a general concept of iron metabolism. As pointed out previously, iron, to be absorbed, must be soluble and ionizable, and in the ferrous state. Unless the element can be converted into this form before passing into the lower part of the small intestines, it is excreted. Iron in hematin, the pigment of hemoglobin, is not liberated by ordinary digestive processes and is therefore not absorbed. In general, other natural organic forms of iron behave similarly. According to Hill (l.c.) all of the element in egg yolk is in inorganic form, probably as ferric hydroxide, and is therefore potentially absorbable.

The absorbed iron appears to be taken up by the liver but the role of that organ in the transformation of iron into respiratory pigments is uncertain. Likewise, there is no conclusive evidence concerning the function of the spleen in iron metabolism. However, it is rather generally believed that this organ is important, but not indispensable, in the degradation of hemoglobin from damaged red cells. The extent of the body's ability to utilize iron from catabolized hemoglobin does not seem to have been thoroughly determined. As shown by Stearns and McKinley ('37) the infant is able to utilize some of the iron released during the period of physiological hemoglobin destruction. The mechanism whereby iron is introduced into the pigment of hemoglobin is not known. That the bone marrow is the site of erythrocyte formation is thoroughly demonstrated.

Hahn and Whipple ('36) exhausted the iron reserves of dogs by the production of prolonged anemia due to repeated bleedings. They then performed viviperfusions to free the organs and tissues from blood. Following this the "parenchyma" iron (functional) was determined. In short feeding experiments of 1 to 2 days the iron absorbed was all used for the production of hemoglobin, and no iron store accumulated in the liver. With longer feeding periods the storage was small. The conspicuous feature was the rapid appearance of

ingested iron in hemoglobin. Viviperfusion carried out 24 hours after the intravenous administration of iron showed that 55 to 70 per cent of the iron was stored in the liver and spleen.

The non-hemoglobin parenchymal iron of various blood-free organs was relatively constant. Average values per 100 gm. of tissue were: 1-2 mg. in liver, kidney and pancreas; 3 mg. in lung; 5-6 mg. in spleen; and 10 mg. in red marrow. Striated muscle averaged 3.1 mg., of which 1.6 mg. was present as myohemoglobin and 1.5 mg. as parenchymal iron. These figures for muscle are constant and do not increase when iron is given intravenously or decrease when anemia is severe and prolonged. In view of this it is suggested that hemoglobin formation is subservient to the needs for non-hemoglobin iron. This is supported by Josephs' ('32) observation that if a diet of milk is continued beyond the normal nursing period, all the iron is used to maintain the "functional iron" at a constant, though minimal, level.

According to Whipple and Robscheit-Robbins ('36), in standard anemic dogs (vide supra) an optimum dose of iron (40 mg. per day for 14 days) will give a net output of about 55 gm. hemoglobin. The utilization is therefore about 35 per cent. The iron was utilized with equal facility when given orally whether in the ferrous or ferric state.

The Content of Available Iron in Foodstuffs.—As we have pointed out previously, only ionizable iron reacts with $\alpha\alpha'$-dipyridyl. Since this form of iron is biologically available while other forms are not, the dipyridyl method has been employed to estimate the available iron in foodstuffs. The following table, prepared from data given by Sherman, Elvehjem and Hart ('34a, l.c.), indicates the marked differences between biological materials as respects available iron in comparison to total iron:

AVAILABLE IRON ESTIMATED BY DIPYRIDYL METHOD

(Results expressed in mg. per 100 gm. of sample)

MATERIAL	TOTAL Fe	AVAILABLE Fe	PER CENT AVAILABLE
Pork liver (dry)	65.22	43.48	67
Beef liver (dry)	26.08	18.26	70
Pork heart muscle (dry)	15.31	13.26	87
Beef heart muscle (dry)	24.00	16.80	78
Beef skeletal muscle (dry)	14.08	7.04	50
Oysters (dry)	31.92	7.98	22
Soy beans (roasted)	10.00	6.00	60
Soy beans (non-roasted)	9.04	7.29	80
Spinach (dry)	56.23	10.94	20
Alfalfa	12.78	3.52	27
Blood (rats)	37.50	4.00	11

Other useful data are those of Shackleton and McCance ('36) who have determined the ionizable iron in a large number of English foodstuffs. The following table was prepared from their data:

AVAILABLE IRON ESTIMATED BY DIPYRIDYL METHOD

(Results expressed in mg. per 100 gm. of sample)

MATERIAL	TOTAL Fe	AVAILABLE Fe AS PER CENT OF TOTAL Fe	MATERIAL	TOTAL Fe	AVAILABLE Fe AS PER CENT OF TOTAL Fe
Cabbage, raw	0.98	72	Grape, dry (raisins)	3.80	97
Carrot	0.56	100	Grape, black, raw	0.27	85
Carrot, cooked	0.41	98	Grapefruit, raw	0.11	92
Celery, raw	0.14	100	Lemon juice	0.07	80
Lettuce, raw	0.80	63	Orange juice	0.20	85
Onion, raw	0.40	100	Peach, fresh, raw	0.39	100
Parsley, raw	10.00	50	Pear, raw	0.21	100
Pea, split, cooked	1.84	71	Pineapple, raw	0.22	91
Potato, cooked	0.62	97	Plum, dry (prunes), raw	3.20	72
Spinach, cooked	4.15	57	Tomato, raw	0.37	64
Apple, raw	0.24	100	Peanut	1.19	100
Apricot, dry, raw	4.08	98	Molasses, black	9.17	100
Apricot, fresh, raw	0.37	95	Salmon, canned	0.89	94
Banana, raw	0.47	100	Sardines, canned	3.44	65
Blackberry, raw	0.95	40	Beef, roast	5.20	19
Cherry, raw	0.48	100	Mutton, roast	5.10	24
Cranberry, raw	0.70	70	Ham, cooked	4.45	15
Date, dry	1.71	82	Veal, roast	1.35	55
Fig, dry, raw	4.17	96	Egg, raw	2.50	100

It will be noted that the iron of eggs is entirely available. Egg albumin is almost free of iron. As shown by Elvehjem and Hart (l.c.) the yolks of hens' eggs contain about 0.0143 per cent iron. The value could not be increased by feeding large amounts of iron, or of iron and copper. In the yolk there is a Cu:Fe ratio of about 1:19. In rats made anemic by restriction to a milk diet the iron of egg yolk is poorly utilized unless copper is furnished (Sherman, Elvehjem and Hart '34b). When anemic rats received 0.3 mg. of iron daily in the form of ferric chloride, hemoglobin regeneration was normal; when this amount was provided as egg yolk, regeneration was poor. Supplementation of the egg yolk with 0.05 mg. of copper, as copper sulfate, was not as effective as 1 mg. daily of copper. As discussed in the section on availability of copper, it is apparent that some of the copper was converted into the sulfide, by hydrogen sulfide from the egg yolk, thus becoming unavailable.

Ferric chloride, ferric pyrophosphate, and ferric hypophosphite are 100 per cent available. The value for ferric glutamate is about 88 per cent. Elvehjem, Hart and Sherman ('33) have pointed out that ferric pyrophosphate possesses several desirable qualities as a dietary iron supplement. It is quite soluble in water and not astringent as are most soluble salts of iron.

The distinction between total and available iron should be maintained clearly in the subsequent discussions of iron requirements.

Metabolism of Copper.—Prior to the discovery that copper is essential for iron utilization, Bodansky's ('21) few analyses of human brain had indicated that fetal brains contain more copper than those of adults. This suggestion of intrauterine storage was further indicated by the studies of McHargue ('25) who found that the liver of a newborn calf contained 8 times as much copper as did that of a full grown animal. Since milk is deficient in copper it is obvious that intrauterine storage of this element is a necessary provision of nature. Various studies in recent years have indicated that the newborn mammal is furnished with enough copper in the liver and other tissues to meet its needs during a large part of the normal suckling period.

In order to develop some understanding of the role of copper in hematopoiesis it is necessary to learn something of its metabolism. Lindow, Elvehjem and Peterson ('29) found that the absolute amount of copper in the body of a normal rat increased from 0.0108 mg. at birth to 0.4422 mg. at 210 to 240 days. This level could not be raised by subjecting the mother to a high copper intake either before or after parturition. The observation confirmed that of Elvehjem, Steenbock and Hart ('29) that diet does not appreciably affect the copper content of milk. But copper added to the diet was readily stored. Rats 75 to 85 days old on a normal stock ration with added copper, contained twice as much of the element as similar animals receiving the unsupplemented diet. Copper feeding increased the copper content of the skeleton, kidney, spleen, and liver, 1.6, 2, 5, and 20 times, respectively. About 98 per cent of the excreted copper was in the feces when copper was added to the ration. With the unsupplemented ration only 65 to 70 per cent was in the feces. Since practically all of the excreted iron is in the feces it is indicated that a difference exists in the partition of iron and copper.

Some indication of the distribution of copper in adult rat tissues is afforded by the data of Lindow, Peterson and Steenbock ('29, l.c.), given on page 225.

It is apparent that of the tissues examined only liver, kidney, and spleen are important as storage organs. But, as shown by Schultze, Elvehjem and Hart ('36), young rats restricted to a milk diet (deficient in Cu, as well as Fe, Mn, and perhaps other essentials) become

Tissue	Mg. of Cu per Kg. Dry Tissue		Tissue	Mg. of Cu per Kg. Dry Tissue	
	(a)	(b)		(a)	(b)
Bone	2.50	4.25	Lung	6.21	10.67
Brain	9.14	11.16	Muscle	2.00	2.10
Heart	9.92	12.25	Skin	3.57	4.00
Kidney	12.41	27.20	Spleen	3.41	17.47
Liver	11.43	213.32	Testicle	8.65	10.00

(a) = Stock ration alone; (b) = Stock ration plus added Cu for 46 days.

markedly depleted of bodily copper stores. In spite of this the feeding of copper and iron for 7 days caused a retention of only 5 per cent of the copper, although maximum hemoglobin formation occurred.

The relationship between copper in tissues and age is indicated by Cunningham's (l.c.) data in which the copper content is expressed as mg. per kg. of dry substances:

	Liver	Heart	Lungs	Spleen	Kidney	Pancreas
Adult bovine . . .	77.0	15.6	5.3	2.9	19.7	3.8
Calf newly born . .	470.0	14.8	4.9	4.8	15.7	5.5

According to Sheldon and Ramage's ('31) spectrographic analyses, copper occurs in all tissues. Chou and Adolph ('35) state that the average adult body probably contains 100 to 150 mg. of copper.

Role of Copper in Hematopoiesis.—It is significant that rapid hemoglobin formation occurs when copper and iron are given to animals, depleted of bodily copper stores, although only a small amount of copper is retained (Schultze, Elvehjem and Hart, l.c.). Schultze and associates were impressed by the observation that blood was the only tissue studied in which a large accumulation of copper occurred when copper was fed to an animal previously depleted of this element. This suggested that the hematopoietic action of copper is purely catalytic, therefore requiring but extremely small amounts in the actual process of hemoglobin formation. Schultze et al., who have worked with young swine, believe that rapid, continued hematopoiesis cannot occur unless the content of blood copper is maintained above a minimum level. This level is regarded as about 20 micrograms per 100 cc. of pigs' blood. It is perhaps significant, in view of this, that Sachs and coworkers ('37) have noted a tendency towards the accumulation of copper in blood of patients with pernicious anemia,

while the iron content is low. As shown by Elvehjem and Sherman ('32), pure iron added to the milk diet of anemic rats is deposited in the liver and spleen to some extent, at least, and hemoglobin production does not occur. But when the iron supplement is replaced by copper, rapid blood formation results, causing a reduction in liver iron to a level slightly less than that in severely anemic rats.

Although the various types of evidence suggest that copper acts in the introduction of inorganic iron into the organic complexes which form hemoglobin, there is no information on the actual mechanism of the process. Elvehjem ('35, l.c.) in a review of the biological significance of copper, states that "It is entirely possible that copper is concerned with the normal functioning of general body activities, and that the body's inability to form hemoglobin (in Cu deficiency) depends upon changes in these activities." Cohen and Elvehjem ('34) believed that copper might act through certain respiratory enzymes. Cytochrome was investigated since it is composed of three hemochromogens, *a, b,* and *c*. In rats depleted of iron and copper by restriction to a milk diet the *a* component was absent or much reduced in heart and liver tissue, but only slight reduction occurred in the *b* and *c* components. When copper alone or iron and copper were added to the milk, the *a* component was greatly increased. It was necessary to feed both copper and iron to increase the *b* and *c* components. Since iron alone was not effective in restoring the *a* cytochrome, while copper was effective, it was indicated that copper has a catalytic role in the formation of hemochromogens other than that required in hemoglobin. These findings are in agreement with Elvehjem's ('31) observations concerning the effect of copper on cytochrome production in yeast. Since copper is extremely active in the oxidation of ascorbic acid (Mawson, '35) and sulfhydryl compounds, in addition to indications of involvement in carbohydrate metabolism (Keil and Nelson, '34), there is reason to believe that it does play other roles in the body aside from hemoglobin production.

Copper Deficiency and Depigmentation.—As early as 1931 Keil and Nelson ('31, l.c.) noted that dark coated rats made anemic by restriction to a milk diet underwent marked changes in the pigmentation of hair. Black hair became a silvery gray. Since iron supplementation of the diet did not restore the original color, whereas iron and copper together were effective, it was indicated that copper has a role in pigmentation. This effect was noted by Cunningham (l.c.) who found that the copper content of skin from black rats and rabbits tended to be higher than that of albinos. He suggested that copper might serve as a catalyst in the formation of melanin pigments and referred to the possibility that copper in the ink sac of the octopus

may be related to the formation of melanin in the ink. Gorter ('35) also confirmed this finding and found that of various metals tested only copper was effective. He claims that the dosage must be much higher than for the cure of anemia. The relation of these findings to questions of pigmentation in skin and hair of humans has not been determined.

Biological Availability of Copper.—Scarcely anything is known concerning the forms in which copper exists in plant and animal tissues. At present only four organic forms of copper are known definitely to exist in nature. These are (a) turacin, a uroporphyrin compound which occurs as a highly colored copper pigment in the feathers of a South African bird, Turaco; (b) hemocyanin; (c) polyphenol oxidase; and (d) a compound recently isolated from ox blood by Mann and Keilin ('38) who have proposed that it be called haemocuprein. In the latter 3 compounds copper is united to protein, apparently in specific combination. It is reasonable to suppose that some compounds of copper are practically unavailable to the body while others are utilized with considerable efficiency. As shown by Schultze, Elvehjem and Hart ('36), copper of wheat germ, alfalfa, brewers' yeast, pork heart, pork liver, cystine cuprous mercaptide, copper aspartate, copper citrate, copper nucleinate, and copper pyrophosphate is utilized efficiently by severely anemic rats. Copper porphyrin and copper sulfide were poor sources of copper. It is possible, therefore, that any foods, such as egg yolk, which release considerable amounts of hydrogen sulfide in the digestive tract, might cause the formation of insoluble copper sulfide, thus interfering with copper utilization. This possibility should not discredit eggs or similar foods in this respect, since the dietary of man apparently contains sufficient copper to meet these possible contingencies.

The Content of Copper in Foodstuffs.—Lindow, Elvehjem and Peterson (l.c.) have reported the copper content of many animal and vegetable products, and give references to earlier analyses. They list as foods containing less than 1 mg. per kg. of fresh material the following: apples, beet greens (tops), cabbage, cantaloupe, carrots, celery, sweet corn, cranberries, cucumbers, gooseberries, grapes, grapefruit, kumquats, lemons, lettuce, muskmelon, onions, oranges, pineapple, pumpkin, rhubarb, squash, strawberries, tangerines, tomatoes, turnips, watercress, and watermelon.

Foods rich in copper, according to the analyses of these investigators, are on the fresh basis: liver (adult beef), 21.5; calf liver, 44.1; Brazil nuts, 14.8; butternuts, 11.7; chocolate (bitter), 26.7; cocoa, 33.4; molasses, 19.3; mushrooms, 17.9; pecans, 13.6; walnuts, 10.0; wheat bran, 11.7; and wheat germ, 12.7 mg. per kilogram, respectively. They have listed the average, minimum and maximum content of copper,

iron, and manganese in different classes of foods as shown in the following table:

DEGREE OF VARIATION IN IRON, MANGANESE, AND COPPER CONTENT OF DIFFERENT CLASSES OF FOOD MATERIALS

CLASS	NO. OF SAMPLES	AVERAGE	MINIMUM	MAXIMUM
		IRON IN FRESH MATERIAL		
		mg. per kg.	*mg. per kg.*	*mg. per kg.*
Fresh fruits . . .	23	6.6	2.3 Watermelon	22.8 Grapes
Nuts	12	41.0	21.4 Walnut	79.2 Pistachio nut
Roots and tubers .	14	11.0	3.0 Onion	23.6 Beets
Vegetables, leafy .	7	69.0	3.4 Cabbage	192.1 Parsley
		MANGANESE IN FRESH MATERIAL		
Fresh fruits . . .	13	4.0	0.2 Watermelon	22.9 Blueberries
Nuts	3	13.3	6.3 Pistachio	18.0 Walnut
Roots and tubers .	7	3.2	0.5 Onion	13.5 Beets
Vegetables, leafy .	8	6.6	0.8 Cabbage	12.6 Beet greens, tops
		COPPER IN FRESH MATERIAL		
Fresh fruits . . .	27	1.0	0.2 Strawberries	3.4 Olives
Nuts	10	11.6	6.0 Chestnuts	14.3 Hickory nuts
Roots and tubers .	11	1.4	0.8 Carrots	2.7 Oyster plant
Vegetables, leafy .	14	1.2	0.4 Watercress	3.1 Artichoke

The content of copper in many foods will be found highly variable, since the widespread use of copper-containing sprays on fruits and vegetables adds much copper to these products. Elvehjem and Hart ('29) raised the copper content of lettuce 148 per cent by adding 500 lbs. of copper sulfate per acre of the soil on which it was grown.

Iron and Copper Requirements of Infants and Children.—At birth normal infants contain considerable stores of iron and copper, largely in the liver. Premature infants contain much less iron and copper indicating that storage occurs late in *in utero* development. Also, the blood supply of infants at birth is larger than necessary for the first few weeks of life since there are no placental tissues to maintain after birth. Consequently a "physiologic" destruction of blood occurs during the first period of infancy. In this period, according to

Stearns and Stinger ('37), about 75 mg. of iron are excreted above that ingested. They have estimated that to provide the iron necessary during the remainder of the first year of life the infant would need to retain 0.75 to 1.0 mg. daily after 2 months of age. Since milk is poor in iron as well as copper it is necessary to have other sources of these nutrients early in life. The normal bodily stores alone are not able to provide for adequate hematopoiesis throughout the entire nursing period.

Various procedures have been employed to estimate the normal iron and copper requirements of infants and children. Among these the metabolic balance method has had a prominent place but, as we have pointed out elsewhere (Chapt. X), the method is deficient from the standpoint of both theoretical and practical considerations. However, it is not without some definite value. By means of this procedure it is possible to determine the approximate iron balance in a few subjects over a limited period of time and deduce from the data some facts concerning iron and copper metabolism. Rose and associates ('30) studied a 31 months-old child and observed a negative iron balance with a daily ingestion of 0.23 mg. of iron per kilogram. Leichsenring and Flor ('32) found, in 4 healthy children between 3 and 6 years, retentions of 0.07 and 0.18 mg. per kilogram at daily ingestion levels of 0.19 and 0.36 mg., respectively. They estimated the daily minimum requirement to be 0.32 mg. per kilogram, and suggested that a 50 per cent margin of safety should be provided. It would seem that such data should be accepted with caution, in the light of variability in the availability of various forms of iron, and of the inability of the body to utilize available iron unless a certain, at present unknown, minimum amount of utilizable copper is also present.

The practical question is: How much iron and copper of a given availability must an infant or child ingest regularly to maintain normal hematopoiesis? The amount excreted is of secondary importance in determining requirement. According to Elvehjem, Siemers and Mendenhall ('35), the average hemoglobin value for average infants and children between 6 months and 2½ years of age was between 11 and 12 gm. per 100 cc. of blood. When 25 mg. of iron and 1 mg. of copper as ferric pyrophosphate and copper sulfate, respectively, were used to supplement the regular diet of infants, the hemoglobin was raised from 9-11 gm. to 12-13.5 gm. per 100 cc. of blood. The use of 12.5 mg. of iron and 0.5 mg. of copper did not give as uniform or consistent results as the larger doses of these nutrients. The importance of copper is fully demonstrated since Josephs ('31), Elvehjem, Duckles and Mendenhall ('37), and others, have shown that the response of anemic infants to iron is enhanced by copper administration. There is no definite information concerning the copper needs. As shown by

Daniels and Wright ('34) in metabolic balance studies of children 4 to 6 years old, the average daily copper retention was 0.026 mg. per kilogram when the average ingestion was 0.086 mg. per kilogram. Higher retentions were found when 0.090 to 0.093 mg. per kilogram were provided by the diet; hence, for safety they set the requirement of this age group at 0.100 mg. per kilogram. Hodges and Peterson ('31), on the basis of their numerous estimations of copper in foods, have calculated the daily copper intake to vary from 0.8 mg. for a child of 3-4 years to 4.81 mg. for an adult. On this basis the copper intake of children scarcely exceeds the requirement suggested by Daniels and Wright. The limitations of the metabolic balance method have been indicated.

Mackey ('31) and Josephs ('36, l.c.) have adequately reviewed the subject of nutritional anemias in infancy and early childhood. These reports reveal the high incidence of such anemic conditions where the dietaries are low in iron and copper and also ascorbic acid. They emphasize the importance of supplementing milk with iron and copper.

The iron and copper needs of older children, especially adolescents, are relatively unknown. Various data show that the average hemoglobin and erythrocyte levels in adolescence tend to be definitely lower than in adulthood but higher than in young children. As shown by Osgood and Baker ('35), there is no appreciable sex difference in the prepuberal period. This is significant since in adults men generally tend to have higher values than women (Wintrobe, '30). An explanation of this fact cannot be given yet, but it appears to be related to differences in dietary practices, menstruation with the consequent loss of iron, and physical development. It would seem that the iron and copper requirements in adolescence are definitely higher than in adulthood but precise values or standards cannot be stated.

Iron and Copper Requirements of Adults.—In an investigation of the poorest classes of the population in Aberdeen and the northeast of Scotland, Davidson, Fullerton and Campbell ('35) found that the total daily iron intake was about 10 mg. per person and an intake of only 5 to 8 mg. per day was not an uncommon finding. Thirty-five hundred individuals of both sexes and at all ages were studied. On the basis of their reasonable standards anemia was found in 41 per cent of infants under 2 years, 32 per cent of pre-school children, 2 per cent of school children, 16 per cent of adolescent women, and 45 per cent of adult women. It was generally absent in adolescent and adult males.

An indication of a satisfactory method of estimating the iron requirements is afforded by the data of Widdowson and McCance ('36) who evaluated the total and "available" iron intakes of 63 men and

63 women of the English middle class living on freely chosen diets. The average intake for men was 16.8 mg., but 37 per cent of the men were ingesting less than 15 mg. per day. The amount of available iron for men was 10.8 mg. per day. Thirty per cent of the women were receiving less than 10 mg. daily and the average value for available iron was only 7.9 mg. The hemoglobin values, obtained on about one-half of the subjects, indicated normal levels on the basis of current standards. But the ingestion of 100 mg. of iron daily as ferrous sulfate or ferric ammonium citrate increased the hemoglobin values for women by 4 to 17 per cent. This supplement had scarcely any effect on men. The studies indicate, therefore, that the level of ingestion in men was approximately adequate, but inadequate in the women.

Orr ('35) found that men maintained normal hematopoiesis when the total iron intake ranged from 5.3 to 23.2 mg. daily. But women ingesting as low as 6 mg. daily tended to be anemic. Also, Farrar and Goldhamer ('35) have reported that a male student 26 years old maintained a normal blood picture throughout an investigation period of 316 days, although the total daily iron intake was approximately 5 mg. Moreover, 2 other young men had normal levels of hemoglobin and erythrocytes, although the total daily intake was only 7.1 and 7.8 mg., respectively. The three subjects remained in positive iron balance. It is important to note, however, that it takes more than three subjects to establish a dietary standard. Also, each of the individuals reported here were ingesting at least 1 quart of milk daily. We have already discussed the significance of adequate calcium and the importance of the Ca:P ratio in iron utilization.

Several years ago Sherman formulated a standard of 15 mg. of iron (total) for normal adults. Although it is possible that normal hematopoiesis can be maintained on levels of ingestion considerably below this, especially when the other factors are favorable for utilization, it would seem that a safe practice would be to follow Sherman's standard. If much of the iron is furnished by foods containing a low percentage of available iron, the level should be raised.

Nothing definite is known concerning the copper requirements in adulthood. Chou and Adolph (l.c.) believe that the copper intake should be about 2 mg. daily. Since the copper content of most common foodstuffs ranges from 2 to 25 mg. per kilogram of dry material, it is probable that the average mixed diet supplies adequate amounts of this mineral.

Iron Requirement in Relation to Menstruation.—Much has been written concerning the effects of menstruation on the content of hemoglobin in blood, the general assumption being that this physiologic process places a burden on the body owing to the iron losses. But as shown by Leverton and Roberts ('37) "the low hemoglobin values

which are accepted as normal for women because of the drain due to menstruation may be a direct reflection of the use of diets habitually low in iron rather than due to the small periodic loss in the menses." In four normal young women who were studied the average menstrual losses of iron were 14.26, 22.84, 11.13, and 13.80 mg. respectively. The losses were relatively constant from period to period for the same subject. The values reported by Gillett ('18) and by Ohlson and Daum ('35) are somewhat higher. The former observed a loss of 34 to 42 mg. of iron per menstruation and the values given by the latter were 18 to 42 mg. On the basis of Leverton and Roberts' data it is necessary for a sexually mature woman to absorb and utilize approximately 14 to 20 mg. of iron during each menstrual period, in addition to that required to meet the other requirements which, presumably, are similar to those of men. This means, however, that the diet must furnish more than the given amount of iron to meet this particular need, in addition to other requirements, since there is not 100 per cent efficiency in the utilization of dietary iron even though it might be in a form which permits absorption.

In the group of young women studied by Leverton and Roberts the average daily intakes of iron for the four subjects were 13.61, 11.87, 10.03, and 11.71 mg. Presumably most of the iron was available since the foodstuffs used were relatively low in organic iron. On this regimen the hemoglobin values increased from 12.95, 12.54, 11.62, and 13.06 gm. per 100 cc. for the first menstrual cycle of the four subjects to 14, 13.92, 13, and 14.3 gm. for the last cycle studied (Leverton and Roberts, '36). These increases in hemoglobin, in spite of the iron intakes that approached the minimum of safety, are significant. They suggest that the previous dietary was poorer in available iron than the experimental regimen which was planned to represent a well-chosen mixed dietary with no emphasis on foods rich in available iron. On the basis of these studies it is possible to conclude that menstruation does cause a significant increase in the requirement for available dietary iron, but this physiologic function does not constitute a large burden, probably no larger than a severe nosebleed. Also, it illustrates the tendency of ordinary dietaries to provide less than enough iron to permit maximal hemoglobin production in sexually mature women.

Iron and Copper Requirements of Pregnant and Lactating Women.—The iron and copper requirements are definitely higher in pregnancy and lactation. It is generally observed that in pregnancy an anemia occurs which, in women who have had no apparent defects in diet or gastric function, tends to be abruptly alleviated within a few days after parturition. As stated by Strauss ('34), there is a steady decline in both hemoglobin and erythrocytes from early in the preg-

nancy until the end of the sixth month, after which there may be no further change, or a slight increase might occur. There are various causes of anemia in pregnancy, among which are infections and an inadequate intake of available iron, but according to Strauss the so-called "physiologic anemia" is mere hydremia.

Alterations in blood volume, with a consequent reduction in the concentration of red cells and pigment, do occur as shown by various workers including Beard and Myers ('33) and Van Donk, Feldman and Steenbock ('34) using rats, and Schultz ('33) and Smallwood ('36) who studied human subjects. Adair, Dieckmann and Grant ('36) state that in their pregnant subjects the mean hemoglobin concentration was 11.56 gm. per 100 cc. of blood and there were 3.77 million erythrocytes per cmm. Their minimum standards for pregnancy were stated to be 10 gm. per cent of hemoglobin and 3.36 million erythrocytes per cmm. On the basis of this standard 11.6 per cent of the pregnant patients were anemic, but on the basis of standards for non-pregnant adults 63.2 per cent were anemic. These findings are to be compared with those of Davidson, Fullerton and Campbell (l.c.). They used the Haldane hemoglobinometer (100 per cent = 13.8 gm. Hb. per 100 cc. of blood) and the Price-Jones standard of 98 ± 10 per cent. In 819 pregnant women, aged 15 to 44 years, the average hemoglobin was 78.1 per cent (10.78 gm. Hb. per 100 cc. blood). In 603 parous non-pregnant women, aged 15 to 44 years, the average hemoglobin was 81.3 per cent (11.22 gm. Hb. per 100 cc. blood), and about one-half of the values were below 85 per cent. The hemoglobin tended to be lower in the older individuals. Values for the pregnant women were below those of non-pregnant women of the same age range. The data suggest that there is a progressive anemia throughout the reproductive years which is increased by pregnancy.

Beard and Myers (l.c.) have suggested that the physiologic anemia of pregnancy is somewhat analogous to the anemia occurring during early growth. Whether this is a valid analogy has not been fully decided, but the evidence does not suggest that hydremia is as pronounced in growing persons as in pregnant individuals. The literature on physiologic anemia has been reviewed by Garry and Stiven ('36).

Since large amounts of iron and copper are stored by the developing fetus, it is apparent that the diet must supply generous amounts of these nutrients if marked hypochromic anemia is to be avoided. Corrigan and Strauss ('36) conducted an important study in which it was demonstrated that the blood of apparently normal pregnant women can be considerably improved by supplementation with iron. Half the patients in the antepartum clinic were given coated tablets containing ferrous sulfate and the other half were given tablets similar in appearance but containing lactose and no iron. The difference was

not divulged to any of the patients. The treated group received an average of 0.5 gm. of ferrous sulfate daily for an average of 162 days. Of the 100 women who received no iron, 24 had less than 70 per cent hemoglobin (100 per cent by this standard is normal) post partum. Of the 100 women who received iron none had less than 70 per cent hemoglobin post partum. The significance of these data is apparent.

The extent of the demand for iron by the human fetus is made clear by Coons and associates ('35) who summarized the results of 9 published analyses. The average iron content of the fetus at birth was 395 mg., and the maximum was 937 mg. It was calculated that the content of blood iron would be between 238 and 437 mg. if the blood volume of a 7.5 lb. infant should be 14.7 per cent of the body weight and the blood hemoglobin were 103 and 189 per cent. They estimated a possible iron store of 160 mg., exclusive of that in the blood. On this basis the iron contribution of the mother during pregnancy would be 400 to 600 mg. They suggest that the diet should provide for the absorption and utilization of 500 mg. of additional iron by the pregnant woman. Coons and associates found a daily iron intake of 15 mg. just sufficient to maintain a positive iron balance in pregnant women, but Toverud ('35) found negative balances when only 15 mg. of iron were ingested daily. According to Macy and Hunscher ('34), the desirable intake is 20 mg. daily during pregnancy. This refers to available iron.

Scarcely anything is definitely known about the iron and copper requirements of lactating women. It is certain, however, that they are larger than in non-lactating mothers since the milk contains some iron and copper, although the amount is relatively low.

Anemia in Domestic Animals.—Anemia in suckling pigs has been reported to occur in Scotland, Sweden, Ontario, and elsewhere. It has been the source of great economic loss due to unthriftiness and excessive mortality. The condition appears to have been first described by McGowan and Crichton ('23). They found, as have others, that access to a supply of iron, e. g., ferric oxide, caused an immediate return of appetite and improvement in condition, the hemoglobin rising almost to normal in about three weeks. Since the discovery of an anemia resulting from cobalt deficiency, and of the necessity of copper for iron utilization, it is not certain whether the anemia of young swine is, in all cases, due specifically to iron deficiency or to other causes. The sources of iron used to treat anemic swine were undoubtedly impure. The details of the blood picture in this condition, attributed to iron deficiency, have not been described. Davidson and Leitch ('34) have reviewed the literature on nutritional anemias of various domestic animals and man.

The type of anemia in cattle and sheep known as "bush sickness,"

"coast disease," and "pining," will be shown subsequently to be due to deficiency primarily of cobalt (Chapt. XI). This disease is apparently identical with the "salt sick" of Florida. In certain instances the latter condition has been reported to be cured by the provision of iron and copper, but not by iron alone. This suggests that in some instances, in the reports of this disease, deficiency of copper may have been the cause, except when the efficacy of cobalt was established. It would seem that anemia in animals is in most instances referable to deficiency of iron, copper, or cobalt.

Anemia in poultry appears to be very uncommon under farm conditions. It may be induced by a milk diet as has been shown by Hart and associates ('29-30). The hemoglobin level, which varies from 5 to 19 gm. per 100 cc. of blood, tends to fall during periods of high egg production (Cook and Harmon, '33). Graham and Smith ('29) reported the results of incubation tests of more than 40,000 hen's eggs from the same genetic stock, which showed consistently low hatchability during February, March, and April, whereas the hatchability rose markedly during May, June, and July. This change corresponded with range freedom and accompanying improved quality of the diet (grass, sunshine, insects, etc.). They attributed the results particularly to deficiency of the fat-soluble vitamins, but it is possible that a deficiency of copper or iron was at fault. Studies of similar diets by McFarlane and associates ('30) showed that the iron content of the egg was little, if any, influenced by the food of the hen, but the copper content was greatly affected by the diet. Many of the chicks, or embryos, from eggs of low hatchability were anemic, especially when tankage or meat meal were fed to the hens. These studies are mentioned because they suggest need for investigations concerning the relation of iron and copper to hatchability of eggs.

Pernicious Anemia.—Physicians long ago recognized that certain cases of anemia were accompanied by parasites, chronic hemorrhage, dysentery, malaria, syphilis, or bacterial toxins, etc., and that recovery quickly followed when the cause was removed. The anemias were, therefore, "caused" or "secondary." Iron tonics, or chalybeate waters were found useful in promoting prompt recovery. Addison's description of the disease since known by his name, in 1849, has been already mentioned. The symptoms first recognized were loss of weight, pallor, lassitude, anorexia, soreness of the tongue, gastric disturbances, and numbness of the extremities. Since there was no known successful treatment, the syndrome became known as "pernicious anemia." In 1892 Ehrlich introduced the technic of staining blood smears by aniline dyes. This brought about rapid increase in knowledge of the characteristic details of the blood picture in different types of anemias. After 1890 there was a wave of enthusiasm for analysis of stomach

contents, and as data accumulated on gastric analysis it emerged that there was a relationship between lack of free hydrochloric acid in the stomach secretion and pernicious anemia. Patients with this disease not infrequently showed lack of peptic activity. Unlike the secondary anemias, pernicious anemia was apparently not due to any superimposed disorder; hence it became known as "primary" anemia. Some investigators continued to believe that pernicious anemia was not essentially different from other anemias, and that the cause should be discoverable. The researches which finally led to the present understanding of the etiology of pernicious anemia constitute one of the most remarkable achievements in the history of nutritional investigations.

Red blood cells are produced in the bone marrow, and in the normal individual they are passed into the blood stream where they are buffeted about during 30 to 40 days, by which time they become considerably damaged and are disposed of as waste material. At a given time the number of red cells in the blood represents the balance between formation and destruction. In pernicious anemia the bone marrow is overcrowded with immature red cells, almost ready to be released into the blood stream, but for some reason they are not liberated. The decreased production of new cells may be accounted for by supposing a lack of a nutrient principle which is essential for the maturation of the new cells, thus causing cessation of their development.

The results of Whipple and Robscheit-Robbins in their studies of hemoglobin formation had shown liver and kidney to be especially valuable for this purpose. Acting on this suggestion, Minot and Murphy ('26) tested the therapeutic value of liver and kidney in pernicious anemia. Their observations showed that in every one of 45 cases the patients improved almost at once under liver therapy, and continued in good health as long as liver was regularly eaten in sufficient quantities. The best results were obtained when raw liver was eaten in large amounts, but patients experienced great difficulty in consuming enough of it continuously to sustain normal blood regeneration. Subsequently Cohn and Minot et al. ('28) succeeded in preparing a concentrate from liver which contained most of the active principle and was much easier to ingest. Cohn and associates ('28) succeeded in fractionating liver to the extent that 140 mg. administered parenterally were clinically effective.

Having once developed pernicious anemia, it is necessary for the subject to continue taking the antipernicious anemia factor or complex during the rest of his life. It was difficult, therefore, to understand why normal persons who never ate liver or kidney did not develop pernicious anemia in a short time. Castle (Castle and Locke, '28) was

impressed with the fact that administration of liver or liver concentrate alleviated all of the major symptoms of the disease except one, namely, the inability of the stomach to secrete hydrochloric acid and enzymes which normally produce hydrolytic cleavage of the proteins of the food in gastric digestion. He postulated that the gastric secretion of the normal individual contains an "intrinsic factor," which acting on an "extrinsic factor," in association with the proteins of some foods and especially abundant in meats, could develop a principle, "anti-pernicious anemia factor," necessary for the maturation of the red blood cells and bring them to a condition suitable for discharge into the blood stream. His hypothesis implied, but did not specifically state, that liver and kidney are simply storehouses of this latter factor which had its origin in normal gastric digestion.

Castle tested this hypothesis by treating pernicious anemia patients with a diet of raw, ground muscle meat which had been predigested in the stomach of a normal individual. Under this treatment all the improvements characteristic of liver therapy were observed. Feeding the same kind of meat, without the previous digestion, produced no remission on other patients, but these responded well to liver therapy later. A third group of patients were given pure gastric juice obtained from normal persons. They did not benefit by this treatment. The conclusion drawn from these observations was that the antianemia substance is not present in either meat or in normal gastric juice, but is the product of the interaction of these substances during digestion.

The extrinsic factor has been shown to be present in a number of common foods, among which are beef muscle, eggs, wheat germ, autolyzed yeast, rice polishings and tomatoes. The nature of this principle is still unknown. It was later demonstrated that when the gastric mucosa of pigs was incubated with beef muscle the mixture was effective in producing a hematopoietic response in patients with the disease. Fresh gastric mucosa alone or whole desiccated stomach were, however, ineffective.

Greenspon ('36), on the basis of these observations, postulated the theory that "an erythrocyte-stimulating hormone resides in the gastric mucosa. In pernicious anemia, in which atrophy of the gastric mucosa takes place, a loss of this hormone occurs, coinciding with the disappearance of acid from the gastric juice. Thus, in addition to the well-known external secretion (digestive), the glands of the gastric mucosa produce an internal secretion (hematopoietic); this suggests an analogy to the pancreatic gland. This hematopoietic hormone may control the level of erythrocyte production in normal individuals."

Guided by this hypothesis Greenspon made experiments on one patient which resulted in the conclusion that pepsin is antagonistic to the antipernicious anemia factor in stomach juice (Castle's intrinsic

factor). This view rested on the finding that pepsinized ventriculin was ineffective in pernicious anemia, whereas, feeding depepsinized desiccated gastric mucosa was effective. The depepsinization was accomplished by extraction of the pepsin from frozen gastric mucosa, using 2 per cent hydrochloric acid at 0° C. and precipitating the pepsin from the filtrate by means of acetone. The filtrate was then combined with the residue so that a preparation containing all the constituents of gastric mucosa except the pepsin resulted. Depepsinized gastric mucosa, incubated with dilute hydrochloric acid, without addition of beef muscle, was effective in pernicious anemia. Feeding of depepsinized gastric mucosa, incubated with dilute hydrochloric acid and pepsin, was ineffective. Feeding normal gastric juice, peptically inactivated, was effective in causing remission of pernicious anemia, without the addition of beef or other source of extrinsic factor. These observations are opposed to the existence of an extrinsic factor as postulated by Castle.

Greenspon interpreted the normal process in the stomach as follows: The beef (or other source of extrinsic factor) when incubated with normal gastric juice binds the pepsin and prevents it from inactivating the antianemia substance. He believes these findings support the view, therefore, that it is no longer necessary to postulate the existence of an extrinsic factor.

These findings have been controverted by Ungley ('36), Castle and Ham ('36), Helmer and Fouts ('37), and others, on the basis of convincing observations that depepsinized gastric juice is ineffective in the treatment of pernicious anemia. The recent literature on the value of different preparations has not clarified the problem of the factors involved in remission of the disease.

The most notable attempts at purification and identification of the antipernicious anemia factor (hypothetical reaction product of the extrinsic and intrinsic factors) have been those of Dakin and West. Dakin, Ungley and West ('36) believe that the substance "is, or is associated with, a peptide, possessing many but by no means all the properties of an albumose." The evidence suggests that glucosamine is not an active component of the molecule. The peptide, on hydrolysis, yields arginine, leucine, glycine, proline, hydroxyproline, aspartic acid, and an acid resembling hydroxyglutamic acid. The molecular weight probably is between 2000 and 5000. A few years ago the factor was believed to be a component of the vitamin B-complex and that it might be the substance now called riboflavin. According to Ashford, Klein and Wilkinson ('36) riboflavin is neither the antipernicious anemia factor nor the extrinsic factor. Trager, Miller and Rhoads ('38) have reported that urine from normal persons or patients with aplastic anemia or leukemia contains a substance which

enhances the growth of mosquito larvae. But this substance is either lacking, or present in much smaller amounts, in extracts from the urine of pernicious anemia patients. It is found in the urine of such patients after the institution of adequate pernicious anemia treatment. Since investigations of pernicious anemia are greatly handicapped by the lack of suitable experimental animals, it would seem that mosquito larvae might become of some use for this purpose.

Of interest are the studies of Williams, Macy et al. ('37) on the chemical composition of the blood in pernicious anemia. They find the serum minerals to be unaffected, but the plasma lipids are characterized by an increased amount of neutral fat and a deficiency of cholesterol esters and phospholipids, which return to normal level after therapy. According to their studies, the macrocytes of pernicious anemia are slightly more spherical and the degree of sphericity, the cell volume, and corpuscular hemoglobin are related. Contrary to the prevailing belief, the chemical composition of the erythrocytes appears to exhibit striking abnormalities which indicate a deficient corpuscular structure. In relapse the red cells contain excessive amounts of cholesterol esters and low amounts of phospholipid and free cholesterol. The cation and anion content of the red cells are elevated, the former due chiefly to increased potassium, and the latter to greater hemoglobin content. It is pointed out that physiologic activity of any tissue or organ is associated with an increased content of phospholipid and of free cholesterol, whereas lowered activity, degeneration and retrogression are accompanied by a decreased content of these lipids and an increase in the amount of neutral fat and cholesterol esters. According to this view, the red cells in pernicious anemia are in a state of lowered activity, or they are degenerating and retrogressing.

Limited space does not permit further discussion of this subject. The literature on the nutritional aspects of pernicious anemia has been adequately reviewed up to 1935 by West ('35).

Sprue.—Whereas pernicious anemia appears to be characterized generally by a deficiency of the intrinsic factor, sprue is probably due, in most instances, to a deficiency of the extrinsic factor in the diet and complicated, perhaps, by an inadequacy of iron and perhaps some components of the vitamin B-complex, in addition to poor absorption in the intestinal tract. This view is supported by the extensive investigations of Castle, Rhoads, Lawson and Payne ('35), whose observations show that the blood picture in sprue bears considerable resemblance to the macrocytic anemia of pernicious anemia, and that sprue is indeed a deficiency disease. Also, the studies of Wills and associates ('37) indicates that the counterpart of tropical macrocytic anemia, sprue, can be produced in monkeys by means of a diet devoid of

appreciable sources of the vitamin B-complex and low in protein. Wills et al. believe that the anemia is due to a deficiency of Castle's extrinsic factor in addition to some other substance. Thus the problem of nutritional anemias not involving iron or copper is indeed complex.

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CHAPTER

X

Iodine and Its Relation to Thyroid Function

ALMOST from the time of its discovery by Courtois, in 1811, iodine has been recognized as an important element in the maintenance of health, particularly in relation to thyroid function. For various reasons, to be discussed briefly in this chapter, cretinism, endemic goiter, exophthalmic goiter, and some other types of thyroid disturbances, have been the source of human distress from time immemorial. These have been largely problems of diet, for laboratory experimentation and human experience during the past century have shown that most cases of thyroid dysfunction are attributable to inadequate amounts of iodine in the diet. It is necessary, therefore, to consider here the more important aspects of iodine in nutrition. Since the present questions of significance are grounded in the early human experience with thyroid diseases, some attention will be given to the historical background before discussing the current opinions and data of this subject.

Early Views on the Cause of Goiter and Cretinism.—Hirsch (1885), in his *Geographical and Historical Pathology,* gives an extended account of the history of endemic goiter and cretinism. He states that medical writings of antiquity contain many references to "tumors" of the neck. Undoubtedly the "tumors" were goiters. In the light of our accumulated information some of the early views were bizarre. For example, Wharton, who gave the first anatomical description of the thyroid in 1656, suggested that the gland fulfilled a cosmetic function in women since it gave grace to the contour of the neck. Hinkmar's *Life of St. Remi* (5th century) states that the disease was believed to be a punishment from God, and cites cures effected by touching the King's hand.

Endemic goiter was known from ancient times to be confined

to certain regions, usually mountainous districts far removed from the sea. Writers of the first century A. D. mention endemics of goiter in the Alps, and in the 13th century Marco Polo gave an account of its prevalence in the Central Asia plateau. As exploration increased it became evident that the disease was world-wide, but the general impression of the type of regions in which it was likely to occur remained unchanged. Moreover, the prevailing view was that the cause lay in environmental factors and most probably in some abnormality of the water.

The seriousness of goiter and cretinism as health problems naturally occasioned much speculation and attempts at inquiry as to their cause. These are all discussed at some length by Hirsch, whose account of the diseases will be found well worth study. Among some of the opinions were its relation to the geology and mineralogy of the affected locality. It tended to occur more frequently and with greater severity in regions characterized by older geologic formations than in the newer. Also it tended to occur only in regions of sedimentary formations which are composed of detritis of older rocks. Several writers attributed the prevalence of goiter to the habitual use of water rich in calcium and magnesium, but no generalizations were found to hold true. Neglect of personal hygiene, pressure on the thyroid gland, some unknown poison, an infective agent, heredity, and the deficiency of iodine in air and water, were all brought forward as causes of goiter and cretinism. It is interesting to note that Hirsch, although the year was 1885, states that "It would then remain a question, and a very doubtful one, whether iodine has a prophylactic power against goiter, as well as a curative."

Observations on the Relation of Iodine to Thyroid Function.— It is generally believed at present that almost from time immemorial, burnt sponge was more or less a popular folk remedy for goiter and cretinism. However, Harington ('35) writes that "The earliest reference to this treatment which I have been able to find occurs in the writings of Arnaldus Villanovanus (A. D. 1280)." Certainly medieval European physicians were generally aware of the remedy for reference to it occurs repeatedly in their writings. But it was at least over 500 years after Villanovanus that any inkling was found of the specific substance which caused the beneficial effects possessed by burnt sponge.

Following the accidental discovery of iodine in burnt seaweed, by Courtois in 1811, Fyfe, in 1819, isolated the new element from the sponge. About this time Coindet (1820), a physician in Geneva where goiter was very prevalent, although unaware of the isolation of iodine from sponge, postulated that the element discovered by Courtois was the goiter-curative principle in burnt sponge. As stated by

Harington ('35, l.c.), "The success which attended the first tests which Coindet made of his theory was dramatic; large goitres of long standing disappeared with incredible speed in response to administration of iodine, and the enthusiasm aroused by the success was such that people took to carrying little bottles of the magic element hung round their necks like charms."

Boussingault, in 1825, stated definitely that "until now iodine is the only specific known for goiter." But the immediate wave of enthusiasm, as is so often the case, was accompanied by injudicious use of the new substance. In many instances entirely too large amounts of the element were used with the consequent production of severe hyperthyroidism, the baleful nature of which was soon noted. Thus, iodine therapy soon fell into some degree of disrepute, although Coindet had pointed out that toxic effects could be avoided by discrete regulation of the dosage.

During succeeding years evidence continued to accumulate which indicated the relationship between iodine and thyroid function. In 1833, according to McCay ('35), Boussingault described the value of salt rich in iodine as a preventive of goiter. Chatin's extensive studies, about 1850, demonstrated a significant correspondence between the amounts of iodine present in water and foodstuffs in different areas with the regional incidence of goiter.

Although by this time the work of Coindet, Boussingault, Prévost, Chatin, Koestl, Lombroso, and others, had clearly demonstrated the definite goiter-curative effect of iodine, the adverse report of Rilliet, in 1860, to the French Academy of Medicine, swerved the medical thought of that time to unreasonable points of view and caused the essential abandonment of iodine in the treatment of goiter. The bases of Rilliet's conclusions were the results from experiments in which iodine was given to large groups of school children in France. Since the amounts employed were immoderately high, Rilliet and others concluded that the toxic effects observed warranted the exclusion of iodine from the armamentarium of medicine.

The subject remained controversial until Baumann (1896) discovered that the thyroid gland, in comparison with other tissues, is very rich in iodine. Once again there was a revival of interest in iodine deficiency as the cause of goiter. The renewal of prophylactic and therapeutic treatment with iodine was based this time on more careful control of dosage. Consequently, iodine in goiter prevention became firmly established and is now sound medical and nutritional practice.

In this country Marine and his associates began to study the subject soon after Baumann's important discovery. In studies of the histological changes which accompany goiter, Marine and Williams

('08) found that a fall in the iodine store precedes any cellular changes. They demonstrated that pups born of a mother with three-fourths of the thyroid removed, the mother being kept on a diet low in iodine, had enlarged thyroids with the histological changes characteristic of goiter. The same animal when fed iodine during a subsequent gestation, delivered pups whose thyroids were normal in all respects. Following this early work of Marine, McClendon, and others, the number of investigators increased rapidly so that at present the literature is extensive and in general agreement that iodine is essential to normal thyroid function.

Effects of Iodine Deficiency.—The principal anatomical effect of iodine deficiency in experimental animals is thyroid hyperplasia, but diets highly purified with respect to this element have not been studied. It appears that the increase in size, incident to iodine deficiency, is essentially no different from that in normal growth of the gland. However, adenomata appear early in the goitrous thyroid. It should be remembered, of course, that the thyroid gland is perhaps the most vascular organ in the body and that changes in size may be not only of considerable magnitude but rapid as well. As stated by Orr and Leitch ('29) ". . . the normal histological picture and the rich vascular supply appear to indicate a mechanism by which the normal physiological requirements for the thyroid hormone are supplied by colloid absorption over relatively small areas with the preservation of a relatively large store of colloid capable of rapid mobilization. This in turn indicates that the body from time to time may make urgent calls on the thyroid for a supply of the hormone." Since hormone production is dependent upon an adequate amount of iodine, this element must be constantly supplied to maintain a normal functional state.

As emphasized by Marine ('35a), ". . . hyperplasia indicates hyperactivity but not necessarily hyperfunction. Myxedema and cretinoid states may occur in individuals and animals with typical hyperplasia. It is more accurate to consider all functional hyperplasia as indicating relative or absolute iodine deficiencies and colloid goiter as the recovered, resting and physiologically normal stage." The normal gland appears to be composed of many closed (ductless) alveoli, filled with the jelly-like material known as *colloid,* and lined with a single layer of cuboidal epithelium. In 1901 Oswald noted that the iodine was contained in the colloid. Also, he found that the iodine content of the thyroid varied in general with the amount of visible colloid. These relationships were amplified by Marine and Lenhart ('09) who showed that the iodine store in general varied inversely with the degree of active hyperplasia and that in extreme degrees of thyroid hyperplasia the iodine store was almost completely exhausted. The

relation of iodine store to histological structure of different species is shown in the following table prepared by Marine ('35, l.c.):

RELATION OF IODINE STORE TO HISTOLOGICAL STRUCTURE
(Iodine in mg. per gm. of dried gland)

SPECIES	NORMAL	HYPERPLASTIC STAGE			COLLOID OR RESTING STAGE
		Early	Moderate	Marked	
Man	2.17	0.88	0.71	0.32	2.00
Dog	3.32	0.62	0.37	0.11	1.99
Sheep	2.47	...	0.40	0.01	3.00
Ox	3.46	1.65	...	0.19	...
Pig	2.51	1.10	...	...	2.35

As shown by Marine and Lenhart, enlargement of the thyroid does not occur if the diet contains adequate amounts of iodine, but in iodine deficiency the gland begins to increase in size as soon as its iodine content falls below 0.1 per cent of the dried gland tissue.

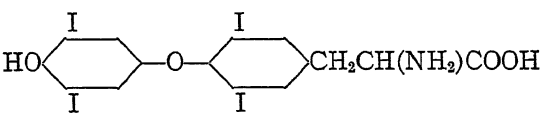
It is not profitable to discuss here the classifications of thyroid disease in medical literature; it suffices only to indicate the broad types of the disease. These may be regarded as having their origin in hypofunction or hyperfunction of the gland. *Myxedema* is a general type of hypofunction. It may result from any factor which prevents the adequate production of thyroid hormone, including, of course, iodine deficiency. Reduced metabolic rate, thickening of subcutaneous connective tissue, alopecia, general physical and mental sluggishness, are the chief symptoms of myxedema, or deficiency of thyroid function. If the deficiency occurs in childhood, infancy, or in the embryonic stage, the condition is called *cretinism*. These conditions are generally accompanied by an enlargement of the thyroid, or hyperplasia. When the resultant swelling of the neck is noticeable it is called *goiter*. If the goiter is enlarged without hyperactivity it is usually referred to as *endemic* or *colloid goiter*, while if hyperactivity does accompany it, the condition is called *exophthalmic goiter, Graves' Disease*, or *Basedow's Disease*.

It is generally believed that exophthalmic goiter is attributable to hypersecretion of the thyroid hormone and that this may be caused by excessive ingestion of iodine. Although excesses of iodine are very injurious, there is no conclusive evidence of this. It would seem that the functional relationships with other glands of internal secretion are more important in exophthalmic goiter.

As pointed out by Evvard ('28), domestic animals, raised in areas of iodine deficiency, may show marked degrees of iodine insufficiency. In pigs, hairlessness is a common symptom. It is a peculiar manifestation since the alopecia is rarely complete, many stages being represented in a single litter. The pigs are often of good size and are generally carried *in utero* somewhat longer than normal. The skin is thickened, pulpy, and very tender. Usually the thyroids are enlarged. At times colloid may be observed in the thyroids, but usually there is degeneracy, showing widely separated and irregular tubules with the intercellular spaces filled with serum, leucocytes, fibroblasts, extravasated blood, and exfoliated follicular or acinic cells. These symptoms, and others, are quite variable but there seems to be no general species-differences in the manifestations of iodine deficiency.

Compounds of Iodine in the Body.—The unique capacity of thyroid tissue to accumulate iodine is shown by Marine and Rogoff ('17), who found that when 38 mg. of potassium iodide were administered to a dog by mouth, 18 per cent of the iodine was recovered from the thyroid gland whose ratio to body weight was only 1:687. The average normal iodine content of the human thyroid is reported to be about 2 mg. for each gram of dried substance. The amount in other tissues is very much less, being expressed in γ per cent (1 γ = 0.001 mg.). It seems probable that all of the iodine in the thyroid is combined with globulin. The complex is commonly designated as *thyroglobulin*. According to Harington ('35, l.c.) the iodine complex, upon appropriate hydrolysis, can be accounted for between thyroxin and diiodotyrosine. He believes that ". . . the molecule of the complete active secretion of the thyroid contains both thyroxine and diiodotyrosine and that some linkage between these two is ruptured during hydrolysis which is a necessary initial stage in the isolation of thyroxine."

Thyroxin was first isolated by Kendall ('15). It was later synthesized by Harington and Barger ('27), who gave it the following structural formula:



(Thyroxin)

It has been known for several years that thyroxin is biologically very active while diiodotyrosine is inactive. However, it is quite certain that the hormonal function of the thyroid is not mediated, in entirety at least, by thyroxin. As shown by Lunde et al. ('29), blood

iodine may be separated into two fractions by alcohol extraction. Dodds, Lawson and Robertson ('32) claim that the insoluble fraction is increased in all patients with toxic goiter and that the level may be reduced by orally ingested iodine.

Further investigation probably will reveal the more precise nature of iodine compounds in the body. Certainly one may conclude that the functions of iodine in bodily processes are not accomplished simply through the action of thyroxin alone.

Geographic Areas of Iodine Deficiency.—It is necessary to consider the iodine content of soil and water since these are important factors in determining the status of iodine nutrition in man or animals. McClendon ('27) has written an extensive review of the geographic distribution of goiter in relation to the amount of iodine in soil and water. Areas of notable iodine deficiency are the Alpine mountain regions of Southern Europe, including all Switzerland, Northern France, Northern Italy, and the Balkan States; the Himalayan mountain region of Eastern and Southern Asia, the Gilget district of Northern India, and the plateau regions of Western China and Eastern Mongolia; the Andes mountain region of South America, the most noted section of which is the Peruvian plateau; parts of New Zealand; and in North America, the entire basin of the Great Lakes, the basin of the St. Lawrence, various areas in the region of the Rocky Mountains and the Pacific Northwest. In a well-known valley of British Columbia called Pemberton Meadows it was difficult to raise farm animals owing to the marked deficiency of iodine. Following the supplementation of foodstuffs with iodine, goiter is said to have practically disappeared from that region.

New Mexico, West Texas, Kansas, and the area northward into South Dakota, are relatively rich in iodine. At one time a gulf or inland sea covered this region, which left vast salt deposits rich in iodine. The Piedmont region of South Carolina also contains large areas rich in iodine. But it is only in a few substances such as the nitrate deposits of Chile and Bolivia, and in some sponges and a few other marine products, that concentrations as high as 0.1 to 0.2 per cent occur. In McClendon's ('33) world survey, Japan was found to be the only non-goitrous country, there being about one goiter per million Japanese. The relatively high consumption of seaweed, which is rich in iodine, is regarded as the cause for practical absence of goiter from that country.

It is estimated that sea water contains about 25 parts of iodine per billion. Seaweeds, sponges, and other marine plants and animals have great capacity for the absorption of iodine from sea water. *Laminaria,* a common seaweed, contains iodine equivalent to 0.5 per cent

of its dry weight. Organisms in the soil, as well as the vegetation, absorb iodine and often contain much greater concentrations of this element than the rocks from which the soil was formed by weathering. The soil of glaciated regions contains little iodine, because the ice carried away the ancient soil with its vegetation and microorganisms, and the formation of new soil has not been in progress long enough to accumulate as much iodine as is needed by animals and man dependent on such soils for food. This is believed to account for the deficiency of iodine in much of Canada and the northern part of the United States.

There are certain other factors which deplete soils of iodine. Clay and humus soils tend to retain the element better than sandy soils or those poor in humus. Also, calcium is said to antagonize the retention of iodine since it disintegrates humus and renders the soil porous, in addition to its alkalizing effect. Remington found that potatoes grown on sandy soil contained little iodine, whereas those produced on clay soils were much richer in this element. Organic matter oxidizes rapidly in sandy soils, as pointed out by McClendon, thus liberating iodine. The problem of soil composition and physical status, particularly as respects the dispersion of components, is indeed important. Desirable factors for certain needs are undesirable for others.

Also, it is possible that the mode of combination of iodine in soils, as well as the amount, is important in determining the concentration of this element in foodstuffs. This is indicated by a report of the Medical Research Council of Great Britain (Committee on Iodine Deficiency and Thyroid Disease, '36) in which it was shown that the only appreciable difference between the iodine content of water, milk, and pasture grass of two different English counties was in the water. But this was not marked and scarcely explained the large difference in goiter incidence in the two counties. The observation suggests that the availability of soil iodine might be a significant factor in the occurrence of endemic goiter.

Use of Iodine in Prevention and Treatment of Goiter.—As pointed out previously, iodine prophylaxis was used as early as 1820, by Coindet, in conditions of iodine deficiency. But because of overdosage the early procedures were not very successful, and it was not until the present century that the administration of iodine, for such needs, became established on a wide and secure basis. The first large scale experiment in goiter prevention with iodine in man was begun in 1917 through the effort of Marine and Kimball ('17). They studied the effects of administering sodium iodide on the incidence and course of goiters in girls in the public high schools of Akron, Ohio. Two grams of the salt, in 0.2 gm. doses distributed over

a period of two weeks, were given to each girl. This procedure was repeated each spring and autumn. Re-examination of the pupils at the end of the first year revealed practically the same dramatic preventive and curative effects that had been noted in animals by Marine and other investigators. With the early results of this experiment available it became evident, as recently expressed by Marine ('35a, l.c.), that goiter is the simplest, easiest and cheapest of all known diseases to prevent. The results of the study, at the end of the first year, are given in the table which follows:

	Pupils Taking Prophylactic Treatment		Pupils Not Taking Prophylactic Treatment	
	Pupils	Per Cent	Pupils	Per Cent
Thyroids remained normal.	283	100	637	74.0
Increased from normal to slight goiter	0	0	259	26.0
Small goiters (unaltered)	287	66	759	87.0
Small goiters (disappeared)	141	33.5	10	1.2
Small goiters (increased).	2	0.5	103	11.8
Large goiters (unaltered)	34	66.7	106	99.5
Large goiters (decreased)	17	33.3	5	4.5
Total	764	...	1879	...
Total number of girls examined. .	4415	...	...	...

In 1917 Kimball sent his first publication to Professor Klinger in Zurich, Switzerland, who, in 1918, applied iodine therapy to the school children of that city. In some schools 100 per cent of the children were goiterous. Klinger used an iodized fatty acid combined with chocolate and made into tablets. The method was later employed in the schools of the cantons of St. Gallen, Berne, and Zurich, with very striking results. Thus the value of iodine prophylaxis has been unequivocally established for at least twenty years, but the proof of its usefulness has been extended by more recent investigations. The most notable perhaps are those made in Michigan and Ohio. In 1924 a special committee was appointed by the State Medical Society of Michigan to cooperate with the Michigan State Department of Health, in a state-wide program of goiter prevention. The State Department of Health made a goiter survey to determine the incidence of goiter in school children, and the amount of iodine in the water supply of each section. The Wholesale Grocers' Association and the Salt Manufacturers' Association cooperated in putting upon the market a

table salt containing one part of potassium iodide in 5000 parts of salt, and in urging customers to buy this salt. The program contemplated the recording of goiter incidence during a ten-year period, as shown by records of operations for goiter in hospitals, and by a re-survey of school children. The results of this grand experiment are now available. They are best stated in the words of Dr. R. D. McClure ('34), Chief Surgeon of the Henry Ford Hospital:

1. Iodized salt as used in Michigan did at first apparently increase the number of thyroid operations.
2. The increase was in the nodular goiter or adenoma group, and we believe the iodized salt may have activated a group of quiescent adenomata, producing toxic goiter symptoms.
3. The increase reached its peak in the second year after the introduction of iodized salt.
4. An increase in the death rate from goiter as shown by the Board of Health statistics reached its peak in the second year after the introduction of iodized salt.
5. There was no increase in hyperthyroidism, excepting in the nodular goiter or adenomata group.
6. The number of operations for toxic, diffuse and toxic nodular goiter has rapidly and steadily decreased after the apex of the second year increase had been reached.
7. The incidence of endemic goiter or enlarged thyroid has been reduced almost to nil since iodized salt has been so widely used.
8. We now see no cases which show the slightest ill effects from the use of iodized salt.
9. Toxic nodular goiter and toxic diffuse goiter are less apt to occur when there has been no previous enlargement of the thyroid (endemic goiter); at least this would seem a safe conclusion based on our experience.

As a basis for further evaluation of the Michigan experiment, we have the records from Ohio. In a significant paper by Kimball ('37) the experiences of these two states in goiter prevention are effectively presented. It so happened that The Ohio State Department of Health in 1925 made a rather comprehensive study of the incidence of goiter throughout the state and planned to support the general use of iodized salt. But owing to active opposition to this method of prophylaxis on the part of some persons in the State, the public health measure was essentially abandoned. Hence, it is enlightening to compare the Michigan data with such of those which are available from Ohio.

In 1936 a survey was made in the Cleveland parochial schools to determine the incidence of goiter and the use of iodized salt. A modification of the tabulated results, given by Kimball, are as follows:

	Total	Normal Thyroids	Goiter	Per Cent Goiter
Using iodized salt	2752	2503	249	9.0
Not using iodized salt	2785	1752	1033	37.1
Indefinite	798	624	174	21.8
Total	6335	4879	1456	23.0

In comparison with these data we have those from Midland County, Michigan:

	Total	Normal Thyroids	Goiter	Per Cent Goiter
Using iodized salt	3089	3031	58	1.9
Not using iodized salt	1184	1009	175	14.5
Indefinite	618	593	25	4.0
Total	4891	4633	258	5.3

Thus, we have factual evidence that iodized salt, as a prophylactic, is effective. Moreover, there are other aspects of health than the prevention of goiter. Hypothyroidism or conditions of hyperplastic activity, owing to iodine deficiency, are accompanied by defects other than deformity of the neck. Mental development, sexual maturation, and the development of positive personality are retarded. Hence, iodine supplementation, insofar as it corrects or prevents a goiterous condition, promotes these functions, and contributes to the maintenance of good health.

Effects of Iodine in Large Amounts.—The advisability of adding iodine to the diet of man and animals has long been debated, as shown earlier in this chapter. Unfortunately much of the contention, both pro and con, has had no basis in facts since clear-cut experiments on the effects of iodine in various amounts have not been sufficiently numerous. At present, therefore, we are not yet certain as to what constitutes a "large" amount of iodine. Orr and Leitch ('29, l.c.) conclude, on the basis of scanty evidence, that the minimum daily requirement may be about 45 γ for adults and 150 γ for children. On the basis of this, one might presume that one hundred times, or more, the stated requirements would constitute a large amount of iodine.

We know of no very useful objective studies on normal humans, in this regard, but physicians have written extensively of their clinical

findings. We are not particularly concerned with such data and impressions here, since they are essentially medical problems and are concerned with the question of determining what types of thyroid disturbances should be treated with iodine.

The data from animal experimentation are illuminating. Evvard and Culbertson ('25) fed one group of pigs 40 mg. of potassium iodide per head daily for approximately 140 days. In this test and in two others of rather similar nature the iodized animals showed an increased growth rate and a reduction in the food consumption per unit of weight increase, as compared with control animals. In England, as stated by Orr and Leitch ('29, l.c.), pigs and brood sows fed as much as 1 gm. of potassium iodide per day showed no toxic effects. Phillips, Curtis and Erf ('34) have studied a herd of heavy-producing dairy cows which have been fed daily about 70 to 200 mg. of iodine, largely as potassium iodide, fish meal, and kelp, over a period of about 3 years. Physically the cows remained in very good condition. There was no evidence of hyperthyroidism, the milk and butter fat production increased, and the calves were normal.

There are instances, however, of severe injury when several grams of iodine are ingested daily, or even in one dose. The skin particularly is affected by eruptions and occasionally acute eczema develops. But one must conclude that the physiological range of iodine tolerance is fairly wide. Certainly no injurious effects can be attributed to the use of iodized salt, generous amounts of sea food, and other foods naturally rich in iodine.

Secondary Factors Contributing to the Cause of Goiter.—Marine ('35b) has pointed out that there are contributing causes other than deficiency in iodine, which may be relative or absolute. Puberty, pregnancy, the menopause, certain infections and intoxications, exposure to cold, excess of certain dietary constituents such as fats, proteins, and calcium, and deficient oxidation, all increase the needs of the body for the thyroid hormone which contains iodine. The effects of high calcium in the diet appear to be closely related to the levels of this element in proportion to the phosphorus and the magnesium content of the diet. Deficient oxidation may result from anemia or from oxygen deficiency due to living at high altitudes. Little is known about factors which may interfere with the absorption of iodine. McClendon has pointed out that organisms in the soil have a remarkable power to absorb iodides, as has been already mentioned. Hence, it is possible that when certain types of microorganisms, or certain animal parasites, constitute the prevailing flora or fauna of the alimentary tract the iodine taken in the food or water may be diverted to these and escape absorption. It has also been suggested that in certain cases the thyroid gland may have lost its power to utilize

iodine for the synthesis of the thyroid hormone, but this suggestion is not given credence by Marine because it is not in harmony with his extensive observations.

That a high calcium intake may prevent the utilization of iodine by the thyroid gland is indicated by a number of observations which date from studies made over a century ago. Tanabe ('25), Hellwig ('31), and Thompson ('33) have reported evidence which seems to establish the claims that a high calcium intake increases the goiter-producing effects of certain diets. Zondek and Reiter ('23) found that the addition of calcium chloride to the water greatly delayed the metamorphosis of tadpoles. Abelin ('28) found that when rats are given calcium, the metabolic effects (stimulation of metabolic rate) of administered thyroxin was diminished. Rats restricted to a somewhat low iodine intake, and water containing a calcium salt, develop enlarged thyroids, while without excess of calcium the thyroids remained essentially normal. Thompson found that the ingestion of calcium carbonate is increasingly effective as the iodine intake is lowered. The parathyroids are found to be enlarged in experimental low-iodine, high-calcium feeding experiments.

Thus it is shown that there is more in goiter production than iodine deficiency. Several factors other than calcium have been investigated but the results are essentially inconclusive. Some of these are fat, vitamins A, B-complex, C, and D. McCarrison ('33), and others, believe that diets high in fat tend to produce goiter. Some of the data regarding ascorbic acid (vitamin C) will be discussed later. It is not profitable to consider vitamins A, B-complex, and D until less equivocal results are reported.

Chesney, Clawson and Webster ('28) made the remarkable observation that a diet consisting solely of cabbage may produce enlargement of the thyroid gland in rabbits. Clinically, detectable thyroid hyperplasia occurs in 2 to 3 months, and with continued feeding of cabbage very large goiters (up to 45 gm.) were produced. These goiters were associated with a lowering of the metabolic rate, and the administration of iodine raised the metabolic rate and prevented thyroid hyperplasia. The thyroid hyperplasia produced by feeding cabbage is histologically identical with simple or endemic goiter. These observations were confirmed by Marine and associates (Baumann, Cipra and Marine, '31), and constitute definite proof that deficiency of iodine utilization and consequent thyroid hyperplasia may be caused by some positive agent.

Marine and associates state that boiling or steaming cabbage for 30 minutes increases its capacity to produce goiter. The maximum effect of steaming is reached under thirty minutes. The fibrous material left

after pressing steamed cabbage is as effective as whole cabbage fed in equivalent amounts. The juice pressed from steamed cabbage is only slightly effective in causing goiter.

Rabbits previously iodized and fed fresh cabbage up to 75 calories per kilogram per day developed palpable thyroids in about 30 days, while with steamed cabbage the glands were comparably enlarged in 10 to 15 days. Rabbits normally have a metabolic rate of about 2.4 calories per kg. per hour, and after thyroidectomy, it falls to 1.75. This degree of lowering of metabolic rate was accomplished by feeding steamed cabbage in about half the time required when fresh cabbage was fed. Winter cabbage was more effective than summer cabbage. Steamed winter cabbage was effective in producing thyroid enlargement when fed in the amount of 25 calories per kilogram per day. Fresh or steamed carrots and mangel roots have no thyroid hyperplasia-producing qualities. Fresh turnip is ineffective, but steamed turnip is moderately goiter-producing. Brussels sprouts resemble cabbage in this respect.

Marine reasoned that since steamed cabbage is more goiterogenic than fresh, this property increases as the ascorbic acid content decreases during heating. Sterile fractions from the suprarenal glands, containing ascorbic acid, not only failed to produce goiter in rabbits, but had the opposite effect of causing involution. Marine concluded from the observations recorded that cabbage contains a powerful goiterogenic substance which acts by exhausting the thyroxin store of the thyroid gland. This is destroyed by enzymes in cabbage, but is stable when these are destroyed by heat. When present in the diet in sufficient amount, the goiterogenic substance rapidly depletes the iodine store, which would have been sufficient, in its absence, and hyperplasia results.

It was shown that cabbage is inactivated by drying in air at 25-35° C., or *in vacuo* at higher temperatures. Cabbage extracted with alcohol or acetone is quite inactive goiterogenically. The extracts, evaporated *in vacuo,* were likewise inactive. Prolonged boiling with access to air, or in acid or alkaline solution, or autoclaving, does not destroy the goiterogenic substance. Cabbage was extracted with ether and the fatty material obtained thereby was definitely goiterogenic.

Marine and associates tested the feeding of mustard oils (e. g., allyl isothiocyanate) to rabbits but with negative results. Injection of small doses of acetonitrile daily for 21 days caused striking thyroid hyperplasia, as did likewise hydrogen cyanide. Rats and mice were much less affected by the latter reagent than were rabbits but in spite of these numerous investigations the goiterogenic substance in cabbage has not yet been identified.

The Antigoiterogenic Effect of Ascorbic Acid.—The fact that heat treatment which destroys the antiscorbutic principle (ascorbic acid) in cabbage likewise increases its goiterogenic property, suggested to Marine and associates that ascorbic acid might have a protective, or antigoiterogenic action. They found that the antigoiterogenic property of fresh plant and fruit juices was in general inversely proportional to their iodine reducing capacity. They (Marine, Spence and Cipra, '32) maintained immature rabbits on a diet of alfalfa hay and oats, which diet is goiterogenic, and showed that the goiterogenic effect was greatly enhanced by methyl cyanide. They showed that rabbits fed this diet, which were injected with the thyrotropic extract of the hypophysis, first prepared by Loeb and Bassett, and simultaneously given 100 gm. per day of ascorbic acid by mouth, had thyroids about 30 per cent smaller than those given the thyrotropic factor alone. The guinea pig thyroid reacts quickly to the thyrotropic factor, and more slowly to ascorbic acid. The former, when both factors were being simultaneously administered, caused marked hypertrophy of the thyroid by the fourth day. By the eighth day this had disappeared entirely, if the proper amount of ascorbic acid had been given. They found that ascorbic acid directly or indirectly counteracts the thyrotropic factor. It is apparent, therefore, that keeping the tissues saturated with ascorbic acid is a useful procedure for preventing iodine depletion, which may be brought about by several factors already mentioned.

Iodine Requirements.—As we have stated previously, the iodine requirement appears to depend in considerable measure upon the physiological status. Children, adolescents—particularly during puberty—and pregnant and lactating mothers, require considerably larger amounts than normal adults, if the higher incidence of goiter in such groups may be regarded as an indicator of relative needs. Estimations based on "iodine balance" studies may be looked upon with some skepticism. From the standpoint of cost it is essentially impossible to secure such data on more than a very limited number of persons. Moreover, the restrictions necessarily placed on subjects being tested are such that the results can be scarcely regarded as representative of normal conditions. We are therefore without any very precise information on the subject of iodine requirements. It is rather certain, however, that the range of iodine tolerance is wide. Hence, a safe practice would be the free use of iodized salt (under the supervision of public health officials), sea foods, and other natural foods relatively rich in iodine. Seaweed, according to McClendon ('33, l.c.), has about 1000 times as much iodine as any other food. Since it has been a constant constituent of the Japanese diet for many generations, and apparently without any injurious effects, it would seem that even

that food might be used with impunity by people to whom it is palatable.

There has been some question of the utilization of different forms of iodine. Since this may be of some significance with respect to iodine needs, investigations of the question are of definite value. Remington, Coulson and Levine ('36), in studies on the rat, have shown that iodine in the form present in milk, oysters, and dried haddock, was just as effective in the prevention of thyroid hypertrophy and in the promotion of iodine storage in the gland as was potassium iodide. In general, the two forms of iodine were approximately of the same value. Certain forms of iodine, however, are not very available in nutrition. For example, the iodine of iodosalicylic acid was poorly utilized by laying hens (Asmundson, Almquist and Klose, '36). In view of the present evidence there is no real basis for contention that either "food iodine" or "inorganic iodine" is superior.

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CHAPTER

XI

"Trace" Inorganic Elements

CHEMICAL ANALYSES of the animal body and of its products reveal the presence of a large number of the mineral elements, while spectrographic examinations detect traces of many others. Until recent years little significance has been attached to these "traces" of inorganic constituents. Whether certain of these are present because they fulfill some physiological function or are incidental to the ingestion of foods containing them is not yet known. Up to the present time there is very little to indicate the part played by these physiologically rare elements in human nutrition; therefore it is necessary to view the subject from the standpoint of animal nutrition. There are two aspects of the problem of trace elements in relation to health, —one is that of the nutritional need of the animal organism, and the other that which is manifested in industrial hygiene, agriculture, and in foods, namely, the possibility of intoxication due to any one of these minerals.

The mineral content of foodstuffs is subject to many determining factors,—the soil composition, effective rainfall, and other weather elements; for example, aridity appears to depress the phosphorus content of crops and to raise the calcium content.

The continued feeding of rations deficient in one or more indispensable elements, either because of an injudicious selection of feeds, or of abnormalities in composition of foodstuffs related to soil deficiency, drought, or other cause, will eventually lead to the production of pathological symptoms. The excessive feeding of minerals may result in mineral malnutrition often of a severe type. The presence in foods and water of toxic minerals, such as fluorine and selenium, also leads to mineral malnutrition when such materials are consumed in amounts above certain safe minima.

Of the trace elements which are known to play physiological roles and are indispensable in the diet, iodine, iron, and copper have already been discussed. In this chapter will be considered manganese, zinc, and cobalt, which appear to occupy positions of indispensability. In addition, aluminum, strontium, vanadium, bromine, boron, silicon, and nickel, which are believed by certain investigators to play physiological roles, but which appear not to have been definitely proven to be essential for normal nutrition, will be discussed, as well as fluorine and selenium. The nutritional importance of the last two elements is open to question, but they are known to constitute health hazards.

The efforts to secure undisputed evidence for the nutritional role of any particular element are attended with much experimental difficulty. This is particularly true when the element in question may be required by the animal in only small amounts. The experimental approach involves the removal from the diet in so far as is possible of the element to be studied.

Manganese

Manganese is universally distributed throughout the vegetable kingdom. The indispensability of this element for plant development has been well known to experimental botanists for some years. The need for it is now recognized to such an extent that manganese is included as a matter of course in a complete nutrient solution for water-culture work, although toxic effects may ensue if the amount present is very large.

The occurrence of manganese in animal tissues and the example of its necessity in the vegetable world indicated that manganese might be needed by the animal body.

Several experiments had been recorded in which it was sought to test the effects of adding manganese to diets not devoid of this element, but without significant results. Richards ('30) and Peterson and Skinner ('31) had indicated its significance for reproduction in both plants and animals. Orent and McCollum ('31) made the first crucial test of the need of this element by animals. They prepared a diet extremely low in manganese, and observed that young rats on this ration could grow to maturity in an apparently normal manner. The females were shown, by the vaginal smear method, to go through normal estrous cycles. They produced approximately the normal number of young when mated with normal males. These females, deprived of manganese, failed, however, in 97 per cent of the cases to suckle their young. They appeared to be indifferent to them and did not give them the opportunity to suck which is char-

acteristic of female rats on the same dietary formula with small amounts of added manganese.

The manganese-free rats, when given foster young from stock litters, failed in 8 cases out of 10 to suckle them although the young were vigorous and aggressive because of hunger. The 7 young reared by the other 2 females were retarded, apparently from insufficient milk supply.

Lactating female rats of the breeding stock did not show interest in and therefore did not give opportunity for the young of manganese-free mothers to suck. Only 7 of 107 manganese-free young were reared by stock foster-mothers. These were undersized and inferior in appearance although the litters were small and in all likelihood secured all the milk they wanted.

Male rats raised on a manganese-free diet showed no abnormality other than testicular degeneration. This degeneration is well under way by the 100th day on the diet. The atrophy then rapidly progresses until only vestiges remain and complete sterility results. There was little or no obesity in these males. The same manganese-free diet, upon the addition of 0.005 and 0.05 per cent, respectively, of manganese in the form of the chloride, kept males in sexual potency for at least 14 months. Their testes appeared normal at the end of the experiment.

The element was found to be absent, by the spectrographic test, from the young of manganese-free mothers. It is present in newborn and 15-day old rats from mothers on manganese-containing diets; hence the element passes through the placenta if present in the mother's blood. Manganese was spectrographically absent from the organs of rats raised on the experimental diet. It was suggested, from the results of these experiments, that manganese is in some manner concerned with the production of a hormone by the anterior lobe of the hypophysis, which is essential for the functioning of the testes and for the normal development and functioning of mammary tissue.

Kemmerer, Elvehjem and Hart ('31) found that mice restricted to a diet of whole milk supplemented with iron and copper, did not grow so well as when 0.01 mg. of manganese per mouse per day was added. Without manganese addition their mice did not ovulate normally, but when iron, copper, and manganese were added as supplements to milk the animals exhibited normal estrous cycles. In view of this apparent species-difference, Orent and McCollum ('32) repeated their work and confirmed their earlier observation that rats on a manganese-free diet exhibit normal estrous cycles and that deprivation of this element had no appreciable effect on growth. Waddell, Steenbock and Hart ('31) attribute the somewhat slower growth rates and subnormal final weights of rats restricted to milk diets to a low caloric intake because of the high water content of the diet.

Skinner, Van Donk and Steenbock ('32) and Van Donk, Steenbock and Hart ('33) reported that female rats receiving a whole milk diet supplemented with copper and iron did not exhibit normal estrous cycles. Addition of manganese had no effect on the estrous cycle. Their animals on the milk-copper-iron-manganese diet often failed to exhibit estrous over long periods of time and when cycles did occur, they were less frequent than in the normal animal. However, supplementation of the whole milk-copper-iron diet with sucrose or milk solids resulted in improved growth, ovulation, and reproduction in the rats. A deficiency of milk in energy for reproduction is therefore suggested by these experiments. Hence the difference in the type of diets may possibly explain such discrepancies as exist between the observations of the two laboratories (Johns Hopkins and Wisconsin). In addition, it should not be overlooked that milk may be somewhat deficient in unknown nutrients essential for development into fully normal adulthood which were provided in the diet of Orent and McCollum. Also, Keil and associates ('34) showed that the need of manganese for growth or reproduction may depend to some extent upon the store of manganese in the rats when the experiment is begun. These investigators obtained reproduction in first generations of rats fed milk supplemented with iron and copper, but the second generation failed to reproduce unless manganese was added.

Daniels and Everson ('35) employed a milk-iron-copper-manganese diet on which they reared fifth generation young rats with approximately 100 per cent viability. The same diet (i. e., cow's milk) without added manganese contained 0.03 mg. of this element per liter. On the latter diet 8 first generation females produced 103 young; 47 in first litters, 33 in second, and 23 in third. These young were sired by stock males, since males on the manganese-deficient diet are sterile. Of these 103 young, 41 were either born dead or died within two hours; 25 were anesthetized for analysis; and 37 were transferred to stock mothers. Sixty-nine per cent of the latter subsequently died within a few days; 12 were raised by 2 females (6 each) to average weaning weights of 53.7 and 41.5 gm. respectively. In marked contrast to these results were the young of fifth generation females restricted to milk-iron-copper-manganese. One hundred per cent of these were reared with an average weaning weight at 28 days of 69.2 gm., a weight corresponding to young of the breeding stock. The rats must have secured an adequate caloric intake from this particular liquid diet.

Mother rats on this manganese-deficient diet, which had lost their young at birth, were able to suckle 96.9 per cent of foster young from the stock colony to average weaning weights of 58.7 gm.

In 1935 Daniels and Everson (l. c.) using a milk-iron-copper diet containing 0.03 mg. manganese per liter, studied the cause of the high

mortality in the young born of female rats on this manganese-low diet. These young were sired by stock males. More than a third of the young of manganese-depleted mothers were born dead or died shortly after birth. Those that did survive were not active enough to suckle even when transferred to stock mothers; for 69 per cent of them subsequently died within a few days. However, mothers on the manganese-low diet were able to suckle young taken from mothers on a normal diet. The diet of Orent and McCollum was much lower in manganese than that of Daniels and Everson, which may account for the lower capacity of their rats to suckle stock young.

From Daniels and Everson's observation and those made by Orent and McCollum, it is evident that manganese plays an important role in the development of the fetus during gestation. Fertility persists in female rats restricted to a diet so low in manganese that the young are not normally nourished *in utero,* and are debilitated at birth.

Tutt ('34) described 3 cases of sterility in cows, in 2 of which ovarian cysts were found. Injections of colloidal manganese were given and pregnancy followed in 2 animals. In the third, administration of manganese and wheat germ oil were unsuccessful.

It is of interest that attempts to induce symptoms of manganese deficiency in pigs have not been successful even though the concentration of manganese in the ration was reduced to 1/17 of that of a normal ration and the feeding was continued for 10 or 11 months. It appears that the manganese requirement of the young growing pig is satisfied by a concentration of 1 part in 180,000 of the ration. If injury from manganese deficiency is to be produced in this species, it will be necessary to reduce the manganese content of the ration still further.

The role of manganese in the nutrition of birds has recently assumed practical importance. Wilgus and coworkers ('37) have shown that a bone abnormality, called perosis, first described by Hunter and Funk ('30) and by Payne ('30), can be almost entirely prevented by addition of 0.0025-0.015 per cent of manganese to a diet on which the disease regularly occurs in a high percentage of chicks although it already contains 0.001 per cent of this element. The symptoms of this disorder are gross enlargement of the tibial-metatarsal joint, twisting or bending of the distal end of the tibia and proximal end of the metatarsus, and slipping of the gastrocnemius tendon from its condyles. The latter incident causes severe crippling in the affected leg and if both legs are so affected, death soon follows from inability to get food. Excess of calcium and phosphorus in the diet tends to intensify the disease, whereas it has been shown by Titus ('32) that the pericarp of rice, oats, and wheat, tends to prevent it. This action is now attributed to their high content of manganese. Wilgus and asso-

ciates (l.c.) found that zinc and aluminum possessed a similar preventive property, but were less effective than manganese. It would appear that these elements exert their effect through binding phosphate in the intestine and preventing the formation of insoluble and unabsorbable manganese phosphate. A mixture of manganese, aluminum, and iron was shown to be entirely preventive in the presence of limited amounts of calcium and phosphorus. From the evidence now available it appears that deficiency of manganese is the primary cause, but that excessive feeding of calcium and phosphorus aggravates the condition, and such preventive action as other elements may afford is through their influence on the absorption or excretion of manganese, calcium and phosphorus.

Lyons and Insko ('37) observed that eggs from hens which had been fed a manganese-deficient diet gave a hatchability of less than 10 per cent. Those embryos which were sufficiently developed for observation before death showed without exception very short legs, but did not develop slipped tendon. Another group fed the same ration plus 40 p.p.m. each of manganese as sulfate, and 100 p.p.m. of iron as ferrous ammonium sulfate, produced eggs giving good hatchability and normal chicks. Embryos which died showed normal development of appendages and mandibles. Eggs from hens fed the perosis-producing diet contained much less manganese than those from hens fed the supplements named. These observations suggested manganese deficiency as the cause of the abnormality. This was proved to be correct by the injection of 0.03 mg. of manganese directly into the albumen of such eggs just prior to incubation, which resulted in an increase in hatchability and the normal development of the embryos. The metatarsi, tibiae, and humeri of chicks and 20-21 day embryos from the injected eggs were 52, 44, and 40 per cent longer, respectively, than the same bones from the eggs which were not injected with manganese. The injection of a similar amount of zinc salt into the eggs had no preventive effect on the disorder. These results have been confirmed and extended by the studies of Gallup and Norris ('38).

Skinner and associates ('31) found 0.038 and 0.034 mg. of manganese per 100 gm. in the shaft and head, respectively, of fresh rat bone, with only traces in the marrow. By feeding a diet high in manganese they could increase the content of this element to 0.221 mg. Gallup and Norris ('38, l.c.) found 0.060 mg. per 100 gm. of dry matter in chick bones from a manganese-deficient diet, whereas by supplementing the diet with this element they raised the content in bone to a fairly constant value of about 0.200 mg. Partial depletion of the bones in manganese resulted in the characteristic deformities.

Lyons, Insko and Martin ('38) studied the manganese require-

ment of the growing chick. They found that the minimum protective level of manganese under the conditions of their experiment appears to be 35—40 p.p.m. of manganese. These investigators state that the exact minimum protective level of the element cannot be fully established until the relationship between the calcium and phosphorus of the diet and the availability of manganese is known. They suggest that there may be three main factors which determine the protective level of manganese in the chick's diet: (1) the level of available manganese in the diet of the mother hen; (2) the calcium and phosphorus content of the diet; and (3) the availability of the manganese supplement.

From these studies it appears that the amount of manganese which is essential for normal development is greater for birds than for mammals, and also that skeletal defects are produced in birds which do not appear to occur in the rat. This statement is based only on the observation of Orent and McCollum that their rats on a manganese-deficient diet grew normally, and were long and normally lithe animals. No direct observations are available showing the histological changes, if any, in bones of such rats.

Nothing definite is known about the human requirement for manganese. There appears to be no evidence that manganese deficiency occurs in man, but it is quite possible that it may do so. The experiments of Everson and Daniels ('34) on children, which followed their animal studies, are of interest from that standpoint. Their results indicate that there is a definite manganese need in children, which may not be met by diets that appear adequate in all other respects. The amount of manganese retained was found to be proportional to the amount ingested, thus indicating that manganese is essential to the physiological development of children. They suggest, therefore, that the diet of children should contain between 0.20 and 0.30 mg. of manganese per kilogram of body weight.

Chronic manganese poisoning has been recognized both in man and animals. The toxic effects observed in animals are only with doses so large as to cause gastric disturbances. These effects include degenerative changes in the central nervous system, liver, and other organs, and disturbances of the circulatory system.

Becker and McCollum ('38) report that on purified diets supplemented by 0.0499, 0.0998, 0.2495, and 0.4990 gm. manganese, rats grew well and produced healthy young. On a diet supplemented with 0.9980 gm. of the mineral, there was some depression of growth. Reproduction, however, was excellent. No symptoms of manganese toxicity were observed. Evidently the high phosphorus content of the diet prevented such symptoms by reducing the amount of absorbable manganese.

Zinc

Zinc has been shown by a number of investigators to be an essential element for the development of plants. However, owing to its wide distribution in foods, it is unlikely that zinc deficiency ever occurs in animals except when induced experimentally. That it may occur naturally in plant life has been observed by Finch and Kinnison ('33).

Zinc occurs in practically all animal tissues but is most abundant in the liver and pancreas. Only traces of this element are found in lung, brain, or testicle. The zinc content of blood is markedly variable. Colostrum contains three times the amount of zinc present in normal milk, and the zinc content of active mammary gland is twice as high as that of the inactive gland.

The earliest attempt to find whether zinc is important in animal nutrition was made by Bertrand and Berzon ('22), who, however, employed a diet deficient not only in zinc but in several nutrient essentials. They noted that rats receiving zinc survived 25 to 50 per cent longer than their zinc-low controls. Hubbell and Mendel ('27) observed that mice throve better with added zinc than did their controls. Newell and McCollum ('33) could not reduce the zinc content of diets low enough to show that this element was important, but Todd, Elvehjem and Hart ('34) were more successful in this respect, having prepared a diet containing but 1.6 mg. per kilogram of food. Addition of zinc to this diet, which was supplemented with 2 cc. of milk per rat per day improved the growth of the animals. Stirn, Elvehjem and Hart ('35) have reported that zinc is an essential element in nutrition. The only effects produced by deprivation of this element which they described were retardation of growth and interference with the development of a normal coat of hair. More recently Hove, Elvehjem and Hart ('37) pointed out that there are some indications of deficient intestinal absorption in rats on zinc-low diets.

An interesting suggestion concerning the possible action of zinc came from the work of Scott ('34), who showed that zinc was a constituent of insulin crystals. In view of Scott's clear-cut evidence concerning the rather unique effects of zinc salts on the formation of crystalline insulin, and the fact that the pancreas is very rich in zinc, this element may play a significant role in carbohydrate metabolism. However, a definite interpretation as to how zinc functions cannot yet be made. At present there is no definite relation known between zinc and insulin activity.

Maxwell ('34) found that the presence of small amounts of zinc salts in hypophyseal extracts produced a marked augmentation in the ovarian weight increase in young female rats. He also observed the

augmentation with pregnancy-urine preparations to be increased when zinc salts were added to the hypophyseal synergistic component. Hove, Elvehjem and Hart ('37, l.c.) also have suggested a relationship between zinc and the hypophysis. At present there is but little basis for this postulate. Until a better dietary procedure is developed for the production of zinc deficiency it will be impossible to make positive assertions regarding the indispensability of this element.

Cobalt

The demonstration of the biological significance of cobalt is one of the most interesting of recent developments in nutritional research. McHargue ('27), Fox and Ramage ('30), and others reported finding traces of cobalt in biological materials, but Stare and Elvehjem ('33) stated that if this element occurs in animal and plant products it does so in extremely small amounts. They found less than 0.01 mg. of cobalt in 100 gm. of milk. Blumberg and Rask ('33) made a spectrographic analysis of the ash of milk and could not detect cobalt lines in the spectrograms. Stare and Elvehjem found less than 0.01 mg. of cobalt in the entire body of rats reared on milk supplemented with iron, copper, and manganese. The amount in the organs of young swine reared on the same ration was of a similar order.

Waltner and Waltner ('29), while studying the toxicity of various metals, found that a polycythemia was produced when either powdered metallic cobalt, cobalt chloride, or nitrate in amounts of 0.5 to 2.0 per cent was added to the mixed diet of normal rats, or when 0.01 or 0.1 gm. of cobalt chloride or cobalt nitrate was injected subcutaneously. This fact was corroborated by Orten and associates ('32). The following table illustrates the effect on the blood of administration of cobalt:

BLOOD FINDINGS IN RATS FED MILK-IRON-COPPER DIET SUPPLEMENTED WITH COBALT SULFATE

GROUP NO.	SUPPLEMENT TO MILK-Fe-Cu DIET	BODY WEIGHT	HEMOGLOBIN	CELL VOLUME	ERYTHROCYTES
		gm.	*gm. per 100 cc.*	*per cent*	*per c.mm.*
I–A	None	236	12.2	52	7,900,000
II–A	$CoSO_4$	180	17.0	73	10,700,000

Orten and associates ('32, '33) observed in cobalt polycythemia definite rise in blood volume, which was due to increased cell volume rather than to significant change in plasma volume.

Feeding manganese with the milk-iron-copper-cobalt diet seemed to alleviate the toxic effects resulting from prolonged administration of small amounts of cobalt. It also seemed to increase the effect of cobalt in raising the red cell count, which the authors express as "some stabilizing influence on the increased hemoglobin, erythrocyte, cell volume, and blood volume values characteristic of cobalt polycythemia."

Little information is available at the present time regarding the mechanism involved in the production of polycythemia by cobalt. Preliminary work indicates that the spleen does not play an essential role in the phenomenon.

Ascorbic acid seems to assist in the maintenance of a normal level of red blood cells in the circulating blood. When ascorbic acid was injected intravenously, simultaneously with cobalt, into rabbits, polycythemia failed to appear; when ascorbic acid was injected after the production of polycythemia, a decrease in the hemoglobin concentration and red blood cell count resulted, but the effect was temporary. According to these investigators (Barron and Barron, '36), cobalt polycythemia seems to be due to the inhibition by cobalt of the respiratory function of immature red blood cells.

"Coast Disease," "Bush Sickness," "Pines," or "Enzootic Marasmus" in Farm Animals.—In New Zealand, Australia, Florida, and certain localities of the North Gulf and South Atlantic states, there has long occurred a disease of sheep and cattle known as "coast disease," "bush sickness," and "wasting disease." The condition was described in 1896 by Aston, in New Zealand, as a disease affecting cattle. The animals become greatly emaciated, primarily as the result of loss of appetite, and suffer progressive anemia. The anemia suggested iron deficiency, and analysis of the soils and herbage in areas where the disease occurred showed that they were very low in this element. The provision of iron licks, in the form of limonite, a hydrated ferric oxide, in some instances afforded complete relief to the animals, but in other cases it was less effective or not at all. After some years of study it was shown by Underwood and Filmer ('35) that an iron-free preparation made by acid extraction of limonite, was effective, and the prophylactic value of the extract was traced to its content of cobalt. This was the first evidence that cobalt is an essential nutrient for mammals. Marston ('35) and Lines ('35) reported successful cures of sheep suffering from "bush sickness" by means of pure cobalt salts. Lines ('35, l.c.), Askew and associates ('36, '37), and Dixon ('36) have extended these studies, and have fully confirmed the view that deficiency of cobalt in soils and in herbage grown on deficient soils is the cause of the disease. The cobalt requirement of sheep (Lines, '35, l.c.) is apparently about 1 mg. per ewe daily. Denham ('37) has fully reviewed the investigations in this field.

Neal and Ahmann ('37) have pointed out that in Florida, on certain types of soil, ferric citrate or iron oxide and copper sulfate were found effective in preventing "salt lick," an anemia occurring in cattle. On other soils, these supplements were not sufficient because of deficiency of cobalt as well as iron and copper. They found that calves fed a ration of Natal grass hay, corn, and dried skim milk developed the anemia, and were cured by cobalt administration.

They describe the symptoms in cattle to be long, rough hair, scaliness of the skin, listlessness, gauntness due to loss of appetite, muscular atrophy and retarded development of sexual characteristics. The erythrocyte count may be above average, and the hemoglobin concentration equal to or above that in animals receiving cobalt and growing normally. Volume and color indices show that the condition is a microcytic hypochromic anemia. The spleen is shrivelled and fibrous, and the heart flabby, although of normal size. They describe growth studies in calves fed Florida-grown feeds, and show that supplementing these rations with cobalt is essential to successful animal production in this region.

The studies described illustrate how elusive may be the cause of malnutrition in animals or in man in certain instances. The discovery of the indispensability of cobalt is an example closely paralleling the problems in animal and poultry production arising in certain regions from iodine and from manganese deficiency.

Nickel

There is at present no definite evidence concerning the significance of nickel in animal nutrition.

Nickel is present in some foodstuffs. Blumberg and Rask ('33, l.c.) could not detect it spectrographically in cow's milk. Newell and McCollum ('31) reported its occurrence in certain edible fish. Bertrand and Macheboeuf ('25) found nickel in brain, skin, spleen, pancreas, and liver. The fat, muscle, and uterus contained but small amounts of this element. Dutoit and Zbinden ('30) found it by spectrographic analysis in the ash of human blood and they also observed its tendency to accumulate in the human pancreas. Fisher and Scott ('35) were unable to detect nickel in the pancreas of cattle of any age. They state that possibly only traces of these metals are present and that the amount is beyond the sensitivity of the method used in their studies.

According to Bertrand, insulin preparations contain a considerable amount of nickel. He showed that the simultaneous injection of a small quantity of nickel and insulin into a rabbit or a dog greatly increased the action of the insulin on the blood sugar. Blatherwick and Sahyun ('27) did not confirm these findings. They found that there

was a great deal of individual variation in the insulin hypoglycemia resulting from a certain dose per kilo of body weight. They suggest that the results obtained by Bertrand might have been due to this variation rather than the metal.

Boron

Boron has been demonstrated to be essential for plant growth, but there is no experimental evidence in support of the view that it is necessary for animals. Data on the distribution of boron in animal tissues is scant. Bertrand and Agulhon ('13) reported the presence of this element in various tissues of the guinea pig, rabbit, sheep, ox, and horse. Boron was also found in human, cow's (Wright and Papish, '29; Blumberg and Rask, '33, l.c.), and ass's milk, as well as eggs of fowl (Drea, '35), turkey, and goose. This mineral element appears to exist normally in small amounts in all animals, being more common in the species of marine origin.

McCollum and Yue ('37) studied the distribution of boron in rats of their stock colony fed the stock diet, in rats from this same colony fed purified diets containing varying amounts of boron, and also in pathological organs and tissues of humans. They observed that the boron content of the whole rat of their stock diet increases with age, and varies with the size of the animal. The results of these experiments indicate that this element is transmitted to the young through the placenta. Newborn young contain a fairly high concentration of boron, but the element seems to decrease during the nursing period, which leads to the suggestion that the milk of these rats is probably poor in boron.

Boron is invariably found in all the tissues of the adult rat; hair and skin being the richest locales. The boron per cent in muscle and the skeleton is always low, but it seems that some is always present. The boron content of the hair not only increases with age, but also varies with sex and the concentration of the element in the diet. The diet plays a major part in determining the boron content of the rat. Most of the boron ingested is excreted and but a small amount is retained by the animal organism.

In pathological tissues of human subjects, boron is present in nearly all the organs and tissues. As in the rat, the accumulation of this element is greatest in the hair. Heart, stomach, lung, and kidney follow in the order named. Age seems to be an important factor in regulating the concentration of boron in both animal and human tissues. The amount of the element in the human body increases with age and varies with individuals. Apparently the origin of boron in the animal body lies in the food ingested.

Bromine

Bromine is an invariable constituent of marine algae. Land plants contain considerably less of this element. Organs and tissues of all marine and land animals contain it, but in variable amounts. It is thought that the actual bromine content of any tissue is determined by environmental conditions and perhaps selective affinity by the species and cells in the different parts of the individual tissue.

The metabolic significance of bromine has been discussed by a number of investigators. Bertrand ('20) suggests that this element may act as a catalyzer in the body. Various statements (Ucko, '34; Kuranami, '32, '33; Bernhardt and Ucko, '26; Zondeck and Bier, '31, '33; Jacobson, '35) have been made that the anterior pituitary gland is concerned with the metabolism of bromine. These findings, however, have been contradicted by Serbescu and Buttu ('34), Dixon ('35), and Neufeld ('36). The amount of bromine normally present in the pituitary is of the same order as that of the blood. At this time there is insufficient evidence for the belief that the pituitary plays an important role in the physiological function of bromine.

Tanino ('31a, '31b) and Jacobson ('35, l.c.) have considered a possible relation between the thyroid gland and bromine metabolism. They believe that the thyroid probably produces a bromine-containing substance, possibly dibromthyroxin. Neufeld ('36, l.c., '37) has shown that the thyroid contains amounts of bromine somewhat higher than those present in other tissues. The Br:Cl ratio in mammalian tissues in Neufeld's experiments does not show sufficient constancy to suggest the existence of any physiological ratio. However, the ratio for the thyroid is relatively much greater than for other tissues. A larger number of analyses are needed before attaching significance to this finding. From the experimental evidence available it is not yet possible to state that bromine plays a role in the physiology of this gland. Neufeld's results indicate that it is not functionally associated with the essential principle of the thyroid. Further investigation is necessary before any functional significance of bromine to the thyroid gland is ascertained.

Winnek and Smith ('37) studied the nutritional effect of synthetic diets containing 5 p.p.m. bromine. They observed no particular difference between the general appearance, rate of growth or reproduction of the animals on the bromine-low diet, and the diet supplemented with the element. There was no evidence that bromine is essential in the nutrition of the rat. The amount of bromine in the tissues of these animals depends not only on the amount of bromine ingested, but also on the Br:Cl ratio of the diet. The young born to mothers on the bromine-low diet have a greatly diminished bromine content as compared with young born to the stock females.

Silicon

Next to oxygen, silicon is the most abundant element in nature. It is found in plants and in many marine organisms. In small amounts it appears to be a natural constituent of all animal tissues. It is present in hair, skin, thymus, tendon, dura mater, and lens of the eye. The silica content of the individual organs appears to be directly dependent upon their connective tissue content (Schulz, '01). Gonnerman ('18) determined the silica content of bile, blood serum, stomach, intestine, liver, kidney, heart, brain, testicle, skin, and hair. He ('20) also found silica in various concretions, some of which were of human origin; they included biliary calculi, urinary calculi, bezoars, and enteroliths. This mineral has been found in milk (Wright and Papish, '29, l.c.; Drea, '34), in edible fish (Newell and McCollum, '31, l.c.) and in all other tissues from normal chicks (Drea, '35, l.c.). Brown ('27) obtained quite variable results for the silicon content of human skin. A definite, but not marked, tendency apparently existed for a decrease in the silicon content of the skin in older persons. King and coworkers ('33) have detected small but significant amounts in fetal tissue. They also showed that in adult animals a considerable excretion of silicate occurs through the kidneys and that the concentration of soluble silicates in the urine is influenced by the diet. When the urinary excretion of silica was markedly increased there was no significant change in the blood silica, except slight temporary increases, indicating an extremely low renal threshold for silicates. At present there is no experimental evidence to show whether silicon is a normal constituent of protoplasm or whether its occurrence is merely adventitious.

Strontium

Stoeltzner ('08) and Lehnerdt ('10) observed that feeding strontium led to the formation of excessive amounts of osteoid tissue in bones.

Wheeler ('19) found that strontium was capable of replacing calcium to a considerable extent in the egg shell and in the bones. Fewer eggs were obtained from hens fed strontium salts than those fed calcium salts.

Shipley and coworkers ('22) pointed out that this mineral cannot replace calcium in bone formation. Kinney and McCollum ('23) found that the entrance of strontium into the bones was not inhibited by the presence of calcium in the diet. Lesné, Vagliano and Christou ('24) confirmed these observations.

Drea ('35, l.c.) showed that strontium was one of the elements that pass from the feed and water into the hen's blood and egg, and

then into all of the chick's tissues and the chick's blood. Drea believes these findings to be of definite physiological significance. He suggests that this distribution of strontium may be due to the inability of the hen's organism to distinguish it from calcium.

Vanadium

There is little information about the effect of vanadium in the diet. The few studies reported have dealt with the toxic action of vanadium salts and most of these have been pharmacological experiments.

Vanadium is one of the "trace" elements in the hen's blood and egg. It is present in significant amounts in the blood of the chick and hen, in the femur, heart, kidney, and lung. Drea ('35, l.c.) believes that this suggests some function associated with the hemapoietic system.

Several French experimenters (cited by Lyonnet et al., 1899) have recorded the therapeutic effects of small doses of vanadium in various diseases.

Daniel and Lillie ('38) in a study of the nutritional significance of this element report certain basic data as to tolerance levels of vanadium in the diet and the histologic effects produced by the ingestion of the element. They fed the mineral in the form of sodium metavanadate in the following amounts: 11.5, 23, 92, 184, and 368 p.p.m. respectively. The animals on the diets containing 11.5 and 23 p.p.m. vanadium showed no toxic symptoms. On the diet containing 368 p.p.m., death of the rats occurred. In the amounts fed in these experiments they noted no significant indication of a stimulating effect of small amounts of vanadium. There was no evidence of a cumulative effect from vanadium ingestion.

With the high concentration of vanadium, the food consumption of the rats decreased and starvation was superimposed on the condition of chronic poisoning. A definite symptomatology and pathology was produced by the higher levels of vanadium in the diet.

Aluminum

Aluminum is universally distributed in soils and plants, and animals have been in intimate contact with this element throughout the ages. Numerous analyses of plant and animal tissues have been recorded in the literature, but whether or not uncontaminated tissues from either class of substances contain appreciable amounts of aluminum is still a matter of dispute. It is generally agreed that the amounts present, if any, are very small. There are two types

of analytical procedure for its estimation in extremely small quantities: (1) the spectrographic method based upon the number and intensities of lines in the spark or arc spectrograms. As the amount of aluminum in the sample decreases to extremely small amounts, certain lines fade out, and the results may be made roughly quantitative by comparing lines from the material under examination with those of standards prepared with known amounts of the element. (2) The other method depends upon the ability of aluminum hydroxide to absorb dyestuffs quantitatively, with the formation of colored complexes called lakes. These lakes when developed to their maximum intensity by pH control, are compared as to color intensity with standard lakes prepared from aluminum solutions of known content. As little as 0.0005 mg. of aluminum may be estimated by this method. The spectrographic method is even more delicate as a test for the presence or absence of aluminum. The great difficulty with all such determinations is the prevention of contamination of the ash with aluminum during the ashing process. The air in an ordinary room contains considerable floating material, as is evidenced by the visibility of a beam of light falling through the air when viewed at right angles to the beam. All ordinary dust contains a high percentage of aluminum. The usual practices of ashing a sample for hours at relatively low temperatures expose it to dust long enough to contaminate it with quite appreciable amounts of aluminum, and the metal from this source appears in the result.

McCollum, Rask and Becker ('28) employed the spectrographic method and concluded that uncontaminated plant and animal tissues contained not more than 0.5 parts of aluminum per million. Kahlenberg and Closs ('29) likewise employed the spectrographic method and found easily detectable amounts of aluminum in all samples examined. McCollum, Rask and Becker pointed out that Kahlenberg and Closs had mistaken two calcium lines for aluminum lines and that their spectrograms did not reveal the presence of aluminum in their products. This cause of the dispute, which is still going on, is due to faulty technic, usually accidental introduction of aluminum into the sample. Certainly the amounts of the element in natural products (the inner portions not exposed to dirt) are almost vanishingly small.

McCollum, Rask and Becker ('28, l.c.) studied the possible role of aluminum in animal physiology. They concluded that aluminum compounds are not absorbed from the stomach or intestinal tract when present in the diet. Aluminum compounds when present in the alimentary tract do not form any union or compound with the stomach or intestinal walls. Aluminum in the diet in concentrations as high as 600 p.p.m. exerts no noticeably deleterious action on growth, reproduction, or general well-being as judged by external appearance and

autopsy. Similar observations have been made by a number of other investigators using the rat as the experimental animal as well as the dog, rabbit, pig, and guinea pig.

The absorption of aluminum is exceedingly small. No systemic pharmacologic effects can be ascribed directly to absorbed aluminum. It has been demonstrated that the absorption of phosphorus from the digestive tract is governed by dietary aluminum. However, this can be shown only under grossly exaggerated circumstances. For example, depressed phosphorus absorption was observed in an experiment in which 1,400 p.p.m. of aluminum was added to the diet. Under these conditions very severe rickets was produced in young animals due to the inhibition or phosphate assimilation. This exaggerated condition of aluminum concentration with reference to phosphorus can never be encountered with the aluminum naturally present in the diet or derived from aluminum utensils.

That aluminum is not toxic is indicated by the fact that when it was injected instead of taken by mouth, it produced no toxic effects. In these animals growth was not retarded, reproduction and lactation were not impaired; there was no evidence of gross or microscopic pathology in the organs.

All the experimental data lead to the conclusion that no harmful effects can be expected from soluble aluminum occurring naturally in foods or introduced by utensils into a diet of normal phosphorus content. It is highly questionable, therefore, whether aluminum has any physiological significance.

In 1907 President Roosevelt appointed the Referee Board of Consulting Scientific Experts to examine into the alleged health hazard of aluminum. The Board reported in 1914 the conclusion that aluminum compounds, when used in the form of baking powders in foods, were not found to affect injuriously their nutritive value. This referred to the addition of baking powder in the preparation of foods. Aluminum compounds (baking powder containing sodium aluminum sulfate) when added to foods in large quantities (up to 200 mg. per day) may produce mild catharsis; larger quantities usually produce catharsis. This action, the Board recognized, was due to the sodium sulfate produced in the chemical reaction of the baking powder. Sodium sulfate is the modern name for Glauber's salt, which has for centuries been used to some extent as a cathartic drug. The Board reported that "The aluminum itself has not been found to exert any deleterious action injurious to health beyond the production of occasional colic when very large amounts have been ingested," and "When aluminum compounds are mixed or packed with a food the quality or strength of said food has not been found to be thereby reduced, lowered, or injuriously

affected." The Board membership included some of the most famous physiologists and eminent medical men of America.

The above investigation was instituted because of the suspicion that baking powder containing sodium aluminum sulfate as its acid constituent might be detrimental to health. Fear has been created in the public mind largely through the advertising of competing baking powders not containing aluminum. With the advent of aluminum cooking utensils the trade war has been largely shifted to this field. We have repeatedly seen literature which was distributed to homemakers, informing them that the cooking of foods in aluminum vessels would cause cancer. There are numerous expressions of opinions by eminent medical authorities that there is no health hazard in respect to aluminum. Space limitations permit only a few references.

In an editorial in the Journal of the American Medical Association ('26) it was stated: "First, it is not at all certain that there has been an alarming increase in the incidence of cancer. Secondly, it is reasonable to believe that the use of aluminum cooking utensils has increased because they were found to be desirable for cooking purposes. Thirdly, investigations made in Great Britain under the auspices of the Medical Research Council indicated that cooking, even of acid fruits and vegetables, for long periods of time in aluminum ware showed so little aluminum in the juices after cooking that it required the most delicate chemical tests to indicate its presence. Indeed, not only the fruits but the actual acids themselves were boiled in aluminum ware, without the giving off of more than slight traces of aluminum."

In an editorial in Hygeia ('29), published under the auspices of the American Medical Association, occurs the statement: "Due to the advent of aluminum cooking utensils the sale of other types of ware for this purpose has been greatly injured. As a result there has been considerable propaganda during the past few years that the cooking of food in aluminum was a common cause of cancer. There is no scientific evidence for such statements."

In a publication of the U. S. Bureau of Standards ('27) it is stated: "There is no evidence available which would indicate that aluminum cooking utensils represent a potential danger to health."

Similar statements from responsible scientific men and eminent medical authorities in England, Germany, and France could be cited. Those wishing to pursue this subject further are referred to a report (Bulletin No. 3, '23) of the Mellon Institute of Industrial Research, where will be found numerous abstracts from scientific publications and many editorial comments from medical and other reputable publications. From these sources the conclusion is very strongly supported that aluminum is not a health hazard.

Fluorine

The element fluorine has been known to occur in animal structures since 1802, when Morichini first demonstrated that fumes which etched glass were given off when elephants' teeth were treated with sulfuric acid. However, because of its great reactivity the element was not isolated until 1886.

Fluorine is widely distributed in nature, i. e., soils, rocks, and water in the form of insoluble minerals, the most important of which is fluorspar (CaF_2). It is present generally in very small amounts in plants and in animal tissues. Fluorine is found chiefly in bones and teeth, more particularly in the enamel, in skin, cartilage, brain, blood, and striated muscle. No convincing evidence has been as yet produced to show that it performs any useful function in animal nutrition, or that it is an essential nutrient in animal metabolism. The literature dealing with the distribution of fluorine in biological products and the physiological effects of this element has been reviewed by McClure ('33).

Because of its physiological effects rather than its nutritive value, fluorine demands consideration in any discussion of mineral malnutrition. Its occurrence above certain minimum concentrations in waters, and in minerals commonly used as calcium supplements, brings about derangements of structure in function in men and in animals.

McCollum, Simmonds, Becker and Bunting ('25) first showed the detrimental effects of the inclusion of fluorine (0.01 per cent of sodium fluoride) in the diet of rats. Compared with normal specimens, the skulls of these animals were white and more porous and lacked the luster of normal bone. The rami of the mandibles were thinner and less prominent than in the controls. There was osteoporosis affecting the alveolar process. The rats receiving fluorine developed incisors whose anterior surfaces were bleached, whereas normal rat incisors have a bright orange color on this surface. The incisors grew backwards so that occlusion was lost, and the teeth could no longer be chiseled out by attrition to maintain their cutting edges. The teeth were poorly calcified and were brittle, causing the lower incisors to break off at the gum line, whereas the upper incisors, having no occlusion, overgrew into almost complete circles and in some cases penetrated the roof of the mouth. The effect was interpreted as a retrograde disturbance in tooth development rather than a stimulation to over-activity. The molars were well developed, the only abnormality apparent on gross inspection being abnormal whiteness and lack of the normal luster.

It had for years been the practice of farmers to feed finely ground rock phosphate as a phosphorus and calcium supplement to farm

rations. Such natural phosphate may contain as much as 3 to 4 per cent of fluorine. Following the observation of McCollum and associates, Tolle and Maynard ('28) at Cornell, Reed and Huffman ('28) at Michigan State College, and others, demonstrated that feeding rock phosphate caused loss of the reddish yellow color of normal teeth of cattle, and thickening and roughening of the mandibular bones. McClure and Mitchell ('31) did not observe any outward changes in the teeth of rats fed 0.03 and 0.06 per cent of fluorine. However, the results of this investigation led them to the conclusion that fluorine, particularly in the form of its more soluble salts, may cause its deposition as an abnormal constituent in the bones, as demonstrated by an increase above normal in the bone ash of the experimental rats. Smith and Smith ('32) demonstrated the same outward abnormalities in rats fed 0.05 per cent of sodium fluoride, and showed that the teeth did not differ significantly from normal in chemical composition.

It had long been known that mottled enamel occurs in human teeth in certain localities in many parts of the world. In human subjects mottled enamel is characterized chiefly by the presence of dull chalky white or paper white patches distributed irregularly over the surface of the teeth. The teeth may appear dead white and unglazed. Loss of normal translucency causes an unnatural glare in the mouth. Such teeth frequently are pitted and corroded, and are structurally weak; when decayed they do not hold fillings well. The mottling, or staining, of these teeth is a secondary phenomenon, some never becoming stained. The stain varies from dark brown, almost black to yellow, and is usually most pronounced on the upper central incisors. These investigators point out that the stain tends to follow the lip line, and suggest that exposure to light and air may be a factor in its production.

Black ('16) examined mottled teeth histologically and found that the intercementing substance normally present between the enamel rods is lacking. In severe cases the enamel prisms are not well calcified. The defect occurs during tooth development, since the tip, when first erupted, exhibits the typical white, unglazed appearance. Normally formed teeth never become subsequently mottled. In general the deciduous teeth are not affected, but a few mottled deciduous teeth were observed in Indian children in Arizona. This indicates that fluorine passes through the placenta with difficulty, and those teeth which are enameled before birth tend to be protected.

Black ('16, l.c.) and McKay ('30) were the first to attribute this abnormality to something in the water supply. The problem was solved by Margaret Cammack Smith and her associates ('31, '32, '33) who investigated the locality of St. David, Arizona, where mottled enamel was endemic, and found it associated with excessive consumption of

fluorine in drinking water. Simultaneously Churchill ('31) called attention to the presence of fluorine in waters from localities affected with mottled enamel. Smith and Smith ('32, l.c.) found that when the water supply contained 2.7 to 5 p.p.m., the teeth of the inhabitants were mottled in mild to moderate degree; when the water contained more than 5 p.p.m., the mottling was moderately severe to severe, and the teeth showed pitting and staining.

There are localities in every country in the world and in many states of the United States in which mottled enamel has been reported among the human population and most of the native born inhabitants of these sections are so afflicted. The toxicity of various fluorine compounds differing widely in water solubility seems to be the same for all practical purposes. Foods containing as low a concentration as 14 to 16 p.p.m. of dry substance induce this effect. Dean and Elvove ('35, '36, '37) studied the minimal threshold of chronic endemic dental fluorosis. They determined the fluorine in the domestic water supply for each of a group of 10 cities, with the following results expressed in parts per million: Pueblo, Colo., 0.6; Big Springs, Texas, 0.7; Mullins, S. C., 0.9; Monmouth, Ill., 1.7; Galesburg, Ill., 1.8; Colorado Springs, Colo., 2.5; Plainview, Tex., 2.9; Amarillo, Tex., 3.9; Conway, S. C., 4.0; and Lubbock, Tex., 4.4. An analysis of these data indicated a quantitative relationship between the fluoride concentration and the clinical manifestations of dental fluorosis. Continuing their study, they found in the water of Clovis, N. M., 2.19; Webster City, Ia., 1.56; East Moline, Ill., 1.51, and Junction City, Kans., 0.67 p.p.m. They concluded from their observations that amounts of fluorine not exceeding 1 p.p.m. are of no public health significance.

The teeth are by far the most sensitive to fluorine ingestion. The severity of this dental defect, which is generally restricted in its occurrence to the period of development of the permanent teeth, is closely related to the concentration of fluorine in the water consumed. Water containing less than 1 p.p.m. of fluorine appears to be innocuous to at least 90 per cent of exposed children; water containing from 1 to 2 p.p.m. produces a chalky white enamel, the incidence being about between 40-50 per cent; concentrations of 2 p.p.m and over induce first a deeply stained, and later a pitted and corroded enamel. The incidence of mottled enamel among exposed children increases to 100 per cent when the fluorine in the drinking water attains a concentration of about 6 p.p.m.

Aside from the dental dystrophy, which is the first symptom to appear, chronic fluorine toxicosis induces cellular hyperplasia of the thyroid, retarded ossification of the connective tissue and a greater transparency of the bones, anorexia, loss of weight, estrual disturbances, decreased milk production, diuresis and polydipsia. Impairment of

milk production and fetal nutrition by toxic doses of fluorine may be a direct result of a depressed appetite. There is a marked disturbance of the osseous metabolism, resulting in great thickening and exostosis of the long bones and the mandibles, although the gross chemical composition of the bone may not be disturbed except for an increased content of fluorine and of magnesium and a decreased content of carbon dioxide. In sheep, the bone changes have been reported as simulating those of osteomalacia. The fluorine content of milk is not readily affected by the concentration of fluorine in the ration, but in the case of the laying hen the egg is not well protected against fluorine invasion. The coagulation time of the blood of the chickens has been observed to decrease; the Ca, P (inorganic), and hemoglobin of the blood do not seem to be affected.

Detrimental effects of fluorine upon calcium and phosphorus utilization have been observed upon various species of animals, depending upon the concentration of fluorine in the ration and probably other factors. Only rarely have fluorine dosages been observed to affect the concentrations of calcium and inorganic phosphorus in the blood plasma, nor does fluorine exert its damaging effect on tooth and bone by altering significantly the concentration of the enzyme prominently involved in calcification, namely, phosphatase. The bone changes ordinarily observed in fluorosis are an increased, unchanged or decreased ash content, possibly dependent on the calcium content of the diet; a depression of the Ca:P ratio; an increased magnesium and fluorine content; a decreased content of carbon dioxide; and a decreased breaking strength. A softening of bones has also been reported, with an increase in volume due to enlarged marrow spaces. Tooth composition is not so readily affected by fluorine as is bone composition, although the changes produced are often in the same direction.

An interesting observation is that of Phillips and associates ('34) of the similarity of fluorosis to scurvy in guinea pigs. Their evidence leads them to believe that ascorbic acid deficiency and the effects of chronic fluorine toxicosis are due to disturbances in specific phases of cellular respiration. These studies suggest that fluorine toxicosis produces its systemic reaction through an interference with cellular respiration and that the primary point of attack is the enzymatic systems of the body. Phillips and Chang ('34) also observed that fluorine toxicity in rats causes the vitamin C potency of the anterior lobe of the hypophysis and the suprarenal gland to increase.

Little attention has been given to the pathways of entrance of fluorine into the organism other than through the drinking water and food. Murray ('36) has recently shown that fluorine can pass through the placenta and gain entrance to the fetus. He found that litters of rats receiving 0.05 per cent sodium fluoride in their diet, killed within

24 hours after birth, contained five times the fluorine content of the control animals in their body ash. In further experiments the relative influence of contact with fluorine during gestation and the consumption of milk from mothers ingesting fluoride was determined by bone analyses. The results show that the young absorb fluorine when it is administered both before birth and after. There is not only placental transmission of fluorine but also excretion through the milk. This indicates that mottling of the temporary teeth may result from antepartum maternal fluorosis and that in regions of endemic fluorosis milk may contain biologically effective amounts of this element. These results have been confirmed by other investigators (Knouff et al., '36).

Removal of Fluorine from Water.—Fink and Lindsay ('36) found that activated alumina is very effective in removing fluorine from water at pH 7.5. Elvove ('37) found that this element may be effectively removed from water by means of calcium triphosphate, magnesium oxide, or magnesium hydroxide.

Sharpless ('36) reported that aluminum chloride inhibits to a large extent the toxic effect of fluorine. He also studied the effect of calcium salts on fluorine toxicosis. These salts had a partial protective action. He found that a mixture of 2.0 per cent calcium carbonate and 2.0 per cent aluminum chloride protected rats from fluorine poisoning.

Nelson ('36) has obtained similar results using aluminum sulfate.

Dispensability of Fluorine.—Sharpless and McCollum ('33) attempted to determine whether fluorine is an indispensable element in the diet. They prepared a diet containing no determinable amount of fluorine, which was fed to young rats, starting at about 50 gm. weight. Their method permitted estimation of a range of values with differences of but 0.01 mg. of fluorine, and added amounts of this order could be accurately estimated.

The bones of rats on their stock diet contained nearly 0.04 per cent of fluorine, whereas those from rats fed the fluorine-low diet gave values of 0.001 to 0.0046 per cent. The highest value of bones from stock animals was 0.08 per cent. The addition of 1 mg. of fluorine per 100 gm. of the fluorine-low diet caused the content of this element in the bones of the animals receiving this diet to rise as high or higher than that of bones of the stock rats. They found somewhat less fluorine in the teeth of rats than in the bones, and no fluorine could be detected in the teeth of the low fluorine rats. The teeth appeared to be excellent in respect to calcification, and there was no indication of caries.

Since rats grew normally on the fluorine-low diet, and reproduction was not adversely affected, although the range of the fluorine content of the bones was reduced to from 6 to 25 p.p.m., and was eliminated

from the teeth so far as could be determined by the method used, it was concluded that fluorine plays no physiological role and is not necessary in the diet.

Selenium

Although there is reason to believe (Beath, '35, '37; Byers, '35, 37; and Trelease, '38) that selenium may be necessary for the development of certain plants, it appears to be the only mineral element known to be absorbed from the soil by plants in sufficient quantities to render them poisonous to animals and perhaps to man.

The injury is greatest in livestock because they are generally fed solely on home-grown products, whereas the human population usually secures its breadstuffs almost entirely from cereals grown in various parts of the country. Likewise much of the canned and other staple foods are obtained from afar. Consequently, the intake of selenium tends to be considerably smaller in man than in farm animals.

The first scientific report of selenium poisoning appears to have been written in 1856 by Madison, a veterinarian of South Dakota, who observed the condition in cavalry horses. He suggested a toxic factor in the local forage as the probable cause of the disease. The occurrence of the disease was reported at various times in different parts of the country. The true nature of this disease did not become generally known until Franke of the South Dakota Agricultural College demonstrated the toxic character of some of the grains grown in sections of South Dakota where the disease in livestock has been more or less prevalent. Following this, Robinson ('33) and Franke ('34, '35) demonstrated the presence of selenium in the grain which had previously been found toxic to animals. Selenium is present in concentrations ranging from mere traces to as high as 0.1 to 0.2 per cent in a wide variety of plants growing upon certain types of soils. Such vegetation may induce a slow chronic disease commonly known as "alkali disease" and "blind staggers," or it may be quickly fatal. This disease, prevalent in the North Central Great Plains of the United States, causes considerable losses of cattle, horses, pigs, sheep, and chickens as the result of ingestion of the selenium-bearing vegetation growing in those regions. Plants heavily contaminated with selenium have an offensive odor and ordinarily are not well liked by livestock. It is thought that any vegetation containing 5 p.p.m. of selenium is potentially dangerous although this appears to be a conservative estimate. This element occurs in greatest concentration in the leaves, in intermediate concentration in the stems, and in least concentration in the seeds. The selenium in plants is derived from and is in concentration proportional to the selenium occurring in the soil.

Whether animals are fed with inorganic selenium compounds or

with the natural plant toxicant, the pathologic and symptomatic features resemble closely those encountered among animals in the toxic areas. Cattle, horses, sheep, and pigs show erosions of the ends of the weight-bearing bones, with hoof abnormalities that considerably impair locomotion. For example, cattle, after losing their hoofs, may walk on their fore-knees. Malaise, anorexia, stunted growth, loss of body hair, loss of weight, emaciation, impairment of reproductive power, and severe anemia develop followed by exhaustion, toxemia, and death. Poultry subsisting largely on seleniferous grains do not grow well, are nervous, and have ruffled feathers. Chickens show a decided impairment of fertility; egg production is delayed and reduced; the eggs have low hatchability. The embryos are generally deformed in those eggs which fail to hatch. They may have short upper beaks and their eyes may be absent. If the eggs hatch the young do not live long, and monstrosities frequently occur among them. Similiar monstrosities are produced in chicks by the injection of selenium salts into fertile eggs. The symptoms encountered experimentally in the guinea pig and the albino rat do not differ essentially from those in other animals. Even if removed from the selenium-containing diet and fed a good ration, selenized animals do not usually recover. The stunting of growth in calves is pronounced and continued, and in cattle the damage to the kidneys and liver seems to be permanent. Hogs seem to be more easily poisoned by selenium than are Herbivora. Franke ('34, l.c.) noted that rats fed on toxic grains for as short a period as 10 days showed a lack of recovery, growth disturbances, and typical pathologic changes even when subsequently fed a control diet for as long as 165 days. It is of interest that the investigations have shown that the experimental animal is able to differentiate between diets of varying toxicity, and although extreme inanition is observed, Franke and Potter ('35) have found that the pathology is caused by the toxicant while the inanition is due to a voluntary restriction of food intake. Young animals seem to be more susceptible than older ones. These observations confirm those in the case of range animals.

The absorption of selenium by plants has been studied chiefly by Hurd-Karrer ('33, '34, '35). Various plants show widely differing abilities to take up selenium from a given soil. The plants which normally absorb large quantities of sulfur were found to contain large amounts of selenium. The addition of elemental sulfur to a soil containing selenium decreases the damage to the plant as well as the amount of selenium absorbed. Hence treatment of selenium soils with sulfates to reduce the toxicity of selenium is indicated.

Selenium disease at present appears to be essentially an agricultural and veterinary problem. Owing to the low content of selenium in the greater portion of commercial crops and the high dilution of toxic

grains in the general market the danger to public health does not seem to be great. So far human selenium poisoning has not been reported in the toxic areas. In the selenium regions, however, analyses have revealed the presence of selenium in food, such as milk, eggs, corn, wheat, barley, rye, string beans, lettuce, cabbage, and turnip leaves in amounts varying from 0.3 to 150 p.p.m. These findings in addition to the results of the survey conducted by Smith and Franke ('36) of the rural population of parts of Wyoming, South Dakota, and Nebraska, where definite proof of absorption of selenium by some of these individuals and an exhibition of a symptomatology resembling the condition in animals was noted, indicate the gravity of this problem at least locally and also indicate that there is no reason to believe that man is physiologically immune.

The researches on the cause of the animal disease now known to be induced by selenium represent one of the most elaborate and painstaking studies in the field of nutrition. Several bureaus of the U. S. Department of Agriculture and the Experiment Stations of South Dakota and of Wyoming participated in the investigation. Moxon ('37) has written the most elaborate account of these studies.

The development of the selenium problem emphasizes the already recognized importance of "trace" inorganic elements in plant and, hence, in animal and human nutrition.

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CHAPTER

XII

Chemical Nature of Vitamin A

EARLY OBSERVATIONS.—From very early times peculiar virtues were assigned to certain foods for specific purposes, and investigators have learned that it is wise to note all such observations, and to test the validity of any and all suggestions. Hippocrates is quoted as advocating an abundance of liver as a remedy for night blindness. Modern experience abundantly proves that liver is curative in this condition. Among the people of Newfoundland night blindness has been common for many years. It is reported that only men suffer from this trouble, which results from the effect of the glare of sunlight on their eyes while engaged in fishing. Natives of Newfoundland have long believed that eating the liver of the codfish, or seagull, promptly cures night blindness. The efficacy of this popular remedy has been fully established.

Magendie appears to have been the first student of those nutrient principles now known as vitamins. He restricted animals to simple diets composed of sugar, olive oil, wheat gluten, starch, etc., and described an eye disturbance now known as xerophthalmia. Livingstone ('05) in 1857 described this eye condition as occurring among his servants in Africa, and stated that "The eyes became affected as in the case of animals fed pure gluten or starch." He was apparently familiar with the work of Magendie. Spicer (1892) observed sloughing of the cornea of nurslings of mothers in Russia, who had practiced long religious fasts. Xerophthalmia was described in 1865 among slaves in Brazil by Gama Lobo (Pillat, '29). An historical resumé of the older occurrences of the symptoms of vitamin A deficiency will be found in a paper by Eusterman and Wilbur ('32).

Before 1913 it was generally believed that all fats had similar nutritive values, since they were regarded as sources of energy only, and

since their digestibility and caloric values differ but little. In that year McCollum and Davis, and Osborne and Mendel, employing diets composed of more or less purified food substances, observed that rats could not grow or remain in good health when lard, olive oil, or almond oil provided the sole source of fat, whereas their condition was much improved when these fats were replaced by butter fat, cod liver oil, or egg yolk fat. These experiments clearly indicated that certain fats carried an essential nutrient which was absent from others. McCollum designated this factor "fat-soluble A," and it is now known as vitamin A.

Steenbock ('19) conceived the brilliant idea, on the basis of such experimental data as was then available, that the vitamin A effect in foods was associated with the yellow pigment in these foods. He prepared carotene, the principal yellow pigment of carrots and of many other yellow vegetables, and tested it on the rat for its vitamin A activity. From his results he concluded that carotene, but not xanthophyll, afforded the same protection to rats as did butter fat, cod liver oil, and egg yolk fat. Carotene is a hydrocarbon, the empirical formula for which is $C_{40}H_{56}$. It derived its name from its having been first isolated from carrots.

A disturbing fact which warranted skepticism as to the correctness of Steenbock's view was the observation that cod liver oil, which is a rich source of this substance, is only faintly yellow. Students of nutrition were, therefore, not convinced, but were inclined to the view that the specimen of carotene employed by Steenbock was not pure but carried the vitamin A as an impurity. Several investigators at once set about testing the validity of his conclusion, and failed to confirm his results. Since knowledge concerning the essentials of an adequate diet in 1919 was incomplete, it is not surprising that experimenters were not able, when feeding diets composed largely of purified foodstuffs, to maintain growth and health in young animals even when the new fat-soluble vitamin was provided, owing to the inadvertent omission of unsuspected nutrients from the food. The failures to confirm the nutritional value of carotene at that time are now easily understood, but were then very puzzling.

The vicissitudes of the nutritional investigator are well illustrated by the experience of Palmer and Kempster ('19), who reported an extraordinarily interesting experiment with chickens, designed to demonstrate whether or not yellow pigment was necessary in the diet. They planned a diet consisting of white maize, white summer squash, and white onions, which they fed to white leghorn chicks. The chicks could not grow on this food. Palmer was an expert on carotenoid pigments, of which more than 20 related substances were known, and had given considerable attention to the study of their distribution. He

had observed that pig liver contained no appreciable amount of yellow pigment. Hence, it seemed safe to include with the white vegetable foods a small amount of pig liver. This combination proved satisfactory for the growth and health of the chicks, and they grew to maturity in a normal manner, except that they lacked the yellow pigment normally found in the skin, beak, ear-lobes, and scales of the shanks, which in chickens is always abundant when they are fed under farm conditions. These experimental birds were thoroughly bleached. A flock of pullets produced some 800 eggs while confined to the colorless diet. The yolks of these eggs were free from yellow pigment, yet they were fertilized and hatched pale chicks, which were, in turn, reared without yellow pigment on the experimental diet. Palmer and Kempster's conclusion that Steenbock's opinion was erroneous seemed fully warranted.

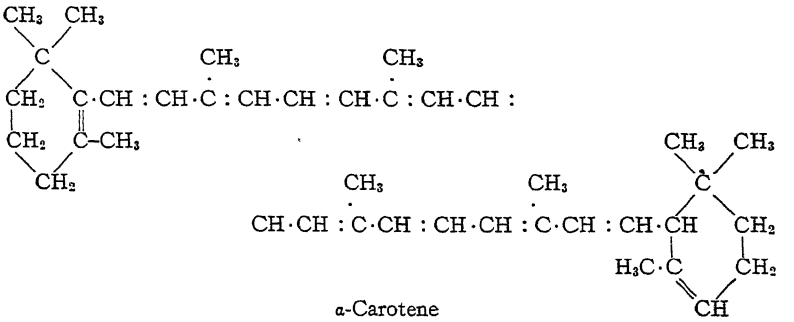
Von Euler, Euler and Hellstrom ('28) confirmed in principle the experiment of Steenbock, using carotene as a source of vitamin A. In 1922 McCollum, Simmonds, Shipley and Park demonstrated the existence of another fat-soluble vitamin (vitamin D), which is essential for the normal development of the skeletal system. This substance had been omitted from the diet which Steenbock had employed, since it had not yet come to light, and this omission was sufficient to cause stunting and early failure of his rats, even when vitamin A was supplied. Von Euler and associates improved their experimental diet by including this factor. They easily demonstrated that carotene was, indeed, a source of vitamin A. Their findings explained why several workers had failed to get satisfactory results with carotene.

It later emerged that the reasons for the success of Palmer and Kempster with their birds were two-fold. The pigs which furnished liver for their study had taken carotene as their source of vitamin A, but their livers had converted the yellow pigment into the vitamin, which is not yellow. They had kept their birds in sunlight which, acting on their feathers and exposed parts, especially the combs and ear-lobes, had formed vitamin D. Chickens generally eat some feathers, and these birds probably secured some vitamin D from this source. They also absorbed some from the exposed skin. Had they kept their birds out of sunlight or under glass, which absorbs ultraviolet rays, the chicks would not have grown and different conclusions would have been drawn from the experiment.

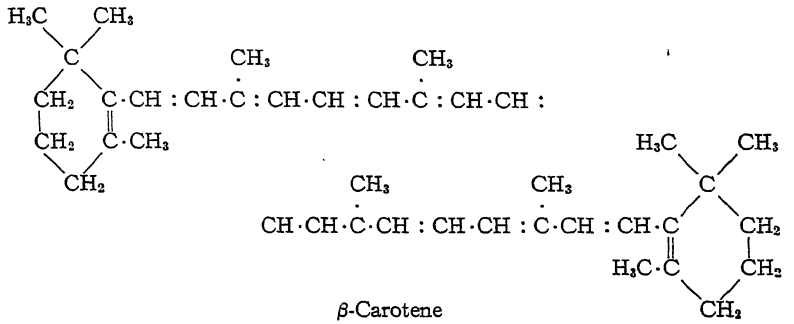
The high vitamin A value of nearly colorless fish liver oils, suggested that carotene was not itself the vitamin. The proof was furnished by the studies of Moore ('29), who showed that carotene is provitamin A, and is converted into the vitamin in the body. Moore fed highly purified carotene, shown to be devoid of vitamin D, to A-depleted rats, whose livers thereupon became rich in vitamin A.

This was demonstrated by (a) the absence of yellow pigmentation; (b) the blue color given by vitamin A with antimony trichloride reagent (absorption band 610-630 mμ); and (c) the presence of an absorption band in the region of 325 mμ. Capper ('31) demonstrated that the chicken, like the rat, converts carotene to vitamin A. The liver oil of rats or birds fed liberal amounts of carotene, is much richer in vitamin A than is cod liver oil.

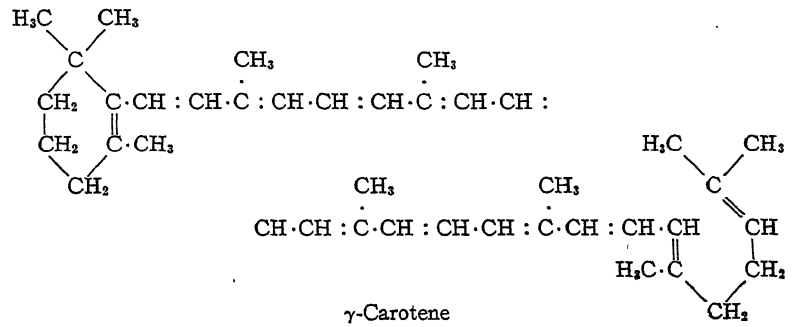
Carotene.—Carotene exists in nature in three forms, α-carotene, β-carotene, and γ-carotene. The structural formulae for these, and many other carotenoids, have been determined. Some of these are:



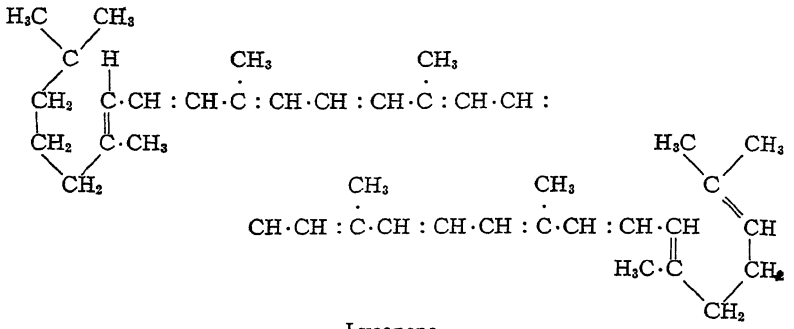
α-Carotene



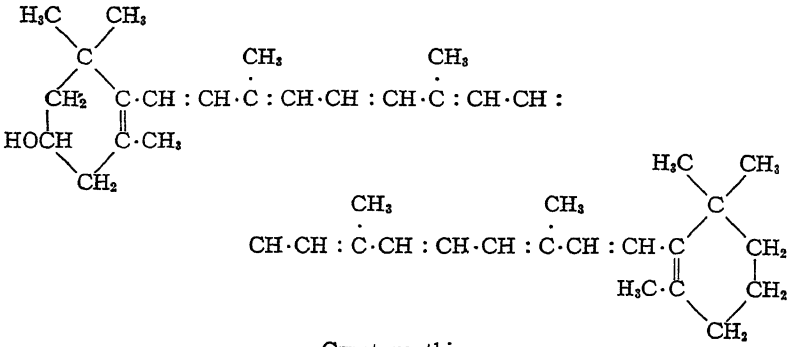
β-Carotene



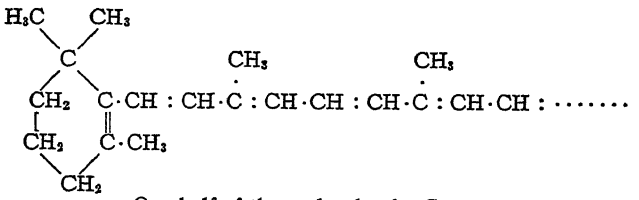
γ-Carotene



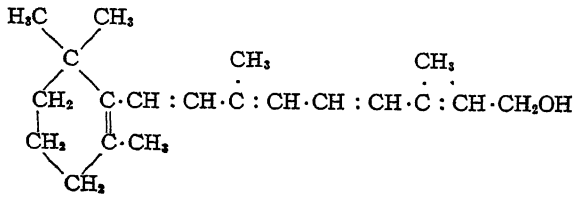
Lycopene



Cryptoxanthine

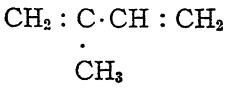


One-half of the molecule of β-Carotene

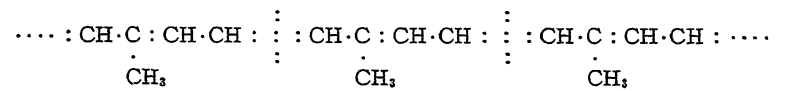


Vitamin A (Karrer et al. '34)

The carotenoid pigments occur widely distributed in plant and animal tissues. They belong to the class of hydrocarbons known as terpenes, of which rubber is a member. These hydrocarbons are all formed in plants from isoprene:

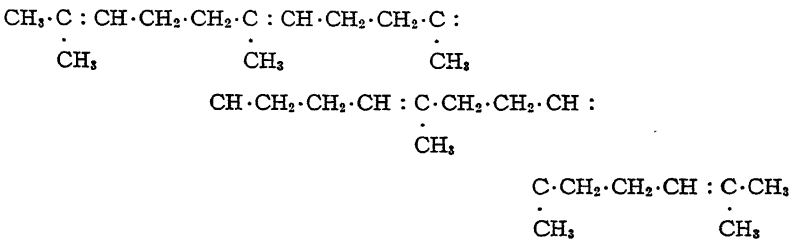


Isoprene readily polymerizes to rubber under the influence of certain catalysts. The formation of the highly unsaturated chain characteristic of the carotenoid pigments may be visualized as taking place through the polymerization of isoprene residues:



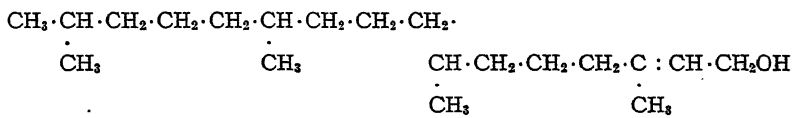
Three Dehydrogenated Isoprene Residues

There occurs in the liver oil of fishes of the shark family a hydrocarbon, squalene, which has the structure:



Squalene ($C_{30}H_{50}$)

It is not known whether this substance plays a physiological role. The alcohol phytol, which occurs in combination with chlorophyll, is derived from four isoprene residues with a terminal alcohol radical:



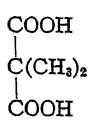
Phytol

Conversion of Carotene to Vitamin A.—Vitamin A may be formed in the liver by the cleavage of β-carotene and the addition of water to each severed end of the molecule to form an alcohol group, as shown above. β-carotene yields two molecules of the vitamin, whereas α- and γ-carotenes yield but one each. The only other known carotenoid pigment which can yield the vitamin is cryptoxanthine, which occurs in yellow maize and paprika, and certain other yellow vegetables.

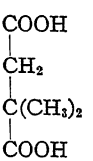
Cryptoxanthine, as shown by its structural formula, is capable of yielding on cleavage, one molecule of vitamin A, as are α- and γ-carotenes. Carotenediiodide, $C_{40}H_{56}I_2$, and dihydrocarotene, are also provitamins. Dihydrocarotene, $C_{40}H_{58}$, is a yellow oil which results from reduction of carotene by means of aluminum amalgam. Diiodocarotene results from the action of iodine on carotene dissolved in ether. It is a dark violet powder, having no definite melting point.

Although it is certain that vitamin A is formed from carotenes in the liver, the mechanism is not well known. Olcott and McCann ('31), employing the ultraviolet absorption test, reported the presence in liver of a carotenase which converts carotene into vitamin A. Ahmad ('31), on the other hand, claimed that carotene is not changed into vitamin A by incubation *in vitro* with liver tissue or by the action of intestinal bacteria. However, it is well established that certain microorganisms synthesize carotene. Von Euler observed that hen's serum can transform carotene into a substance similar to but not spectroscopically identical with vitamin A. Since the liver has an important role in the conversion of carotene into vitamin A, it is interesting that liver oil may contain 100,000 times as much vitamin as the storage fats.

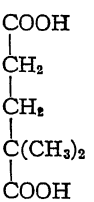
Chemical Behavior of Carotene.—On treatment with hydrogen in the presence of a suitable catalyst, carotene absorbs 22 atoms of hydrogen, showing the presence of 11 double bonds. Treatment with potassium permanganate yields dimethyl malonic acid, dimethyl succinic acid, and αα-dimethyl glutaric acid, as well as 4.5 molecules of acetic acid. Oxidation with ozone yields geronic acid. Oxidation with chromic acid yields six molecules of acetic acid. These substances are the oxidation products of β-ionene.



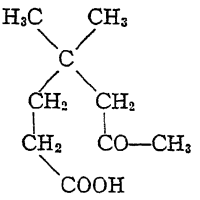
Dimethyl malonic acid



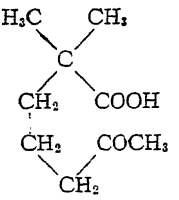
αα-Dimethyl succinic acid



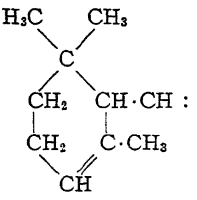
αα-Dimethyl glutaric acid



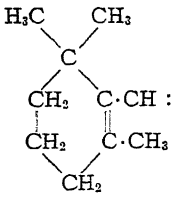
Isogeronic Acid



Geronic Acid



α-Ionone Ring



β-Ionone Ring

On oxidation of α-carotene with ozone neither geronic nor isogeronic acid is obtained. Quantitative experiments with β-ionone and of β-carotene have shown that the amount of geronic acid produced corresponds to two ionone-carbon rings in the latter.

Cholesterol and other sterols, squalene, carotenes, vitamin A and various carotenoids yield blue products when treated with antimony trichloride. The position of the absorption bands serves to identify the particular substance present.

Olcovitch (Olcott) and Mattill ('31) claim that carotene is a pro-oxidant. Six-hundredths per cent dissolved in an auto-oxidizable oil shortens its induction period by one-half. They suggest the possibility that this property may be associated with the biological function of vitamin A.

Properties of Carotene.—Both β- and γ-carotenes are optically inactive, whereas α-carotene in benzol has a specific rotation with cadmium light of +385°. When dissolved in carbon disulfide β-carotene shows maximal absorption of light at 521 and 485.5 mμ; α-carotene shows maxima at 509 and 477 mμ. The melting points of α-, β- and γ-carotenes are respectively, 183°, 183° and 174°. γ-carotene constitutes about one-thousandth part of the mixed carotenes prepared from various plant sources. Kuhn and his associates estimated the α-carotene content of preparations of carotene from different sources. These relations are shown in the following table:

α-CAROTENE CONTENT OF DIFFERENT CAROTENE PREPARATIONS

FROM GREEN LEAVES		FROM OTHER MATERIALS	
	per cent		*per cent*
Palm oil	30–40	Carrot	10–20
Banana	25	Mountain ash berries	15
Stinging nettle	0	Giant squash	1
Spinach	0	Paprika	0
Grass	0	Ovary	0

The carotenes are soluble in acetone, petroleum ether, and other organic solvents, but are insoluble in water.

The carotenes are yellow. With antimony trichloride β-carotene gives a greenish-blue reaction product which shows maximum absorption at 590 mμ.

Isolation of Vitamin A.—In 1925, Takahashi and others claimed to have isolated pure vitamin A. This claim was later challenged by Drummond ('25, '29), who expressed the belief that "the active substance is present in liver oil concentrates in amounts so minute that direct attempts at its isolation by the ordinary chemical methods are of little use."

In 1933 Carr and Jewell, as well as Karrer and his associates, succeeded in preparing rich vitamin A concentrates by different methods. However, in the latter half of 1937 the isolation and identification of crystalline vitamin A from the liver oils of three very different species of fish, *Stereolepis ishinagi,* Atlantic mackerel, and oil No. 1364 (an oil furnished by the Atlantic Coast Fisheries Company) was reported by Holmes and Corbet.

Properties of Crystalline Vitamin A.—The vitamin crystallizes at very low temperatures from methanol, forming clusters of pale yellow needles. It is optically inactive and isotropic. The crystals melt at 7.5-8.0° C. The molecular weight, 294, and content of carbon, 83.28%, and hydrogen 10.44%, correspond closely to the suggested Karrer formula (molecular weight 286; 83.84% C; 10.56% H_2). The extinction coefficient $E\,{}^{1\%}_{1\ cm} = 2100$ is equivalent to a molecular extinction coefficient of 60,000.

In contrast with β-carotene, vitamin A gives with antimony trichloride a vivid blue reaction product with maximum absorption at 610 to 630 mμ. In the ultraviolet, vitamin A shows an absorption band at 328 mμ.

The vitamin has the general properties of an alcohol and esters of

the vitamin have been obtained. However, vitamin A is not precipitated by digitonin, thus providing a means of separation from cholesterol which also occurs in the non-saponifiable fraction of animal fats. By means of such solvents as ether, chloroform, ethyl acetate, or light petroleum ether, the unsaponifiable fraction can be extracted from saponified fats without any loss of the vitamin.

Vitamin A distills in a high vacuum with partial decomposition. Hickman ('36, '37) has described a procedure for separating vitamins A and D from oils containing the vitamins in which they are subjected to an extremely high vacuum. Not only do the free vitamins distill in well-defined fractions under proper conditions, but the esters of the vitamin, as well, behave the same in this respect. By this technique Hickman has shown that in fish oils some vitamins A and D occur as the free alcohols, most of which are esterified with fatty acids, and that most of the fatty acids are represented in the vitamin A esters present. Vitamin A is reducible by aluminum amalgam in ether.

It is adsorbed by Lloyd's reagent and by norite (a charcoal). Silica gel adsorbs the vitamin so tenaciously that in such form it is unavailable to rats, although it may be extracted from the gel with toluene.

In the absence of air, vitamin A is quite stable at high temperatures, but when oxygen is present it is readily destroyed. Vitamin A appears to be less resistant to oxidation when removed from its natural environment, due, no doubt, to the protective action of antioxidants. Certain phenolic substances like hydroquinone are effective in delaying the destruction of vitamin A. Dann ('32) states that vitamin A is particularly stable to oxidation in ethyl alcohol, alcoholic potash, ethyl acetate, n-butyl alcohol, cetyl alcohol, and triacetin. It is readily oxidized by aeration in acetic, caproic, lauric, stearic, and oleic acids, and fairly rapidly in solution in tributyrin, triolein, coconut oil, and peanut oil. It is very stable toward hydrogen peroxide when both the vitamin and the hydrogen peroxide are dissolved in alcohol. Vitamin A is relatively stable to heat in the absence of oxidizing agents. Dunn ('24) reported that vitamin A of cod liver oil was destroyed by mixing the oil with granulated starch and storing it in dark, corked bottles. McCollum, Simmonds and Becker ('27) found that contact of cod liver oil or butter fat with ferrous sulfate caused rapid destruction of vitamin A. Ferric citrate did not catalyze its oxidation, and the action of ferrous sulfate was inhibited by an antioxidant in wheat germ oil.

Light exerts a destructive influence upon vitamin A so that extreme caution must be used in the dispensing and storing of it. Ultraviolet irradiation also destroys the vitamin.

A biological assay of the crystalline vitamin A made by Dutcher

and Guerrant (cited by Holmes and Corbet, '37) indicates a potency of "considerably above 2,265,000 and somewhat below 3,400,000 International Units per gram." A similar assay by Emmet (cited by Holmes and Corbet, '37) indicates that the vitamin "probably has a value of 3,000,000 International Units per gram."

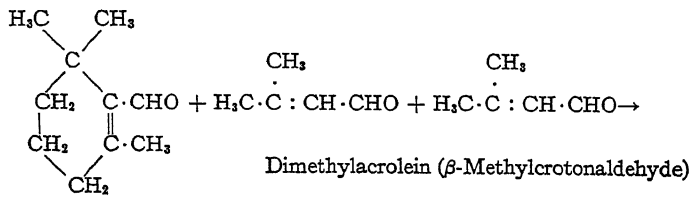
Synthesis of Vitamin A.—The animal body is dependent on the food ingested for its source of vitamin A since animal tissues cannot synthesize the vitamin. Fruits and vegetables are practically devoid of active vitamin A. The carotenoid substances are synthesized by plants, and from them animals derive their supplies of this nutrient, which is converted to vitamin A in the animal organism.

In plant tissues vitamin A exists as the provitamin, carotene. It may be formed in the complete absence of light, but the synthesis of the provitamin in plants is accelerated by light radiations, especially of short wave-lengths. However, the ultimate amount formed is not increased. This synthesis appears to parallel chlorophyll formation, for green outer leaves are better sources of the vitamin than the white center leaves. Chlorophyll does not possess the biological properties of vitamin A. In plants provitamin A seems to be less distinctly associated with the fat, and Pryde ('28) suggests that this may be due to the fact that in the plant it is associated with other molecules which are not fat-soluble.

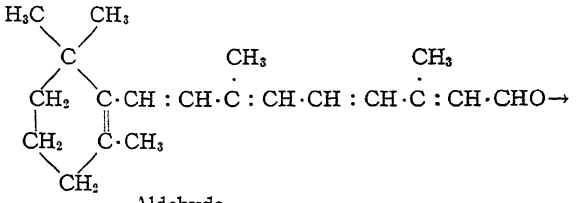
It has been reported that certain unicellular marine organisms are able to synthesize vitamin A. These algae serve as the main source of vitamins for deep-sea animals, and the concentrations of vitamin A attained by them are much higher than those found in land sources of vitamin A.

Although it has been definitely proved that certain microorganisms synthesize carotene (Forster, '31), there is no evidence at this time that vitamin A is formed under the same conditions.

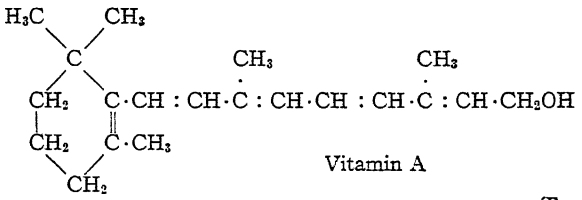
Two groups of workers have reported the chemical synthesis of vitamin A. Fuson and Christ ('36) have condensed two molecules of dimethylacrolein with one molecule of β-cyclocitral to form an aldehyde which, when reduced by the action of aluminum isopropoxide yields an alcohol.



β-Cyclocitral



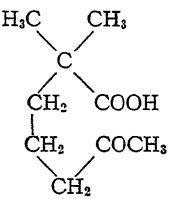
Aldehyde



Vitamin A

(Fuson and Christ—'36).

Although the resulting product has not been synthesized in pure form, it has been obtained in sufficient purity to show analytical data as to its content of carbon and hydrogen, its five double linkages, its alcohol group, and oxidation products corresponding to the structural formula of vitamin A. Like β-carotene, it yields on oxidation geronic

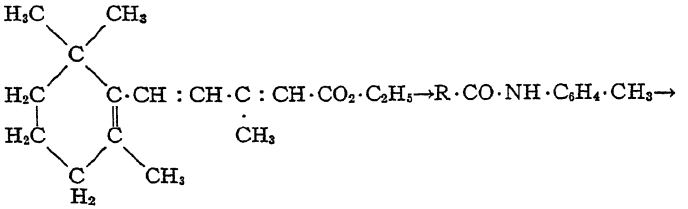


acid and three molecules of acetic acid. This synthesized alcohol reacts with antimony trichloride, forming the characteristic blue color of the standard test for vitamin A. The ultraviolet spectrum shows maximum absorption in the region of 328 mμ. Biological tests for the vitamin A activity of this preparation have not been reported by these investigators.

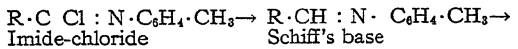
Kuhn and Morris ('37) also succeeded in synthesizing this vitamin by condensing β-ionylidene acetaldehyde with β-methyl crotonaldehyde in the presence of piperidine, and reducing the resulting aldehyde with aluminium isopropylate in isopropyl alcohol solution.

The β-ionylidene acetaldehyde used as the starting product was synthesized from β-ionylidene acetic ester by letting this act on the Grignard product of magnesium methyl iodide and o-toluidine. The o-toluidide was treated with phosphorus pentachloride, and the imide-

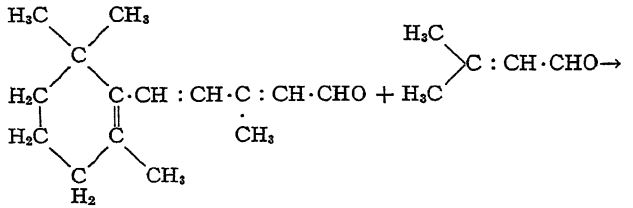
chloride was converted by the action of chromous chloride and subsequent hydrolysis with oxalic acid to the free aldehyde.



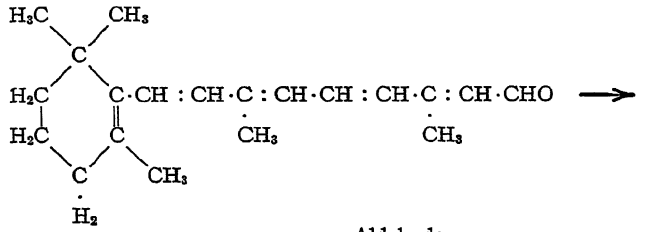
β-Ionylidene acetic ester — o-Toluidide



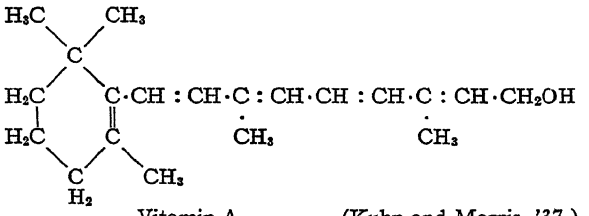
Imide-chloride — Schiff's base



β-Ionylidene acetaldehyde — β-Methylcrotonaldehyde



Aldehyde

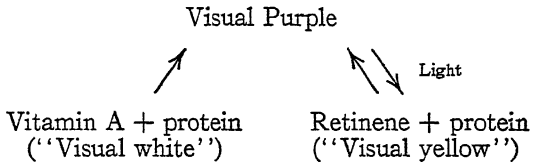


Vitamin A (Kuhn and Morris, '37.)

The physico-chemical properties of their product correspond to those described above, as well as to the vitamin obtained from natural sources. In addition, they report the biological activity of their preparation. Vitamin A depleted rats were fed the synthetic preparation at levels varying from 0.80γ to 8γ * per rat per day. Good growth was obtained by the 1γ dose. With the 0.8γ dose an average gain of 11.7 gm. in 35 days was obtained, which corresponds to the growth activity of 0.9γ of the vitamin A standard. The 0.1γ dose had no effect on the growth of rats. These results are in agreement with the activity of vitamin A from natural sources.

Vitamin A and the Visual Mechanism.—Vitamin A is essential to the normal behavior of the retinal organ of which it is a chemical component. The processes by which the visual purple and other pigments in the eye are elaborated from vitamin A have been studied by Wald ('35) and by Hecht and his associates ('36). Vitamin A is transported to the retina by the circulation. There it combines with the protein to form visual purple which, as a result of a photo-chemical reaction, is then converted into visual yellow. The latter breaks up again, producing vitamin A along with other products. Some of the vitamin A probably is destroyed, since the continuous provision of the vitamin in the diet is essential.

SYNTHESIS OF VISUAL PURPLE (WALD–1936)



The studies of Wald showed that the retina contains a carotenoid pigment, derived from the visual yellow other than carotene or vitamin A, which he named retinene. Wald explains the process of visual response to light as follows: When light strikes the visual purple in the retinal cones the former is bleached to an orange pigment called visual yellow. During this process retinene, which is yellow in color, is formed. The yellow color gradually fades and the retina becomes colorless. When this stage is reached the retinene seems to have been converted into vitamin A. In the normal living animal the vitamin A is resynthesized into visual purple. This cycle appears to be continuous. However, unless the eye continuously receives a supply of vitamin A through the circulation, the regeneration is incomplete. This is the first proven example of a vitamin being an actual structural component of a tissue.

* One millionth of a gram.

A Possible Vitamin A_2.—Recent investigations (Gillam, et al., '37, '38; Edisbury, et al., '37) describe the detection of a substance found in the pigment epithelium of the eye and in the livers, liver oil, and concentrates from fresh-water fishes, yielding with antimony trichloride an absorption band at 683-696 mμ. It is believed that this product may have for fresh-water fishes the same physiological functions as vitamin A has for marine fishes. The evidence is considered sufficient for proposing that this chromogen shall be provisionally called vitamin A_2. It is homologous with vitamin A in the rhodopsin system. Its retinal precursor might correspondingly be designated retinene$_2$. The prophyropsin system is tentatively formulated:

$$\text{porphyropsin} \overset{\text{light}}{\rightleftharpoons} \text{retinene}_2 + \longrightarrow \text{vitamin } A_2 + \longrightarrow \text{porphyropsin}$$

(Wald, '38.)

The International Unit of Vitamin A.—In 1931 an International Conference of nutrition experts met, under auspices of the League of Nations, and adopted standard units for the best known of the vitamins. Samples of pure carotene, prepared by a number of biochemists in different countries, were sent to The Institute for Medical Research in London, where they were pooled, recrystallized, and biologically tested. One gamma (one-millionth of a gram) of this sample was adopted as the International Unit of vitamin A. Further research showed that this sample was a mixture of β-carotene and α-carotene. The biological value of one milligram of the mixture was equivalent to 0.6 mg. of pure β-carotene. Accordingly, in 1934, pure β-carotene was substituted for the International Standard of Reference. Small samples of the β-carotene in coconut oil as a reference standard are available to nutrition workers in many countries. Also a secondary reference material has been prepared. This is a sample of cod liver oil which has been carefully compared by animal experiments with the carotene standard.

The unit of vitamin A activity is, therefore, defined as the amount of activity contained in 0.6γ of the International Standard of β-carotene.

Methods for Estimating Vitamin A in Biological Products.—Three methods,—chemical, physical, and biological—for determining vitamin A activity are available. Although the chemical and optical methods offer advantages of speed and reproducibility, the biological method is fundamentally more accurate and reliable. At present no color reaction is known to be specific for vitamin A activity when correlated with the biological method.

The chemical test, using a Lovibond tintometer or similar instrument, measures the intensity of blue color developed by the anhydrous

antimony trichloride solution in chloroform. This test is only roughly approximate, but it is of some value as an indicator of the vitamin A content in oils of known history, preliminary to their biological assay.

Since vitamin A is characterized by selective absorption in the ultraviolet, the intensity of this band, whose maximum is at 328 mμ, serves as a measure of vitamin A content. The spectrophotometric procedures are of limited applications, because expensive equipment is necessary and their specificity is questionable in oils of unknown origin and also because the carotenes and allied substances of known vitamin A activity fail to absorb at this wave length. However, adaptations of this method have been useful in industrial laboratories in the testing of fish liver oils where the vitamin A activity is due entirely to the vitamin.

Although biological assays are subject to variations due to uncontrollable factors in the animals, at present they afford the most reliable means for estimating the physiological activity of the vitamin. In the biological procedures a diet is employed which is complete in all respects except for the absence of the vitamin. The basal diet is studied with animals which are given a supplement of the source of vitamin A, the results being interpreted on the basis of a particular criterion. The methods which have been studied most thoroughly are the following:

The *Curative Method* is based upon depletion of young rats of their vitamin A reserves, and then observing the rate of growth response which occurs when a known supplement of a substance is provided which contains vitamin A or a provitamin A. Sherman has proposed a unit quantity which is the amount necessary to produce a growth response of 3 grams per week in young rats under specified conditions. This method does not call for a control series of experiments in which the same conditions are adhered to, and in which a reference standard cod liver oil of known potency is fed. The conversion factor of Sherman units into International Units is generally stated to be about 1.4. Owing to variation in response of individual animals and of rats from different colonies, this method is not very reliable in the hands of different workers. Experiments run in parallel to the tests on the unknown substances should always be conducted with a standard reference oil. Failure to do this has resulted in the appearance in the literature of many untrustworthy assay values. The vitamin A test diet is described in the U. S. Pharmacopoeia XI (Revised 1937), and a thorough presentation of recommended experimental details to be observed will be found there, as well as in Coward's ('38) "Biological Standardisation of the Vitamins."

The *Prophylactic Method* is based upon the same type of experiment as is the curative method, but in this case the smallest dose of

the substance under assay is determined which will just prevent the appearance of symptoms of vitamin A deficiency.

In addition, a method based on the changes in the vaginal contents of animals has been described as a criterion for the determination of vitamin A. This method has not proved satisfactory on a quantitative basis, for although the response of the rat to large doses of the vitamin is well marked, it is difficult to estimate slight responses to small doses.

Distribution. Data regarding the distribution and content of vitamin A in foodstuffs and certain other products is extensive. The quantitative distribution of this vitamin is presented in the Appendix.

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CHAPTER

XIII

Nutritional Significance of Vitamin A

THE TWO EARLIEST effects of experimental vitamin A deficiency to be observed were cessation of growth and ulceration of the corneae of the eyes. Goldschmidt ('15) observed keratomalacia in the eyes of rats on Hopkins' multiple deficiency diet. McCollum and Simmonds ('17) described the eye condition resulting from more specific deficiency of vitamin A, as xerophthalmia, a condition which had been previously spoken of by several investigators as "fat deficiency disease." Mori ('22) described keratinization and desquamation of keratinized epithelium of the cornea, and conjunctival and lacrymal gland epithelium. Daniels, Armstrong and Hutton ('23) observed that rats depleted of vitamin A die of bacterial invasion of the ear and nasal cavities before the appearance of xerophthalmia. Following these early observations, numerous studies have been made of the physiology and pathology of avitaminosis A. The results of this deficiency are essentially the same in experimental animals as in man.

Since the significance of vitamin A in relation to nutrition and health is revealed by the effects of the absence of this nutrient from the diet, a review of the extensive experimental nutritional and pathological studies supplemented by the clinical observations available, will form the basis for the consideration of this subject.

General Effects of Vitamin A Deficiency.—A variety of symptoms follows the depletion of vitamin A in the animal organism. Different animals of the same species under the same general conditions may become affected quite differently. Species variations in symptomatology are also quite marked. In young animals interruption of growth is one of the earliest signs of a lack of vitamin A in the diet. The animal becomes emaciated. Its fur is dry and coarse, and the feet are rough and scaly. These are, however, common to several types of deficiency;

consequently, they cannot be considered specific evidences of avitaminosis A.

It is believed that in both adults and older children hemeralopia is nearly always the earliest manifestation of vitamin A deficiency (Frandsen, '35; Sweet and K'Ang, '35). In infants and small children this symptom is frequently overlooked. Hemeralopia may, for a number of years, be the only sign of the deficiency in adults (Frandsen, '35, l.c.; Spence, '31). Even severe degrees of this condition can exist without demonstrable changes being present in the eyes.

As the avitaminosis progresses, gross anatomical changes in the eyes are observed. Patchy dryness, areas of thickened epithelium, loss of luster, and wrinkling of the conjunctiva are all considered to characterize the second stage of the disease. There is also decreased secretion of tears. At this time signs of vitamin A deficiency are noticed in other parts of the body. Keratomalacia is the last stage of the eye manifestations. The corneae are softened, permitting the invasion of bacteria, and often blindness results.

Skin lesions, both in experimental animals and in man, are also early indications of the disease. Nicholls ('33, '34) and Frazier and Hu ('36) found that dryness of the skin preceded the eye changes by several weeks. Bloch ('31) and others have called attention to the dry, scaly, shriveled condition of the skin of infants suffering from deficiency of vitamin A. Pillat ('29) and Sweet and K'Ang ('35, l.c.) state that the skin undergoes more marked and extensive changes than any other tissues excepting the eye. The sebaceous and sweat glands, hair, and nails are affected. Frazier and Hu ('31) first pointed out that in this deficiency the most specific lesions, keratotic papules of varying size, appear, distributed especially over the thighs, arms, and shoulders, which arise from the pilosebaceous follicles. These workers believe that age is a determining factor in the appearance of the skin lesions. The eruption is said to be rare in children, but rather common in adults, and when fully developed probably represents late or severe deficiency. MacKay ('34) and Frazier and Hu ('36, l.c.) believe that skin lesions may exist before xerosis is present, and are a valuable sign of vitamin A deficiency, particularly when the presence of hemeralopia cannot be ascertained, as for instance in the case of infants and young children or in cases where adults cannot be tested. These observations have been confirmed by other investigators (Loewenthal, '33; Goodwin, '34). Sheer and Keil ('34) point out that a complication arises in assessing the effects of A deficiency on the skin because papules due to vitamin C deficiency occur in early and mild cases, and resemble very closely those seen in avitaminosis A. They suggest the combined use of the capillary resistance test and biopsy observations for keratinization in the skin follicles. Youmans ('37) has successfully

treated cases with papular lesions by cod liver oil. Critical clinical studies on human subjects are needed to clear up the discrepancies recorded in the literature. The type of skin lesions described in humans has not been observed in rats. Skin lesions in rats appear when the animals are over four months of age. They are in the form of scabby ears and tails, sores on the nose, sore feet, and ragged hair.

In the latter stages of the deficiency, priapism is frequently observed. By this time abscess formation often takes place in the lymph glands near the base of the tongue in the pharynx, and the rats frequently have hematuria.

Another experimental clinical feature is the strikingly high incidence of calculi and calcareous deposits in the renal passages and urinary bladder in rats long deprived of vitamin A. Less frequent symptoms are muscular incoordination and altered sensitivity.

These characteristic symptoms are not limited to the rat. They have been observed to occur in a variety of other species, including man, when there is a shortage of vitamin A in the diet.

In contrast to the many experimental studies of vitamin A deficiency, clinical investigations have been few, and such as are available deal mostly with infants and children. It is but a short time since the symptoms of this condition in the adult have begun to be looked for clinically.

Visual Disturbances.—After the discovery of vitamin A, investigators for several years depended upon cessation of growth and the appearance of xerophthalmia as signs of deficiency of this nutrient in experimental animals. The xerosis of the conjunctiva suggested possible vitamin A deficiency in human subjects afflicted with this disease. In 1863 Bitot (cited by Blegvad, '24) had described xerosis conjunctivae in 29 patients, all of whom suffered from night blindness. He thought the xerotic covering of the eye cut off some of the light, and thus caused impaired vision in subdued light. In the same year Netter (cited by Blegvad, '24) asserted that he had observed night blindness in patients who did not exhibit xerosis. Eventually ophthalmologists reached the conclusion that night blindness, xerosis conjunctivae, and keratomalacia represent different degrees of the same ailment. De-Gouvea (1883) described night blindness in malnourished slaves in Brazil and noted that they were unable to see when returning from work after sunset, but could see well when starting for work before sunrise, although it was much darker in the morning than in the evening. This is a common experience of persons who suffer from night blindness caused by deficiency of vitamin A. This defect of vision follows exposure of the eyes to light. Night blindness may be due to other causes such as the congenital type, and the types due to retinitis pigmentosa and to detachment of the retina, alcoholism, etc.

Mori ('04), Bloch ('17), McCollum ('22) and others have compiled earlier references to night blindness associated with malnutrition, and the use of liver and cod liver oil as remedies for this ailment. Ophthalmologists have, for obvious reasons, been confused as to the etiology of night blindness. In general, they have agreed that exposure to bright light may cause the disease, and that resting the eyes in darkness may cause remission, while exposure to light may cause relapse.

A new concept was thrown upon this problem by the investigations of Fridericia and Holm ('25) on experimental night blindness in animals, and the chemical observations on the regeneration of visual purple after it has been bleached by exposure of the eyes to light. Night blindness is due to failure of vision in faint illumination. Hess ('07) had studied the effect of previous illumination on visual acuity in hens by watching them pick up grains in light regulated as to intensity. He found that after a stay in the dark they would eat in a light less intense than that in which they would stop eating without previous exposure to darkness. Holm ('25) devised a jumping test for vision of rats, making use of the dislike of the rat to remain on the surface of a table, and its desire to hide under shelter. The animals were placed on a table, the edge of which was about 10 cm. from a shelf on which was a cage that afforded shelter. The cage was marked with a piece of white paper to make it clearly visible. The table was frequently turned to new positions to make the rats depend on sight. Rats released on the table quickly explored its edges and, when they could see the cage, immediately jumped over to it. The degree of illumination could be controlled. Vitamin A deficient rats were compared with normal rats. In dim light the difference between normal and A depleted rats was very apparent. The former would see the cage and immediately run and jump across the gap to it. The depleted rats would explore the table top and perhaps stop at the right corner, run for a leap but give up the attempt. As a rule, they would at last jump, but not infrequently from the wrong corner, and fall to the floor. The attitude assumed in jumping was different from that of normal rats. When a stronger lamp was lighted, the A depleted rats would see the cage and act as did the normally nourished rats. Provision of vitamin A resulted in improvement of vision.

These studies were extended by an examination of the visual purple of the retina of normal and depleted rats, and of rats whose eyes had been exposed to strong light. Most ophthalmologists believe night blindness to arise from an abnormality in the rod cells of the retina, which are the only cells in which visual purple occurs. Vision in daylight, with the perception of colors, is believed to be the function of the cones, whereas the vision of twilight, in which colors are not

distinguished, is the function of the rods, and is dependent upon visual purple. Lythgoe ('37) has pointed out that the deep sea conger has retinae composed almost exclusively of rods, and these are fine and filamentous. He suggests that visual purple, the light-sensitive substance, is adsorbed on their surfaces, and that the large number of rods in the conger by increasing the quantity of visual purple, improves the animal's vision at low illuminations. He also states that day-hunting monkeys have a cone type of retina, whereas night-hunting species have retinae composed mostly of rods.

The Visual Acuity of Dysadaptation Test.—Since the work of Holm ('25, l.c.) showed clearly that vision at low illumination is dependent upon the presence of visual purple in the retina, and Fridericia and Holm ('25, l.c.) showed that the content of this pigment in the retina is decreased in avitaminosis A, and, furthermore, that night blindness is an early symptom of A deficiency, it was logical to suspect that an examination of the ability of the eyes to adapt themselves to vision in light of low intensity might be made a sensitive indicator of incipient vitamin A deficiency. This idea was first put to the test of experiment in this country by Jeans and Zentmire ('34). Using the Birch-Hirschfeld photometer they measured the sensitivity to light of the eyes of 213 children following partial dark adaptation. Forty five were found to have subnormal dark adaptation. Twenty one were kept under observation and were given a good diet including cod liver oil. All who were retained for study regained normal adaptation, the average time for recovery being 12 days. Later, Jeans, Blanchard and Zentmire ('37) described a new biophotometer for this purpose, and suggested further precautions for making the test. With the new procedure they found 35 per cent of 23 children in an orphanage to have subnormal adaptation. They concluded that 3000 I. U. of vitamin A per day meets the normal requirements of children.

Edmund and Clemmesen ('36-'37) used a different instrument, which permitted testing the visual acuity, the visual field, minimum visible, and power of distinction. They studied a long series of normal and of hemeralopic individuals. About half of their pregnant subjects showed low adaptation, which was severe in cases complicated by hepatitis and by vomiting. In patients other than those exhibiting eye disease or pregnancy, the incidence of low adaptation was 5 to 6 per cent. Intramuscular injection of 1 cc. of a preparation said to contain 40,000 I. U. of vitamin A caused improvement which did not persist beyond 7 days. Double this dose produced improvement for more than one and less than two weeks.

These investigators also made quantitative studies on a group of prisoners in Denmark, from which they conclude that 800 I. U. per day per man gave rise to oscillations in the power of distinction,

though rarely dysadaptation proper. Twelve hundred and twenty I. U. per day permitted oscillations in the power of distinction in a few individuals, and in some cases dysadaptation. They believe the minimum protective daily intake of vitamin A to be about 1370 I. U.

Park ('35, '36a) found by means of the dark adaptation test, 83 per cent of A deficiency among Indian children in Oklahoma. Jeghers ('37), who has reviewed the literature on this test, found 34 per cent of young adults deficient in A. On the other hand Snelling ('36) was not able to obtain convincing results with the Birch-Hirschfeld photometer. Palmer and Blumberg ('37) observed marked variability in successive tests, and urge caution in drawing conclusions from the test as it now stands. Their examinations did not indicate that Maryland and the District of Columbia school children were deficient in this vitamin. Hecht ('37) has contributed an excellent review of the literature on rods, cones, and the chemical basis of vision. He points out that, while the primary light reaction is probably simple, visual purple regeneration is complicated, in that it is influenced by pH, and a dark after-bleaching effect. The primary dark reaction probably involves more than the direct reconversion of photoproducts into sensitive material, since visual purple may be formed both by regeneration from the products of bleaching by light, and by the addition of new material. At present there seems to be widespread skepticism as to the reliability of the dark adaptation technic as a measure of vitamin A deficiency.

Friderichsen and Edmund ('36-'37) describe a method for estimating vitamin A nutrition in infants by determining the smallest intensity of light that will produce reflex movements of the face and eyes, when the infant is adapted to complete darkness. Certain artificially-fed infants were less sensitive to light than breast-fed infants, and absorption and utilization of vitamin A was so rapid after administration of fish liver oils, that a normal light response was obtained half an hour later. They point out that treatments involving restriction to water, barley water, or rice water, rapidly exhaust the vitamin A reserves.

Changes in Epithelial Tissues.—The detailed investigations by Wolbach and Howe ('25, '28, '33), Goldblatt and Benischek ('27), and many others, lead to the conclusion that vitamin A is essential in most vertebrate species, and that its absence is indicated by a common effect on epithelial structures. They pointed out that the earliest observable consequence of vitamin A deficiency is atrophy of epithelial structures. These are replaced in many locations, especially in glands and mucous membranes, by stratified keratinizing epithelium identical in appearance in all locations and arising from local proliferation of basal cells. The mucosa of the stomach and

intestines, and the renal tubules, show practically no change. When atrophy occurs, it progresses to a state wherein the cells, while preserving the appearance of viability, become inert physiologically, as well as in their function of covering cell membranes. The basal cells respond by active mitotic division, with the production of new epithelial cells as a reparative function. Regardless of previous function and morphology, the new cells continue to grow and undermine the original epithelium, and form into a stratified, keratinizing epithelium. In the liver and renal tubules, in addition to their physiological activity, the mature epithelial cells possess the power to multiply. These new cells do not respond as do other epithelia by keratinization. Why the reparative activities of basal cells of many different epithelia lead in vitamin A deficiency to a common product, an epidermis-like structure, cannot be answered, they believe, unless keratinization represents a reversion to a more primitive type than those having secretory activities. On the provision of vitamin A, the epithelium characteristic of each region returns to its normal type. The cells which have undergone keratinization are either cast off or disintegrate. Cornification of the external layers of the skin is a normal process, and takes place independently of the vitamin A supply. There is likewise a cyclic keratinization of the vaginal epithelium which appears to be under hormonal control and constitutes a phase of the estrual cycle.

Wolbach ('37) points out that the striking gross pathological condition of vitamin A deficiency in animals and in human infants is the outcome of accumulation of keratinized epithelial cells in many glands and their ducts, as well as in other organs. In glandular organs, cysts of considerable size may form, which contain yellow, cheesy masses of keratinized cells. In the lungs of human beings, and in animals, A depletion leads to cyst formation, bronchial occlusion, and sequelae such as bronchiectasis and atelectasis. Such cysts occur early at the base of the tongue, and have been described as abscesses. Daniels et al. ('23) described the accumulation of cheesy material in the sinuses and internal ear. Wolbach states that although the cysts, and the plugs of desquamated epithelial cells in ducts, bronchi, and trachea, being open to invasion by bacteria from without, serve as a culture medium for those bacteria, there is very rarely invasion of the underlying tissues, presumably because of the protective function of the keratinized layer of epithelium.

In the rat, Wolbach observed the order of response to avitaminosis A by metaplasia to be: (1) salivary glands, and accessory glands of the tongue, buccal cavity and pharynx; (2) respiratory tract, including nares, maxillary sinuses, Jacobson's organ, trachea and bronchi; (3) genitourinary tract, including renal pelves, ureters, bladder, epididymis, prostate, seminal vesicles, coagulating glands, uterus, oviducts,

and accessory glands of the vulva; (4) eye and parocular glands, corneal and palpebral conjunctiva and the harderian, intraorbital and extraorbital lacrimal, and meibomian glands. In the guinea pig the eyes and related structures are refractory respecting keratinization as compared with the rat, whereas keratinizing metaplasia in the uterus and bladder occur earlier and are more severe than in the rat.

In human infants Wolbach observed keratinizing metaplasia in the conjunctiva, nose, and accessory sinuses, trachea, bronchi, pancreas, renal pelvis, ureters, salivary glands, uterus, and periurethral glands. The commonest and earliest appearance of keratinization of epithelium in infants is in the trachea and bronchi. Next in frequency and sequence is the kidney pelvis. In infants deficient in vitamin A, the changes in the mucosa of the respiratory tract appear to account for the frequency, severity, and persistence of pneumonia. In the human subject, as in the rat, the eye lesions occur late. The cornea becomes vascularized, edematous and infiltrated with leucocytes. Infection of the cornea is favored by the accumulation of keratinized cells, and by paucity of tears for washing the eye and may lead to hypopyon, and ulceration of the cornea.

Manville ('37) counted the goblet, or mucous secreting cells of comparable areas in the stomach, pylorus, and large intestine, and found a marked reduction in the number of these in vitamin A deficient animals. He points out that reduction of mucous secretion exposes the lining of the gastrointestinal tract to injuries caused by friction with coarse particles of food. The presence of occult blood in the feces, caused by bleeding from such injuries, he believes is the earliest evidence obtainable of this deficiency. It is well known that early in A depletion there is an increase in the number of keratinized epithelial cells in washings from the conjunctival sac and in urinary sediment. Manville points out that since the protein moiety for mucous formation is not deficient in the A free diet, paucity of mucous production may be related to lack of glycuronic acid, which is a prosthetic group of mucin, the glycoprotein of mucous. He fed animals menthol, which is conjugated in the body with glycuronic acid to reduce its toxicity. When the doses exceeded the supply of glycuronic acid for conjugation, toxic symptoms appeared, and animals surviving for a few days showed ulceration of the stomach, pylorus, small and large intestines, and gall bladder. These ulcers bore a marked resemblance to those seen in vitamin A deficient animals. It is logical to conclude that impairment of function of the goblet cells would account for the reduction of mucus secretion as well as deficiency of glycuronic acid as a structural unit for the formation of mucin.

Changes in the Nervous System.—Nervous symptoms in this type of avitaminosis may result from alterations within the central nervous

system or in the peripheral nerves. These symptoms depend on the nerves affected.

Hart and McCollum ('14), and Hart, Miller and McCollum ('16) pointed out that swine suffering from lack of vitamin A exhibited symptoms of muscular incoordination, which could be cured by feeding butter fat. They showed that there were marked degenerative changes in the nervous system. Similar observations have been reported by Steenbock, Nelson and Hart ('22), Hughes et al. ('28, '29), Mellanby ('26), and Aberle ('34).

Rats depleted of vitamin A show no striking nervous symptoms; incoordination occurs as a late symptom. In the vitamin A depleted rats, Aberle observed clumsiness, incoordination, and finally spasticity, with complete loss of control of the affected limbs, but not until the weight increment was reduced to zero, or until xerophthalmia supervenes late in the course of the syndrome.

Zimmerman and Cowgill ('36) recently described the lesions of the nervous system produced by this deficiency. These investigators found the myelin degeneration in the sciatic nerves greater than in the brachial plexus. No lesions were demonstrable in the cerebrum. In the spinal cord, degeneration of the medullary sheaths in both sensory and motor tracts was quite extensive.

In swine, muscular incoordination is, according to Hughes and coworkers ('28, l.c.), the outstanding symptom, while eye symptoms, which are so prominent in the rat, may be absent. In swine there is watering of the eyes, but no severe conjunctivitis, and keratinization of the tissues about the eyes seems not to be pronounced. However, impaired vision occurs.

One form of posterior paralysis in hogs is due to degeneration of the medullary sheaths. Guilbert, Miller and Hughes ('37) observed that the development of this paralysis is usually secondary to night blindness. Prior to this condition there is incoordination of movements. Back muscles on one side only may become involved, resulting in more or less marked scoliosis. Incoordination and spasms in swine, due to A deficiency, suggest nerve involvement. The findings of Hughes et al. are not contradictory to the view expressed by Wolbach, that nerve lesions are a late manifestation in avitaminosis A. The time required to produce incoordination in swine is not stated by Hughes. His work confirms earlier studies which show that in advanced A deficiency, degeneration of nerve cells occurs in the cord, optic, sciatic, and femoral nerves. Hughes found that the nervous symptoms in swine could be prevented by feeding cod liver oil, butter fat, yellow corn, or alfalfa leaf meal; hence, deficiency of water soluble vitamins was presumably not involved. Vitamin A therapy, if instituted early, results in marked improvement of general muscular in-

coordination, weakness and even posterior paralysis, but complete recovery is rare. The lesions in the nerves once developed are usually permanent and probably often progressive (Hart and Guilbert, '37).

E. Mellanby ('29-'30, '31) presents a different interpretation of the morbid histology of avitaminosis A. He has given special attention to the degeneration of the gasserian ganglion and the trigeminal nerve, which is distributed in the skin and structures of the face, tongue, teeth, eyes, etc. He supposes that xerophthalmia is an expression of degenerative changes in the nerve supply of the cornea, and he suggests that hyperplasia or metaplasia of the various epithelial membranes may be related to changes in their afferent nerve supply. Mellanby and King ('34) suggest that the periodontal metaplasia which occurs in avitaminosis A, and which may be the cause of pyorrhea, and possibly of dental caries, may be an expression of loss of neurotropic control.

E. Mellanby ('34) proposes vitamin A administration in prophylaxis and treatment of septicemias and of infectious diseases, and suggests vitamin A deficiency as the cause of nerve degeneration in beriberi, pellagra, disseminated sclerosis, pernicious anemia, lathyrism, and convulsive ergotism. He cites 13 patients with disseminated sclerosis, who were greatly improved by vitamin A therapy. Wolbach advises caution in acceptance of Mellanby's observations because they depend upon the "capricious Marchi technic" and because degeneration of the myelin sheath is common to many disorders which result from several vitamin deficiencies and diverse causes.

That caution is indicated in drawing conclusions concerning a specific relation between avitaminosis A and nerve degeneration is suggested by the studies of Zimmerman and Burack ('34), who observed degenerative lesions believed to be identical with those seen in human pellagra, in animals receiving sufficient vitamin A, and deficient only in the "black tongue-preventive factor." Clinically, thiamin (vitamin B_1) without A is successfully used in the treatment of beriberi, in which nerve injury is the outstanding feature. Investigations are greatly to be desired in which an uncomplicated A deficiency is studied with respect to nerve lesions and other signs of avitaminosis A, such as the skin manifestations, keratinization of conjunctival scrapings, keratinized cells in the urinary sediment, dysadaptation of vision, etc. It is seldom that even the informed reader can be certain that experimental diets have been complete as respects all factors other than vitamin A.

Lathyrism is a condition commonly seen in India and Northern Africa and occasionally in Southern Europe, which is ascribed to a neurotoxin in lathyrus peas. Spasticity and rigidity of the large muscles and degenerative changes in the spinal cord are its chief features.

Mellanby ('30) has produced a similar condition in dogs by a diet composed of a variety of pea (Akta) and deficient in vitamin A. Animals receiving this diet supplemented with A showed but slight degeneration of the spinal tracts. Further supposition that this vitamin may furnish protection against lathyrism is supported by the experiments of Geiger, Steenbock and Parsons ('33). They produced experimental lathyrism in both young and adult rats by a diet of the flowering sweet pea, itself rich in vitamin A. Young animals were not protected against toxicity of the sweet peas by cod liver oil or cod liver oil concentrates. However, adult animals were protected to some extent.

Effects on Growth.—In the early days of vitamin research the growth promoting property of vitamin A was emphasized. A number of investigations demonstrated that deprivation of this nutrient results in cessation of growth and that the requirement of this vitamin for growth and health is greater in the young animal. It has also been observed that growth varies with the amount of the vitamin in the diet and the amount stored in the tissues of animals. However, growth failure in itself is not specific evidence of vitamin A deficiency; it is believed that interruption of growth resulting from lack of vitamin A is a secondary result of a disturbance in the metabolism of epithelial tissues. This interruption of growth, common both to infants and to laboratory animals, is associated with loss of weight due largely to loss of fat in all storage depots, and also to atrophy of musculature and many organs, *e. g.,* liver and kidney, which do not undergo keratinizing metaplasia.

Changes in Bone Tissue.—Extensive changes are found in the bones in A deficiency. Tozer ('21) found that the bones of animals deprived of vitamin A were fragile and exhibited minute ridges at the rib junction. Histologically, after 14 days on the deficient diet, the changes in the costochondral junction were indistinguishable from those occurring in the ribs of scorbutic animals. After 21 days of depletion, considerable reduction was observed in bone length, both of the trabeculae and the rows of cartilage cells. These changes were progressive and by 30-40 days the rows of cartilage cells and trabeculae had almost disappeared. The cartilage often ended in a thin, ossified band, which was apparently laid down to support a weak joint. After a period of 50 days, due to the marked fragility of the bones, fractures of the ribs occurred and sections showed proliferation of cartilage cells. The cartilage and bone itself are densely calcified. Harris ('26) has allocated a special function to vitamin A in connection with the growth of bone. He considers ossification proper a specific function of A.

Wolbach ('37, l.c.) has shown that in this avitaminosis there is a

cessation of proliferative activity of the epiphyseal cartilages. Those cells which are in the vesicular stage disappear, and there remains only a thin line of atrophic cartilage cells embedded in a calcified deposit which forms a plate of bone on the diaphyseal side. This gives sections of immature bones an appearance similar to that of adult bones. When vitamin A is supplied, there is a prompt response by the capillary blood vessels, which, arising from the bone marrow, penetrate the bony deposit enclosing the vesicular cartilage cells, causing its resorption, following which active cartilage cell regeneration takes place and normal bone growth is resumed.

Effects on Teeth.—The tooth lesions in this condition, which are considered by Wolbach and Howe ('33, l.c.) to be the most important dental changes of any of the deficiencies, are striking and characteristic. The ameloblasts, or enamel forming organs, are of epithelial origin. Avitaminosis A causes them to atrophy and undergo metaplasia, with consequent formation of hypoplastic enamel. Mellanby ('28), who has studied the influence of vitamin deficiencies on tooth development, has shown clearly that teeth formed in animals completely deprived of vitamin A have no enamel; while in less severe deprivation the enamel is defective, of poor structure, and contains pits and fissures which predispose the teeth to decay. Similarly, normal dentin formation is dependent upon an adequate supply of vitamin A. There is no reason to doubt that this vitamin plays a more important role than does any other specific nutrient in maintaining the normal function of the enamel-forming organs.

Wolbach and Howe ('33, l.c.) have described the changes which occur in the incisor teeth of rats and guinea pigs in avitaminosis A. The atrophy of the ameloblasts is accompanied by keratinization. This is followed by atrophy and loss of polar deposition of dentin matrix by the odontoblasts, the dentin-forming cells. The odontoblasts remain morphologically normal and functionally active on the labial side of the tooth in apposition to the enamel organ long after the complete disappearance upon other surfaces. With complete atrophy of the ameloblasts in the rat the odontoblasts disappear also on the labial side. Although they survive, the odontoblasts lose their columnar shape and continue to deposit dentin, but in a centrifugal pattern like osteoblasts or bone-forming cells, instead of at the outer pole only as in the normal cells.

Effects on the Hematopoietic System.—In addition to the interruption of growth, degenerative lesions of the skeletal muscles, lymphoid hypoplasia of the spleen, and an anemia which is accompanied by the accumulation of hemosiderin in the liver and spleen, and by atrophy of the spleen and bone marrow, are secondary results of the disturbance in the metabolism of epithelial structures due to vitamin A

depletion. Berglund, Keefer and Yang ('29) believe that other factors appear to be necessary to produce this form of anemia. Similar observations have been reported by Keefer and Yang ('29), Pillat and Yang ('30) and John ('31). Koessler et al. ('26) suggested that there is a relation between vitamin A deficiency and pernicious anemia, but Sure, Kik and Walker ('31) were unable to substantiate these observations.

Koessler, Maurer and Loughlin ('26, l.c.) have reported a marked decrease in the red cells and hemoglobin in A deficient rats. There apparently is no reduction of the leucocytes in the blood stream of these animals. In fact, a polymorphonuclear leucocytosis has been reported (Findlay and McLean, '25) to be not uncommon after the onset of keratomalacia.

Cramer, Drew and Mottram ('22, '23) and Bedson and Zilva ('23) noted a diminution in the number of blood platelets in A depleted rats independent of their growth propensities. This change becomes markedly manifest before infective conditions appear. Cramer and Drew ('23) suggest that platelets are formed by the endothelial cells of lymphatics and blood vessels. Administration of A stimulates the production of platelets and delays the development of bacterial infection. Falconer and Peachey ('26), on the other hand, find the changes in the relative number of platelets, red cells, and white cells not constant enough to constitute a specific lesion in vitamin A deficiency.

Lesions in the leucoblastic bone marrow are rarely found in A deficient animals, except occasionally in a very few adult animals depleted for a prolonged period. An aplastic condition of the marrow occurs (Findlay and Mackenzie, '22). In the early stages of the deficiency, some slight atrophy, but no hemorrhage of the bone marrow, is found (Tozer, '21, l.c.). Later, progressive atrophy occurs, and in extreme cases little but the delicate reticulum of the connective tissue groundwork and the blood vessels remains. Upon the provision of vitamin A these pathological effects are arrested and the tissues tend to return to normal, provided infectious processes do not intervene. The addition of this nutrient brings about hyperplasia of the lymphoid tissues, increased bone marrow, and complete blood regeneration.

Metabolic Changes.—Sure and Smith ('31) found that in various stages of vitamin A deficiency, as indicated by the severity of the eye lesions, there were no significant changes in the concentration of blood sugar. The carbon dioxide capacity of the blood remains normal in this condition. The content of liver glycogen in A deficient rats was normal. There was reduced food intake, and excessive water consumption in their animals.

Synthesis of purine bodies is diminished in vitamin D depletion

(Morgan and Osburn, '25). According to these investigators, in the normal animal the excretion of allantoin diminishes with a gain in weight and increases as the weight falls. Exactly opposite results are obtained in the case of the vitamin A deficient animals. Emmett and Peacock ('25) observed a disturbance of uric acid metabolism in this deficiency. Kimura ('28) reports that experimental hypercholesterolaemia in the rabbit returns to normal slowly upon administration of vitamin A in the form of biosterin, Takahashi's name for vitamin A.

Green ('34) observed that depletion of vitamin A in the rat produced a marked decrease in the esterase content of the blood serum. Fat metabolism is not altered since fat is still absorbed, mobilized, desaturated, and oxidized in the absence of vitamin A. Administration of vitamin A produces a marked rise in the serum cholesterol.

Urolithiasis.—Osborne and Mendel ('17), Van Leersum ('28), McCarrison ('31), Higgins ('33), and many others have reported the finding of urinary calculi in rats deficient in vitamin A. Higgins ('35) found urinary calculi in 85 per cent of A deficient rats which survived 250 days. These stones were composed principally of calcium phosphate with a trace of calcium carbonate. In studies on both human subjects and on rats, he finds the sequence of events in calculus formation to be: (1) keratinization of epithelium of the genitourinary tract, (2) urinary infection, and (3) alkalinuria. The low solubility of calcium phosphate in alkaline media accounts for the separation of crystalline phosphate. In rats, bladder infection usually occurs after 30 days and renal infection after 60-90 days of deprivation of vitamin A. Addition of ammonium chloride to the diet causes a decrease in the formation of calculi, owing to its acidifying effect. Higgins asserts that precipitation, per se, causes sediment and not stones. Desquamated epithelial cells are present in the urinary tract. Stone formation, however, is facilitated because keratinized epithelium creates sufficient irritation and local lesions to produce fibrin and mucin which form a frame work for the deposition of crystalline sediment and subsequent development of stones.

Oppenheimer and Polack ('37) contested the claims of Higgins that high vitamin A intake and acid ash diet will prevent the formation of phosphate stones. Most investigators of this subject believe that vitamin A deficiency is but one of several causes of urolithiasis. The regular adherence to a diet affording an alkaline ash would certainly increase the likelihood of deposition in the kidney or bladder of calcium phosphate stones because of the alkalinity of the urine, and the insolubility of calcium phosphate in alkaline media. The present day emphasis upon "alkalizing" the blood is based upon fallacy. Another cause of stone formation probably is hypochlorhydria. When the stomach does not secrete sufficient hydrochloric acid, the

urine tends to become and remain alkaline. This condition is corrected therapeutically by the administration of acid. Infection of the bladder, ureters, and kidneys, with ammonia production from urea fermentation, quite apart from avitaminosis A, would presumably cause stone formation. All considerations point, however, to the probability that avitaminosis A does cause loss of power of the mucosa of the urethra, bladder, and other areas in the genitourinary tract, to prevent invasion of the tract by microorganisms which are normally present about the urethral orifice but which, in health, are unable to thrive on a mucous surface. The excessive growth of fuso-spirochetal organisms in the mouth of dogs restricted to a blacktongue-producing diet, demonstrated by Smith and coworkers ('37), is a case in point. Only the etiology of phosphate stones is suggested in relation to avitaminosis A. From the studies of O'Conor ('35), it appears that deficiency of this vitamin is significant in promoting the formation of calcium carbonate stones, especially in subjects restricted to alkaline diets for therapeutic purposes. He states that recurrence of these stones is frequently prevented by a diet rich in vitamin A.

Hou ('35), Lelesz and Przezdziecka ('35), and others, have asserted that rats depleted of vitamin A develop urinary calculi more readily when given large doses of vitamin D. The relationship in humans between vitamin A depletion and the formation of kidney stones still remains controversial.

Disturbances of Reproductive Function.—Long and Evans ('20) described the cell changes in the vaginal mucosa during the estrual cycle of the normal rat as follows: stage 1) small, nucleated epithelial cells only; stage 2) large, cornified, non-nucleated cells; stage 3) intensification of stage 2—a great many cornified cells; stage 4) polymorphonuclear leucocytes, cornified cells and large epithelial cells or only cornified cells and leucocytes, or occasionally, large nucleated epithelial cells and cornified cells; and stage 5) polymorphonuclear leucocytes and epithelial cells. In 1922 Evans and Bishop reported a change in ovarian function in vitamin A deficiency. This change is characterized first by the appearance of abnormal numbers of cornified epithelial cells (keratinized cells) and the disappearance of leucocytes. This cornified phase becomes further and further prolonged until the entire cycle is characterized by cornified epithelial cells. If gestation ensues, animals occasionally exhibit resorption of the fetuses when depletion of the vitamin is severe. Usually they carry their young to term or slightly beyond, exhibiting an obscure impairment of the birth mechanism which ordains fetal death. Lesser severity of vitamin A deficiency may permit rhythmic estrus, obscured by the continuous cornified smear, but evidenced by the recurrence of copulation. Even though conception does not occur, the

estrual cycle is not appreciably lengthened, so there is obscure involvement of that endocrine mechanism which establishes pseudopregnancy after infertile copulation.

Macy and coworkers ('27), Evans ('28), and others, conclude that the presence of cornified cells in the epithelial layers of the vagina is the first and most delicate symptom of vitamin A exhaustion. This view is not shared by Parks and Drummond ('26) and Coward ('29). However, recent investigations (Mason, '35) seem to establish these findings.

Aberle ('33) found that continual cornified peripheral vaginal cells appeared in 100 per cent of rats on a diet deficient in vitamin A, and that this symptom invariably preceded other symptoms of avitaminosis A. The time elapsing was positively correlated with the amount of the vitamin contained in the diet or stored in the body.

Sherwood, Brend and Roper ('36) gave rats massive doses of carotene in oil for 15 days, and observed that such doses prevented a normal vaginal smear picture. They examined 18 rats at 8 hour intervals through eight complete estrus cycles. These rats were then given 1500 I. U. of vitamin A in the form of carotene daily for a period of 15 days. A second group of 16 rats were similarly observed, and were then given 3750 I. U. of carotene for 15 days. Control animals on the same diet were given 0.5 cc. daily of cottonseed oil, or of corn oil, and showed normal vaginal smears throughout the period of observation. In the experimental animals receiving massive carotene, the smears did not progress from the nucleated epithelial to the cornified cell stage. There was an excess of nucleated epithelial cells regardless of the phase of the estrus cycle. This was due to a rapid cell growth. These results confirm those of Mason and Ellison ('35). Simpson and Mason ('36) treated thirty cases of senile vaginitis in elderly women who were subsisting upon poor diets, by oral administration of cod or halibut liver oil. Vaginal repair was rapid and complete. This study seems to point the way to dietary prophylaxis against this condition.

Mason ('33) has made the most critical study of the effects of different states of nutritional deficiency on the testes of the rat. In the so-called "incomplete total inanition" or the state induced by feeding a diet containing all essentials, but fed in insufficient amount, when brought about after the animals have attained sexual maturity, the testicular injury resembles in some respects that caused by vitamin A deficiency. There occurs cellular sloughing and gradual reduction of seminiferous tubule size. Depletion of germinal epithelium is never complete.

In uncomplicated A deficiency there is testicular degeneration, with profound injury to the seminiferous epithelium. The residual

germ cells show evidence of continued attempts at sperm formation even in tubules whose epithelium is reduced to a few layers of cells. Injury to the seminiferous epithelium is not irreparable, as in vitamin E deficiency, since the restoration of an abundance of vitamin A in the diet leads to regeneration and complete reinstatement of the tubular epithelium in a high percentage of cases. Mason states that testicular injury occurs before other external symptoms of A deficiency appear. He believes that testicular injury and vaginal cornification are two early manifestations of disturbed metabolic processes in epithelial tissues. It appears that the more highly organized and physiologically specialized the epithelium, the more readily is it disturbed by lack of any factor necessary for its metabolic needs. Repair of avitaminosis A injury to the testes requires from 5 to 13 weeks. During vitamin A therapy, the repair process is not greatly retarded when the body weight of rats is maintained at a subnormal level, or is even caused to decrease. The testicular damage after A depletion cannot be attributed to an indirect effect resulting from hypophyseal disturbance, but is one of the manifestations of the effect of this deficiency on epithelial structures in general.

Moore and Mark ('36) observed, in a clinical study, metaplasia and inflammation of the prostate in man which resembled the effects of vitamin A deficiency. A series of vitamin A depleted rats showed atrophy of the testes and accessory sex glands which these investigators believe to be indicative of some disturbance in the hypophyseal-gonadal-prostatic hormone relationships. Avitaminosis A in the rat they found to be associated with foci of inflammation and epithelial metaplasia in the prostatic acini and vesicular ducts similar to that reported in other organs. Focal metaplasia and inflammation is occasionally encountered in the prostate in cases of extreme inanition associated with stenosis of the esophagus. It seems probable that this lesion is due to vitamin A deficiency.

Relation to Infection.—The literature on the relation of vitamin A deficiency to resistance to infection is very extensive and conflicting. In 1935 the Council on Pharmacy and Chemistry of the American Medical Association defined its attitude toward the permissible claims which advertisers may make for vitamin A in cod liver oil as follows: "By virtue of its vitamin A content it promotes growth and, as indicated by experimental studies, may be an aid toward the establishment of resistance of the body to infection in general, though it has not been shown to be specific in the prevention of colds, influenza and other such infections." At this time the Council has not altered its position on this subject.

Since it is generally conceded that deficiency of vitamin A impairs the health and functions of epithelial membranes, the possibility that

avitaminosis A might increase susceptibility of the organism to colds and other respiratory diseases, has been tested by several investigators. Gardner and Gardner ('34) studied two groups of carefully paired children from 6 to 14 years of age. The experimental group received 10,000 "units" daily of vitamin A in halibut liver oil. The control group was selected on the basis of a high resistance to colds. In the treated group there was a marked decrease in severity and incidence of colds, accompanied by improvement in health in general.

Cameron ('34) divided 65 women students into five groups. The members of one group took a tablespoonful of cod liver oil daily from November to March. The other groups were given respectively a vitamin A concentrate, a cod liver oil concentrate in tablet form, an inert tablet, and no medication. The number of days with colds reported from the group taking no medication was considerably higher than from any of the other groups.

Holmes et al. ('36) made a five-year study of the effect of administration of cod liver oil to an industrial group on absenteeism caused by colds and other respiratory disease. In the first to the fifth year inclusive, there were respectively, 341, 566, 691, 648, and 785 subjects under observation. Approximately half of these were given about 5 tablespoonfuls of cod liver oil weekly. The results indicate that colds and other respiratory infections were materially lessened in the cod liver oil group as compared with the control group. Necessarily, elements are involved in such studies which make it impossible to appraise quantitatively figures employed in the expression of the results of the observations. Eventually, sufficient data will be accumulated to make clear the general tendency of this form of dietary prophylaxis in its effect on respiratory disease.

Clinical findings are conflicting. From the facts available it is impossible to evaluate these results because the intake of the vitamin was not definitely established and the nutritional condition of the cases studied was extremely varied. In conclusion, investigations indicate that vitamin A is anti-infective merely in the sense that it prevents the typical keratinization of tissues produced by avitaminosis A and secondary infections. Vitamin A does not effect general immunity. The various experiments indicate that there is no basis for the belief that vitamin A therapy is effective clinically in infections caused by specific highly pathogenic organisms. Also it is of no value in infectious diseases which are unassociated with the characteristic structural breakdown of epithelial tissues, and the accompanying localized infection typical of vitamin A deficiency.

Relation to Artificially Induced Infections.—Verder ('29) fed A deficient rats and a normal group, suspensions of *B. enteritidis* for seven days, after which time the animals were killed. A greater num-

ber of the A deficient animals showed positive spleen cultures for the organism than did the controls. Szulo and Kolodziejska (cited by Robertson, '34) report decreased resistance in A deficient rats to the human strain of tuberculosis as compared with controls.

Boynton and Bradford ('31) found that young white rats inoculated subcutaneously with a standard suspension of bacilli of the *mucosis capsulatus* group, after 4, 6, 8, and 10 weeks' depletion of vitamin A, showed marked decrease in resistance to this infection, as compared with the controls receiving cod liver oil.

Finkelstein ('32) found that mice depleted of vitamin A and infected with bovine tuberculosis, ran a more acute course than was the case in mice receiving the same diet plus 0.005 mg. of carotene per day. McClung and Winters ('32) observed that 15 days after the intraperitoneal injection of mouse typhoid I, only about 5 per cent of their A deficient rats were alive, whereas about 95 per cent of the controls which received cod liver oil were alive. As will be shown in the discussion of vitamin D, this appears to have little or no effect on the course of several types of infections. These typical studies, which are in harmony with the earlier work of Daniels ('23, l.c.) and of Mellanby and Green ('29), and Green ('32), must suffice as a fair sample of the rather extensive literature on vitamin A in relation to artificially induced infections.

Effects on Immunological Reaction.—The immunological properties of the blood of A deficient animals appear to remain unchanged. The bactericidal power is lowered, however. Findlay ('25) does not believe this to be a direct cause of vitamin A depletion, since a reduction of this factor occurs in all acute infections. Both Smith and Wason ('23) and Findlay and McLean ('25, l.c.) reported a perceptibly reduced phagocytic activity simultaneous with the appearance of keratomalacia. Tanaka (cited by Robertson, '34, l.c.) states that there was a reduction of agglutinins and amboceptors in rats and guinea pigs deficient in A after injection of cholera and typhoid bacilli, as compared with the controls. v. Euler ('31) found that when carotene was given in liberal amounts to rabbits the amboceptor in the blood was increased. Green ('33) restricted rabbits to a diet deficient in vitamin A and observed that there was no reduction in the natural hemolysin or complement titre of the blood. Upon injecting typhoid bacilli into normal and vitamin A deficient rabbits, she found that the deficient animals produced somewhat less antityphoid agglutinins and bacteriolysins than did the controls. When sheep's red blood cells were used as the antigen, the hemolysin production in the deficient rabbits was much below normal.

Relation to Thyroid Function.—Abelin ('33) and Wendt ('35) observed that administration of vitamin A concentrates to cases of

exophthalmic goiter reduced the basal metabolic rates. They found this treatment especially suitable in cases where the continuous use of iodine is inadvisable. According to Wendt ('36), Abelin ('36), and Sure and Buchanan ('37) thyroxin and vitamin A are antagonistic. They report abnormally low blood concentrations of vitamin A and, to a lesser degree, of carotene in cases of exophthalmic goiter. After successful iodine or surgical treatment the vitamin A and carotene values of the blood were normal. Wendt suggests that in myxedema carotene is not completely converted to vitamin A. These observations have been confirmed by a number of investigators. Abelin ('36, l.c.) points out the merits of using vitamin A as auxiliary treatment in the medication with iodine and with diiodotyrosine and thus lessening the "crisis" and the temporary exacerbations that usually occur in these forms of medication. However, more experimental data are necessary before a definite evaluation of vitamin A therapy in hyperthyroidism is possible.

Role in Healing of Wounds.—A number of clinical publications (Löhr and Unger, '37; Chevallier and Escarras, '37; and others) report beneficial effects of cod liver oil ointments in cases of skin burns and wounds. Healing of wounds produced in experimental animals is accelerated by cod liver oil, the total unsaponifiable fraction of cod liver oil, by vitamins A and D in moderate concentrations, and by the easily-oxidized unsaturated fatty acids, particularly as the free acid and in the presence of vitamin A.

Vitamin A in oily solution, applied to the surface of experimental wounds, causes a marked change in the character of the exudate and a rapid shrinkage of the edges of the wounds.

Functions of Vitamin A.—The functions of vitamin A are not yet definitely established. However, the vitamin appears to be essential for normal growth, for normal function of the eyes, and for normal structure and function of the epithelial tissues. It has been speculated whether vitamin A is an integral part of structures like epithelial cells or whether it acts as a catalyst of certain cellular functions. The symptomatology produced by avitaminosis A offers little insight into the precise function of this nutrient in the tissues.

Absorption.—Due to the close relationship between carotene and vitamin A, and also due to the fact that the vitamin has but recently been isolated and synthesized, experiments dealing directly with the vitamin itself are not available.

The process of digestion is not a requisite for absorption and utilization of this nutrient, but the degree of absorption is widely different for carotene and the vitamin, varying with the intake, manner of administration, and the health of the alimentary tract. Both are soluble in fats and fat solvents and are absorbed by the lacteals of the intestine,

thus becoming intimately associated with the chyle. They enter the circulation through the thoracic duct (Drummond et al., '35). No change in either substance takes place during the absorbing process. The liver plays a significant role in regulating the concentration of the vitamin throughout the body. Examination of normal livers revealed that the amount of A tends to remain constant within certain limits (Hess et al., '33). When the diet is poor the vitamin A reserve of the liver is less than in the normal. Experiments indicate that even complete removal of A from the diet requires a considerable period of time to deplete the liver vitamin reserve.

Assimilation of carotene is extremely variable. Under normal circumstances carotene is somewhat less efficient than the vitamin.

Carotene when administered in small quantities, under ordinary conditions, is utilized to the extent of about 80 per cent. Larger quantities are not as well utilized as vitamin A, particularly if the carotene is fed without being dissolved in oil.

One of the best illustrations of the difference in the absorption of carotene and vitamin A is the effect of mineral oil on the absorption of these substances. Vitamin A appears to be satisfactorily utilized in the presence of this compound, but the absorption of carotene is markedly diminished. The vitamin administered with large amounts of the oil may be largely lost to the body if the concentration of A is very slight, and of the oil is very great. There is very little evidence of such loss when the oil and the vitamin are given separately. The vitamin as well as carotene is less well absorbed in paraffin oil than in vegetable fat and better on a fat-rich than on a fat-poor diet.

The presence of bile is not necessary for the absorption of vitamin A from the gastrointestinal tract of the rat. Greaves and Schmidt ('35) showed that rats whose bile was diverted to the upper part of the descending colon by means of cannula, did not respond to oral administration of carotene as did normal rats. They were responsive, however, when glycodeoxycholic acid or deoxycholic acid was simultaneously given, indicating that these bile acids function as carriers of carotene across the intestinal wall. Variations in the fat content of the diet from 3 to 23 per cent did not materially influence the absorption of carotene from the alimentary tract. On the other hand, the vitamin itself was absorbed by rats with internal bile fistulae. Similar results obtained with jaundiced animals support the view that, whereas vitamin A can be absorbed from the intestine in the absence of bile, the presence of this body fluid is essential to the utilization of carotene given by mouth.

Dietary intake alone of the vitamin is obviously not sufficient to insure its utilization. Reports of secondary cases of avitaminosis A are appearing in the literature, which are due to deficient absorption of

this substance. Among this group are cases of gastrointestinal and hepatic disease, of severe infectious diseases, and of increased requirements due to rapid growth.

Excretion.—Green ('32, l.c.) believes that the urinary excretion does not account for any significant loss of vitamin A, and that if taken in excess, the unstored portion of the vitamin is destroyed in the liver or oxidized in the blood stream. Schneider and Weigand ('37) investigated the elimination of vitamin A in 180 cases. They found that the normal organism does not eliminate it in the urine even after administration of excessively large doses. However, a large percentage of patients with cancer, tuberculosis, or general infections, did excrete the vitamin in urine. The authors believe this to be caused by liver impairment or by a change in the renal permeability.

Vitamin A normally plays an important role in renal physiology. In avitaminosis A, dogs and human subjects are slower in starting and do not maintain diuresis as well as normal animals.

Rowntree ('30) demonstrated in experiments with young children that even after the ingestion of an excess of vitamin A only 2-12% was lost in the feces. Wendt ('37) determined the vitamin A content in the feces of normal subjects with and without vitamin administration and also of patients with various disturbances in the processes of resorption. The cases receiving the vitamin showed a slight increase at first, but later the increase suddenly rose to several hundred or even several thousand units. These results seem to indicate that before an excess of A is eliminated in the feces, the vitamin reserves of the body are filled and that the organism has a regulatory mechanism that prevents an excessive increase in the vitamin content. In the patients with considerable disturbances in the resorption such as icterus and peritoneal carcinosis, the amount of vitamin A excreted from the very beginning was high. The concentration of A eliminated corresponded to the degree of impairment in the resorption.

Storage.—Various experiments (Steenbock et al., '23; Wilson, '27) definitely show that the rat is able to store vitamin A in large amounts in its tissues. The storage capacity for the vitamin is relatively greater at the age of most rapid growth (Sherman and Cammack, '26). The liver appears to be the major reservoir of vitamin A storage, varying in its content with the diet fed. Substantial amounts of A are also retained by the lungs and kidneys. The remarkable powers of vitamin A storage are illustrated by the observation that a quantity sufficient to protect a rat for several months may be given in a single dose. According to the findings of Chevallier and Choron ('36) the feeding of vitamin A results in more regular increments of the bodily store of the vitamin in guinea pigs than does the feeding of carotene.

Baumann and coworkers ('34) showed that 95 per cent of the rat's

reserves were stored in the liver. Only traces could be demonstrated in rats under 3 weeks of age but slightly larger amounts were found in young whose mothers had been fed large amounts of the vitamin during pregnancy. The amounts stored in these animals were inversely proportional to the state of depletion. At no time could more than 10-20 per cent of the vitamin administered be accounted for. Discrepancies between the incidence of deficiency symptoms and the exhaustion of the reserves, suggested the existence of vitamin A in a form which escapes detection by the usual methods. Fecal elimination of the vitamin was small. Other and more recent studies on this subject, using several different species of animals such as cattle, sheep, and swine, show that vitamin A is absorbed more easily than carotene.

Liver diseases (Wilbur and Eusterman, '34) prevent the proper storage of vitamin A as well as the conversion of carotene into the vitamin.

Of outstanding physiological interest are the experiments of Ralli and coworkers ('36), who have studied the effect of administration of vitamin A and of carotene upon the blood and liver vitamin A and carotene of normal and diabetic dogs and humans over periods of from 1 to 4 months. A given vitamin A or carotene intake was found to cause a greater rise of blood and liver vitamin A and carotene in diabetic than in normal subjects, which is interpreted to indicate that the power of conversion of carotene to vitamin A is diminished in diabetes.

Uterine and Mammary Transmission.—Butter fats from different sources show little variation in vitamin A content notwithstanding the varied intake of the vitamin by the cows. Only a small fraction of the total amount of A given to the cows appears in the milk. It seems that if the cow's diet has a normal vitamin A content, further additions to the diet have relatively little effect on the milk.

A survey of the literature (Dann, '32; Wolff, '32) shows that the vitamin A reserve of newborn young of various species is rather low. Although it increases during the suckling period, there is a definite limit to the amount of the vitamin which can be passed on to the offspring through the mother's milk, no matter how great her intake may be. The concentration of fat in the maternal diet has a perceptible, if scarcely important, effect on the transfer of vitamin A into milk.

Normal Requirements.—The exact daily requirement of vitamin A by humans has not been determined. Just as different foodstuffs vary in the amount of the vitamin they contain, so individuals may vary in their ability to absorb, store, convert, and destroy carotene and vitamin A. Nevertheless, a number of estimates of the daily human requirement for this factor are available, but for obvious reasons they vary a good deal.

The American Public Health Committee on Human Vitamin Needs ('34-35) recommend 2800 International Units as satisfactory to protect infants from vitamin A deficiency.

Fraps and Treichler ('33) believe 1400 I. U. to be the minimal daily requirement. This is considered to be rather low. Stiebeling ('36) states that the daily requirement of adults is from 4200-5600 I. U. Growing children are allowed double the amount. Cameron ('35) recommends an optimal intake of 5000 I. U. or more a day. The Committee of Nutrition of the League of Nations ('36) suggests a daily intake of 8700 I. U. for pregnant and nursing women.

Most of the values cited are estimates based on clinical observations. Jeghers ('37, l.c.) recommends the use of normal dark adaptation as an index of normal vitamin A metabolism and hence a means of estimating the minimal daily requirement of healthy adults. According to this method 4000 I. U. represent minimal requirement for a healthy adult. He advises a 50 per cent increase in the daily intake for optimal results.

Requirements in Disease.—The same general principles apply in pathological states as in normal individuals, but there are several further conditions which are important, such as fever, rapid growth (Hess et al., '33, l.c.), general infection (Wilbur and Eusterman, '34, l.c.), elevated basal metabolic rate (Wendt, '36, l.c.), and pregnancy (Edmund and Clemmensen, '36, l.c.), in which general metabolism is increased and where an increased vitamin A intake is required. Any of these conditions can lead to vitamin A deficiency even though the amount of vitamin A in the diet is theoretically adequate. In many diseases special diets are used which may be seriously deficient in A. Loss of appetite, gastrointestinal intolerance, and impaired absorption of vitamin A may further limit the intake of vitamin A-containing foods. Such cases must be given careful consideration regarding the amount of vitamin to be given, as well as the mode of administration.

Effect of Massive Intake.—A number of investigators have reported that administration of large doses of carotene or vitamin A produces no toxic effects. Davies and Moore ('34) found that as much as 1,333,333 I. U. daily per rat showed no injurious effect. Rabbits (Landy, '34) revealed no changes upon consumption of 21,430 I. U. daily. In a study of a group of children ranging from 8-14 years of age, and of both sexes, dosage up to 16,000 I. U. of the vitamin daily had no apparent deleterious effect on their health McBeath, '32). These data indicate that vitamin A in itself is not toxic in doses much above those necessary to prevent avitaminosis A.

Therapeutic Uses of Vitamin A.—Spence ('31, l.c.), Aykroyd ('30), Frandsen ('35, l.c.), Gamboa ('30), Jeans and Zentmire ('36),

Park ('35, l.c., '36, l.c.), Jeghers ('37, l.c.), and others, have reported complete disappearance of the deficiency symptoms after treatment with vitamin A-containing foodstuffs, concentrates of the various fish liver oils, or carotene.

Thus treatment of this avitaminosis, in the absence of intestinal and hepatic diseases which prevent adequate absorption and metabolism of vitamin A, consists in furnishing the vitamin in adequate amounts in the diet.

If oral administration of the vitamin is impossible, it may be administered parenterally or subcutaneously with equal efficacy.

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CHAPTER

XIV

Chemical Nature of Vitamin D

IN DISCUSSING the discovery of vitamin A it has been pointed out that the deficiency state involving this nutrient long antedated any definite knowledge of its etiology, and that, while some success was realized in its dietary treatment, it was not until after the experimental production of the pathological state in animals that rapid progress was possible in the accumulation of exact knowledge of the dietary factor involved. Liver was known before the Christian era to be a specific remedy for night-blindness; Lind and others knew of the virtues of fresh vegetable foods as preventives of scurvy; and Takaki eradicated beriberi from the Japanese navy by instituting a dietary reform. The experimental production of these diseases in animals was promptly followed, in every instance, by extremely rapid advance of knowledge, both of the nature of the missing substance in diet, and of the physiology and pathology of these states. The history of the advancement of knowledge of rickets and of antiricketic substances followed a comparable course.

The use of cod liver oil as a therapeutic agent appears to date from its employment by Darby in 1789, at the Manchester Infirmary. It was then a "medicine" whose usefulness was not well defined. Its use for rickets was first reported in 1824 by Schuette and although it continued to be administered for this purpose, its specific value remained unrecognized for nearly a century. Thus, Howland ('20) held reservations when he said, "Cod liver oil has been used empirically for a long time. It is regarded by many physicians as a specific in the treatment of this disease (rickets). This is perhaps too strong a praise, but there is no doubt as to its usefulness. Its effect, however, is not very prompt or marked." Yet in 1918 Mellanby had produced rickets in puppies, and had compared the value of several kinds of

oils for its prevention and cure, and had proven clearly the marked superiority of cod liver oil for these purposes. Since the merits of the oils tested correlated fairly well with their content of vitamin A, it was natural for him to infer that rickets resulted from a deficiency of this substance. Korenchevsky ('22) and Goldblatt ('23) confirmed the results of Mellanby in demonstrating that deficiency of a fat-soluble vitamin alone would cause the disease, all other factors necessary for growth being supplied. Hess and Unger ('20) pointed out that infants developed rickets while receiving a liberal intake of milk and an ample supply of "fat-soluble vitamin."

In 1921 Sherman and Pappenheimer found that young rats developed rickets when fed a diet containing little phosphorus and a generous amount of calcium. A healing response was obtained by the provision of a suitable amount of phosphorus. Shipley, Park, McCollum and Simmonds ('22) showed that there were two kinds of rickets,—one due to feeding a diet deficient in calcium, but containing a normal or excessive amount of phosphorus; the other due to feeding a diet deficient in phosphorus and containing a normal or excessive content of calcium (low-calcium rickets; low-phosphorus rickets). Shipley, Park, McCollum, Simmonds and Parsons ('21) found that whatever the defects of the salt composition of the diets employed in the production of experimental rickets in young rats, the effect of the addition of cod liver oil was greatly to improve the growth and condition of the animals, and to shift the pathological condition of the bones toward the normal state. In a rapid series of studies involving many modifications of diets, they concluded that the content and ratios of calcium and phosphorus in the diet, together with the presence or absence of some organic factor contained in cod liver oil, constituted the essential factors in the etiology of rickets in rats under experimental conditions. That this organic factor was not vitamin A was shown by the fact that cod liver oil which had been treated with a stream of air bubbles while hot, no longer contained vitamin A, but was still potent in the prevention or cure of rickets (McCollum, Simmonds, Becker and Shipley, '22). The antiricketic factor which they thus demonstrated was called vitamin D.

Formation of Vitamin D. The Role of Sunlight and Other Sources of Ultraviolet Light.—Since the earliest times certain physicians recognized the value of sunlight in the treatment of disease. Park ('23) has reviewed the older medical literature on this subject. Palm (1890) as the result of a topographic study of the distribution of rickets, concluded that sunlight should be regarded as a therapeutic agent. Huldschinsky ('19) reported that ultraviolet rays exerted a curative action on rickets. A knowledge of the fairly numerous reports on the curative effect of sunlight and of ultraviolet rays on

rickets led Shipley, Park, Powers, McCollum and Simmonds ('21) to test light treatment on the development of rickets in rats kept on diets which quickly induced severe and acute rickets in animals kept indoors. They confirmed the earlier findings by the observation that rats on these diets did not show rickets when exposed to summer sunshine in Baltimore. The experimental production of rickets being now on a sound basis, the observation on the effect of light was soon confirmed by others. The manner in which ultraviolet light exerted its effect, however, still remained a mystery. Simultaneously and independently, Hess ('24) and Steenbock ('24) had the happy thought to test the effect of irradiating a rickets-producing diet with ultraviolet rays and made the remarkable discovery that this resulted in conferring on such diets antiricketic properties.

Soon after reporting their fundamental observations both of these investigators studied the effect of irradiating separately protein, carbohydrates, fats, and mineral salt mixtures, each of these materials being included, singly, in the diet of ricketic rats. From these studies emerged the fact that it was the fat fraction which became "activated", in the sense of possessing antiricketic properties. The next logical step was immediately taken, to determine what constituent of fats, viz., fatty acids, glycerol, or cholesterol was acted upon by ultraviolet rays to produce this effect. It was found that the unsaponifiable fraction of fats only was converted by light into an antiricketic substance. Hess ('24, l.c.) found that inert oils, e. g., cottonseed and linseed, acquired curative properties on irradiation. Hess, Pappenheimer and Weinstock ('22) had extended the observation of Huldschinsky, based upon radiographs, that sunlight and the light from a quartz mercury arc could cure rickets. They demonstrated that the effective wavelengths of light are in the shorter ultraviolet region of the solar spectrum and that the activation of foodstuffs depended upon the same short wavelengths which were effective in the direct cure of rickets.

Further study showed that cholesterol and phytosterol became antiricketic on irradiation (Hess, Weinstock and Helman, '25). Steenbock and Black ('25) found that cholesterol, after esterification, crystallization, and regeneration from its ester, could still be activated. For a short time it seemed that cholesterol and phytosterol were provitamin D. Bills ('35, '38) has written excellent reviews of the researches which led to the identification of the chemical nature of vitamin D. No attempt will be made, for lack of space, to mention here all of the contributions to this field of knowledge. The more significant discoveries only will be cited.

Schultz and Morse ('25) showed that the absorption spectrum of

ordinary cholesterol is banded, and that there are two maxima of absorption at approximately 294 to 283 mμ. They noticed that two additional faint inflections on their densitometer tracings, after brief irradiation, gave way to general absorption. This they explained by the alternative postulates that either the cholesterol had become at least half metamorphoses, or else ". . . the substance in which the absorption spectrum is changed may be a small amount of impurity in the cholesterol which is not removed by repeated crystallization from alcohol, and which is exceedingly absorptive."

Bills ('35, l.c.) pointed out the coincidence that on December 10, 1926, three researches from different laboratories confirmed the hypothesis that provitamin D is an "impurity" in ordinary cholesterol. Rosenheim and Webster ('26) reported that cholesterol which had been regenerated from its dibromide no longer showed the characteristic absorption spectrum and could not be made antiricketic by irradiation. They also showed that ergosterol, a sterol found abundantly in fungi such as ergot oil and yeast, could be activated. They did not determine its extraordinary potency as an antiricketic agent and did not recognize it as the "impurity" in ordinary cholesterol.

Heilbron, Kamm and Morton ('26) reported that when cholesterol is repeatedly recrystallized the substance which is responsible for the characteristic absorption spectrum tends to accumulate in the least soluble fraction. They also observed a third absorption maximum at 269 mμ. Irradiation destroyed the three bands, leaving only general absorption.

Pohl ('26) detected three absorption bands in cholesterol, and noted that they faded as irradiation was continued. Since the absorption bands disappeared when only a trivial amount of cholesterol could have been transformed, he concluded that the absorbing substance was present only in minute amount. He also found that purification of cholesterol through its dibromide removed the absorbing substance. These three studies established the fact that the provitamin D of cholesterol and phytosterol is a substance associated with these sterols but in small amount. Pohl ('27) pointed out that the three absorption bands of cholesterol of ordinary purity were also exhibited by ergosterol, which showed them in vastly greater intensity. Almost simultaneously Windaus and Hess ('27) and Rosenheim and Webster ('27) announced observations that irradiated ergosterol is of extraordinary potency as an antiricketic agent, and the conclusion that the "impurity" in ordinary cholesterol, to which it owed its characteristic absorption spectrum, is ergosterol and that this is the precursor of vitamin D.

Bills, Honeywell and MacNair ('28) confirmed these findings and,

employing a more refined technic than any hitherto used, detected a fourth absorption maximum at 260 mμ. They compared the positions of the four bands of ordinary cholesterol with those of pure ergosterol, and found them identical, making the series 293.5, 282, 270, and 260 mμ. They further observed that ordinary cholesterol, and ergosterol-free cholesterol plus added ergosterol, when dissolved in acetone and boiled with potassium permanganate, showed fading of the absorption bands at essentially the same rate. On the basis of these findings the belief was established that ergosterol, which is clearly a form of provitamin D, is the "impurity" in ordinary cholesterol to which it owes its antiricketic properties on irradiation.

Notwithstanding the apparent proof that ergosterol is a constant contaminant of ordinary cholesterol, the assumption proved erroneous. In 1930 Muessehl and Ackerson reported that amounts of irradiated yeast and irradiated ergosterol which, calculated on the basis of bioassays on ricketic rats, were equivalent to 10 to 50 per cent of cod liver oil in the diet, failed to prevent leg weakness in chicks. Massengale and Nussmeier ('30) found that it was necessary to administer the equivalent of 200 per cent cod liver oil in the form of irradiated ergosterol to produce in chicks the effect secured by 2 per cent cod liver oil. Several other workers secured similar results. Bethke, Record and Kennard ('33) made a critical study of the comparative antiricketic values of these two sources of vitamin D, and found that it required 15 to 20 times as many rat units of vitamin D in the form of irradiated ergosterol as of cod liver oil to insure normal bone formation in chicks.

Employing this new method of comparative assays with rats and chicks, Waddell ('34) tested irradiated cholesterol as a rickets-preventive for chicks, and demonstrated that it is much more effective than an equivalent number of rat units of irradiated ergosterol. Irradiated cholesterol, he found, is at least as effective as the vitamin D of cod liver oil, hence the provitamin, which had been presumed to be ergosterol, is actually another substance. 7-dehydrocholesterol, first synthesized by Windaus, Lettré and Schenck ('35), appears to be the principal activatable sterol or provitamin D in cholesterol. This is the form present in the skin and is probably the chief form found in fish oils. Irradiated cholesterol and activated 7-dehydrocholesterol exhibit the same spectral absorption bands as ergosterol. This product of irradiation proved to be a highly potent antiricketic substance. These findings suggest that 7-dehydrocholesterol is the provitamin D in ordinary cholesterol.

Products of Irradiation of Ergosterol.—The elucidation of the chemical changes which ergosterol undergoes upon treatment with

ultraviolet light was brought about by an English group (Askew et al., '30, '32; Angus et al., '31) and by a German group of chemists (Windaus et al., '31). These changes occur in the following order:

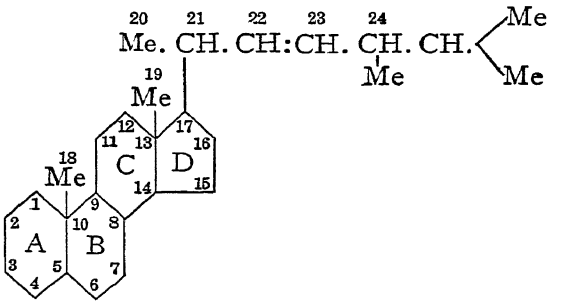
Ergosterol → Lumisterol → Tachysterol →
Calciferol → Toxisterol → Suprasterols
(vitamin D_2) (Substance 248) (I and II)

Lumisterol resembles ergosterol in its absorption spectrum, but has only two maxima instead of four. It appears to be devoid of antiricketic activity, but it is converted by irradiation into vitamin D_2. It forms with calciferol (D_2) a definite addition compound. Tachysterol shows an absorption band at 280 mμ. It appears to have no antiricketic action, but may have a slight toxic effect. Toxisterol, Substance 248, is a product which shows absorption of great intensity at 248 mμ, the band which coincides with the destruction of antiricketic potency. This compound is related to the isoergosterols but has one point of difference, namely, that it does not precipitate with digitonin. It is most readily formed when ergosterol in alcohol is irradiated. Suprasterols I and II represent the products of over-irradiation. These substances are not antiricketic and are slightly toxic. Each of the intermediate irradiation products in the above series has been isolated and studied, as have also the suprasterols.

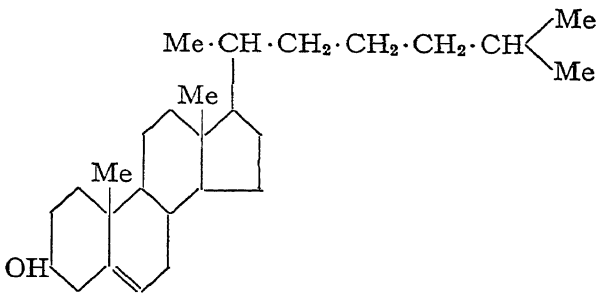
Vitamin D may be formed by other means than by irradiation with ultraviolet light. When cholesterol is exposed to cathode rays it becomes antiricketic (Knudson, '27; Knudson and Coolidge, '27; Knudson and Moore, '29; Hoffman and Daniels, '36, McQuarrie, Thompson, Stoesser and Rigler, '37). The activating effect of cathode rays upon ergosterol has been shown not to be the result of exposure to ultraviolet radiation but is due chiefly to the rays themselves. The vitamin D potencies attainable by the action of cathode rays are lower than those attained by ultraviolet light irradiation. Maisin and coworkers ('30) have reported that ergosterol may be converted into vitamin D by radium emanation.

Extraordinary advances have been made in the study of the structural relationships of the sterols, to which class of compounds vitamin D in its several forms belongs. The formulae for the more important compounds related chemically to vitamin D are presented to show their relationships:

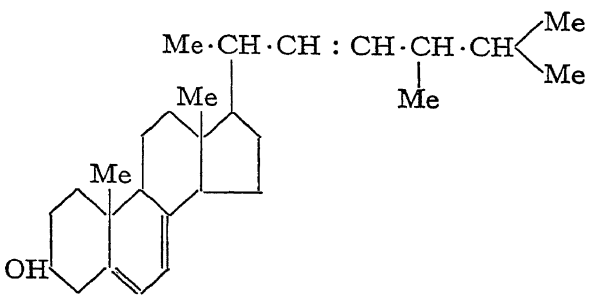
Vitamin D Formulae



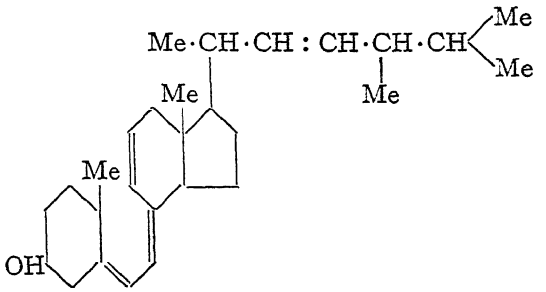
The sterol ring structure. Side chain shown as in ergosterol.



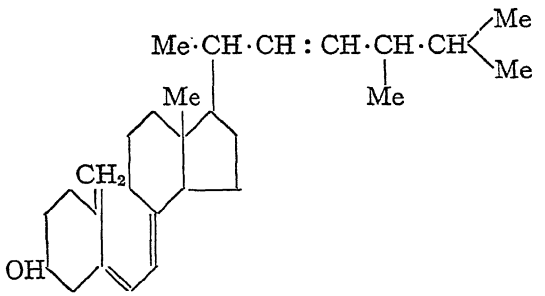
Cholesterol.



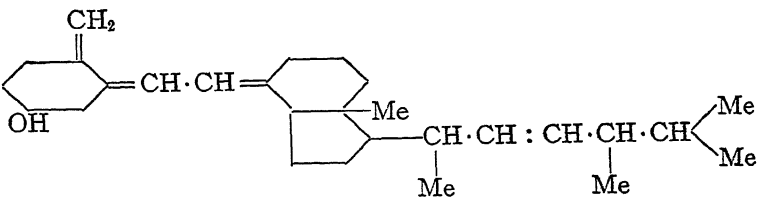
Ergosterol, lumisterol, pyrocalciferol and isopyrocalciferol.



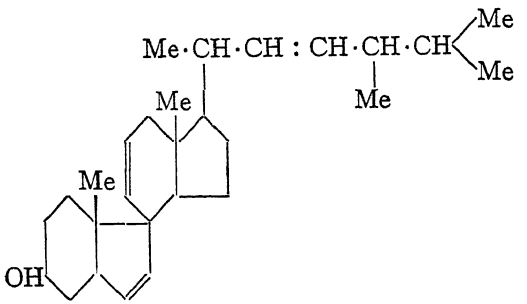
Tachysterol (a tentative formula).



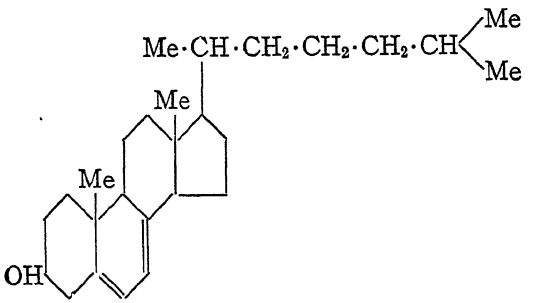
Calciferol (vitamin D_2). Usual, or steroid, formula.



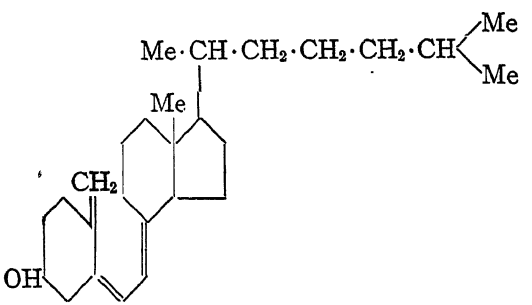
Calciferol, stilbenoid formula.



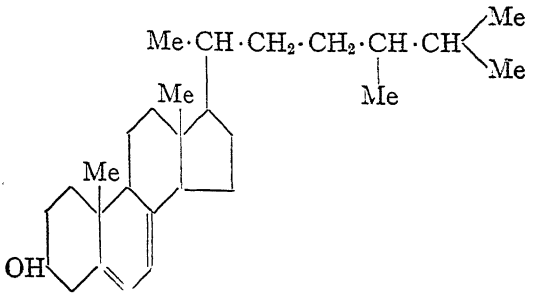
Suprasterol I (a tentative formula).



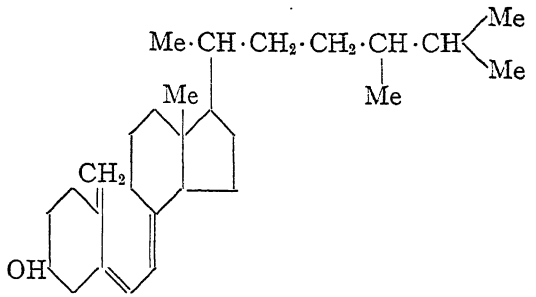
7-Dehydrocholesterol.



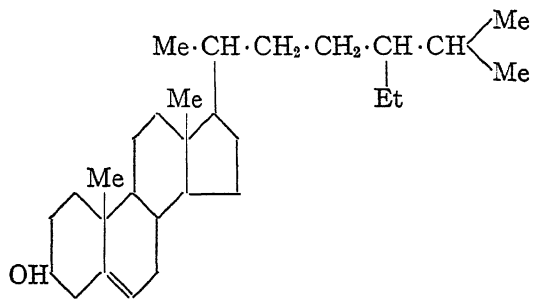
Activated 7-dehydrocholesterol (vitamin D_3).



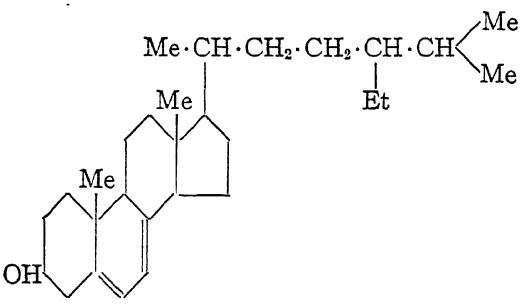
22-Dihydroergosterol.



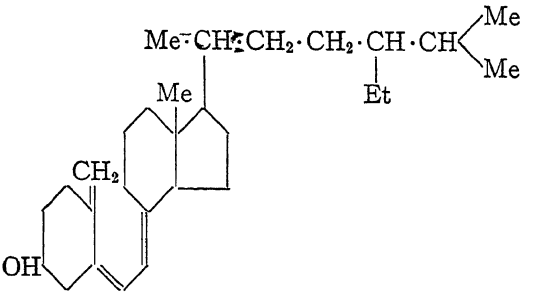
Activated 22-dihydroergosterol (22-dihydrocalciferol or vitamin D_4).



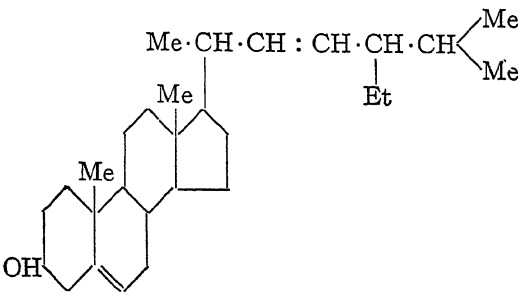
Sitosterol.



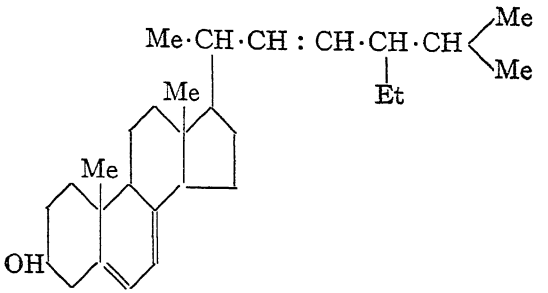
7-Dehydrositosterol.



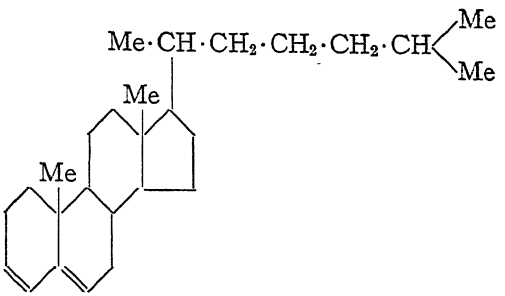
Activated 7-dehydrositosterol.



Stigmasterol.



7-Dehydrostigmasterol.



Cholesterilene.

The Various Forms of Antiricketic Substances.—McCollum and his associates designated the substance in cod liver oil which possesses antiricketic properties "vitamin D." As has already been mentioned, further researches have shown that several sterols possess this property. During the period of attempting to separate the compounds resulting from irradiation of ergosterol, the term vitamin D_1 was given to the first antiricketic substance isolated from irradiated ergosterol which consisted of a molecular compound of vitamin D with a second irradiation product called lumisterol. The molecular compound later proved to be calciferol, now also known as vitamin D_2. Activated 7-dehydrocholesterol is vitamin D_3, and vitamin D_4 is activated 22-dihydroergosterol (22-dihydrocalciferol).

Calciferol is the form of vitamin D which is sold in the pharmaceutical trade as viosterol in oil. Although crystalline esters of the form of vitamin D present in tuna and halibut liver oils have been ob-

tained, no crystalline preparations of the free vitamin from these sources have as yet been described. The evidence is very convincing, however, that the form of the vitamin in these oils is activated 7-dehydrocholesterol. This form is the most important naturally occurring one. It has been isolated by Windaus and Bock ('37a) from the sterol mixture of hog skin, and by Boer and his coworkers ('36) from cholesterol of unstated origin. It appears, therefore, that the provitamin in skin, feathers, wool, etc., is 7-dehydrocholesterol, and it is to the activation of this sterol that the antiricketic effect of ultraviolet rays of sunlight is due. Hence, various texts and other publications are in error when they state that the activatable substance in skin is ergosterol. It has been shown that vitamin D_3 is the form found in irradiated cholesterol obtained from butter, brain, fish oils, etc. It is the form present in irradiated foods of animal origin, including milk. 22-dihydrocalciferol, vitamin D_4, is the form found in irradiated vegetable foods, except in yeast or other fungi, which may be present in certain foods, and which, because they contain ergosterol, would contribute vitamin D_2 on irradiation.

Sitosterol is the name of the ordinary sterol found in plants (Green *sito-*, grain). It has been shown to consist of at least three isomers. Phytosterol was long used as a general name for plant sterols, but sitosterol differentiates those sterols occurring in plants, other than fungi, from the phytosterol of yeast which is composed of ergosterol and zymosterol. Stigmasterol occurs in soy bean oil, the oil of the Calabar bean and probably elsewhere. It is of interest to note that ergosterol was isolated from the oil of ergot by the French chemist Tanret in 1879, but although he described its properties he did not know what an extraordinary substance he had discovered. Of interest also is the report by Windaus and Bock ('37b) that the provitamin D of the higher plants appears to be ergosterol and not 7-dehydrositosterol as had been anticipated.

Physico-Chemical Properties of the Antiricketic Substances.—*Natural Vitamin D:* The physico-chemical properties of the various forms of natural vitamin D found in the liver oils of many species of fish appear to be identical. The vitamin is soluble in alcohol, acetone, and ether; however, the degree of solubility depends somewhat upon the source from which the vitamin is extracted. This factor resists oxidation more strongly than vitamin A, as has been shown by the failure of aeration or treatment with hydrogen peroxide to effect appreciable loss of antiricketic activity. Mild hydrogenation or the action of reducing agents such as hydrogen sulfide, sulfur dioxide, or formaldehyde, do not readily destroy it. Nitrous fumes, on the other hand, produce appreciable loss. The vitamin is thermostable in neutral medium, being able to withstand the direct steaming process

used in rendering fish livers. In acid medium gradual destruction takes place, the rate varying with the reaction and temperature. It is not affected by strong alkali even at high temperatures. Saponification is used to separate the antiricketic fraction of the original oil which is carried in the unsaponifiable portion. This is composed largely of sterols, chief among which is cholesterol. Ergosterol, very similar in composition to cholesterol but more unsaturated, is found in relatively large amounts in mushrooms, fungi, yeast, and especially ergot. Highly purified vitamin D concentrates are optically inactive and show an absorption spectrum in the region 260-270 mμ.

Vitamin D Produced by Irradiation: As has been stated elsewhere, the properties of vitamin D are exhibited by at least eleven different sterol derivatives. The properties of the two considered to be of most practical significance,—irradiated ergosterol and irradiated 7-dehydrocholesterol, will be described here. Before discussing these, the chief physical and chemical properties of untreated ergosterol and cholesterol respectively, will be briefly mentioned.

Vitamin D_2: Ergosterol, the provitamin of calciferol (D_2), is a white, crystalline solid melting at 166°-183° C. according to the degree of hydration. It is sparingly soluble in alcohol and other organic solvents. It is precipitated by digitonin. It is levorotatory and exhibits a strong adsorption in the ultraviolet with well-defined maxima at 260, 270, 282, and 293.5 mμ. Many isomers of ergosterol exist, some of which occur naturally and some of which may be prepared in the laboratory. Of the latter group some are activatable and some are not. Ergosterol itself has no antiricketic activity. After it is irradiated with ultraviolet rays, some of its physical and chemical properties are changed. The crude irradiation product after removal of unchanged ergosterol is a nearly colorless resin. It melts at 166° C. and has a greater solubility in organic solvents than the original ergosterol. It no longer forms an insoluble compound with digitonin. The optical rotation is changed to weakly levo- or even dextrorotatory. The different forms of vitamin D produced by irradiation vary somewhat in antiricketic potency. This may be the result of incomplete irradiation or it may be due to the presence of impurities. The antiricketic potency of activated ergosterol seems to be just as stable as that of the fish liver oils. Even the dry irradiation products withstand storage either *in vacuo* or in air for months without appreciable destruction of the vitamin. Temperatures of 180° C. or higher, or a combination of oxygen with high temperature will, however, destroy it. When ergosterol is irradiated a number of substances are obtained, the compounds formed depending on the wavelengths of light used. Calciferol (D_2) is the form of vitamin D yielded by irradiated ergosterol. Calciferol is a crystalline substance. In appearance it is indistinguishable from

ergosterol. After several months of exposure it changes to a brown resin. It is dextrorotatory and is isomeric with ergosterol, having the formula $C_{28}H_{43}OH$. Like most other products of irradiation, it does not precipitate with digitonin. It shows a strong ultraviolet absorption with a maximum of 265 mμ. Its antiricketic potency is 40,000 I.U. per mg. Like irradiated ergosterol it is inferior, rat unit for rat unit, to cod liver oil for the chicken. Its absolute potency for the rat is enormous, being about 400,000 times that of average cod liver oil.

Vitamin D_3: This form of vitamin D is distinctly different from that produced by the irradiation of ergosterol. It is formed upon the activation of cholesterol or 7-dehydroxycholesterol. Cholesterol itself is a mono-hydroxy-secondary alcohol with a terminal vinyl group with a double bond and containing three saturated hexa-carbon rings. It is a white crystalline substance which melts at 148.5° C., and *in vacuo* at 300° C. it sublimes unchanged. It is levorotatory and is soluble in the common organic solvents. It cannot be saponified and is precipitated by digitonin. Its absorption spectrum consists of the same bands as ergosterol.

The compound 7-dehydroxycholesterol which has been isolated from tuna fish oil, as well as synthesized, is a cholesterol derivative identical with ergosterol except in the side chain. It possesses the same nuclear arrangement of double bonds as ergosterol. It is a C_{27} compound lacking the side chain methyl group as is characteristic of animal sterols which have one fewer carbon atom than the vegetable sterols (C_{28}). In its absorption spectrum, its photochemical oxidation and dehydrogenation as well as its conversion into a series of dihydro-compounds, 7-dehydroxycholesterol closely resembles ergosterol. The changes effected by irradiation of 7-dehydrocholesterol proceed as with ergosterol, i. e.: 7-dehydrocholesterol → lumisterol$_3$ → tachysterol$_3$ → anhydride vitamin D_3 (Windaus, et al., '37). Lumisterol$_3$ closely resembles lumisterol in its spectral and other properties, but gives no addition product with vitamin D_3. Both irradiated cholesterol and 7-dehydroxycholesterol acquire powerful antiricketic properties on irradiation. Vitamin D_3 has practically the same absorption spectrum as calciferol (D_2) and presumably an analogous structure. Of the various forms of vitamin D it has the highest antiricketic activity. Its relative species effectiveness is similar to that of the natural vitamin D found in cod liver oil.

Other Forms of Vitamin D: The various other forms of vitamin D although of theoretical significance to the chemist appear to be of no practical importance because of their low antiricketic activity. They are:

1. (D_4). Activated 22-dihydrocalciferol. (Possibly occurring in plant sterols and hence in irradiated vegetable foods.)

2. 7-dehydrositosterol. It is but slightly antiricketic for rats.

3. Cholesterilene. This was first prepared by Bills ('26) by treatment of cholesterol with fuller's earth. Its antiricketic activity is slight. Yoder ('36) has shown that cholesterilene sulfonic acid and its salts are somewhat antiricketic for rats, but more effective for chicks. It does not require irradiation.

4. 7-hydroxycholesterol (or an impurity associated with it). Studied by McDonald (cited by Bills, '37).

5. Cholesterol, freed from its normal provitamin, but not heated to produce a new one, gives, upon irradiation, a vitamin D which is less effective for chicks, rat unit for rat unit, than the vitamin D which is formed by irradiating either unpurified cholesterol or heat-treated cholesterol (Stavely and Bergman, '37).

6. A product prepared by the treatment of ergosterol with nitrites. It has low potency, and is believed to be different from the ordinary forms of vitamin D, which are destroyed by nitrites (Bills, '35, l.c.).

7. The irradiated product formed by heat treatment of 7-ketocholesteryl acetate, and isobutyl-magnesium bromide (Weinhause and Kharasch, '36).

8. Windaus and Trautman ('37) mention, among other provitamins, 22-23-oxidoergosterol. It is but feebly antiricketic.

9. Bills, Massengale, Hickman and Gray ('38) reported the isolation of a new vitamin D in cod liver oil which may differ from the chemically known forms in the absence of the usual side chain.

The Plant Origin of Vitamin D.—The presence of vitamin D in fish liver oils has been difficult to explain. Steenbock and Black ('25, l.c) suggested that the vitamin might originate from the solar irradiation of microscopic plants and animals living near the surface of the sea which are known as plankton.

Some evidence (Belloc, et. al., '30; Copping, '34) is available indicating the presence of vitamin D in plankton of animal form. Assays of plankton of plant form (Leigh-Clare, '27; Drummond and Gunther, '30) however, have been consistently negative.

An interesting observation has been reported by Darby and Clark ('37). They found vitamin D in *Sargassum* weed, a large marine plant growing in the Caribbean Sea. The probability that this plant might contain the vitamin was suggested by the observation that there is considerable penetration of solar ultraviolet in the very clear sea water at the Torbugas Islands. The *Sargassum* weed collected off the Torbugas Islands yielded oils having definite antiricketic activity for the rat. Since masses of this weed harbor numerous small marine animals of various forms, it is suggested that the vitamin is progressively transferred from the plant to the small animals, thence to the larger fishes.

Vitamin D has not been found in living tissues of land plants. However, since such plants are known to acquire some vitamin D after cutting and exposure to the sun, a possible explanation of the presence of this factor in *Sargassum* is that vitamin D is formed by solar irradiation only in dead portions of the weed.

The Origin of Provitamin D in the Body.—The mechanism is not known by which 7-dehydrocholesterol is formed in the body but it is presumably formed from cholesterol. The function of cholesterol in metabolism is still somewhat of a mystery. Its presence in large amounts in brain and nerves shows that it is an important structural material in these tissues. It is present in small amounts in all tissues, and it was long ago conjectured that cholesterol is convertible into the bile acids, a view which is established by recent researches. Schoenheimer ('31, l.c.) has proved that coprosterol, which is isomeric with dihydrocholesterol, and which was long supposed to be formed by the action of bacteria in the intestines, where it is relatively abundant, actually is formed in the body and excreted into the gut. He isolated the large intestine of dogs, and after repeated rinsing, the anus was closed. The antibacterial action of the intestinal wall was sufficient to destroy the few remaining bacteria and it became sterile. After one or two months the animals were sacrificed and the contents of the isolated colon were examined. From 60 to 70 gm. of a light yellow, wax-like mass, were obtained, which represented the desiccated intestinal secretion, that normally would have been mixed with the feces and have been eliminated. This material contained everything that was secreted into the intestine but which could not be reabsorbed. From it he isolated considerable amounts of dihydrocholesterol, but no trace of coprosterol. It thus appears that coprosterol is the product of bacterial action, not on cholesterol, but on dihydrocholesterol. Coprosterol represents the cis- and dihydrocholesterol the isomeric trans- form of the same substance.

Distribution of Vitamin D in Foodstuffs.—Vitamin D is present in only a few foods which are commonly eaten, namely, in the flesh of certain fish and in eggs. Although fish liver oils have food value as sources of energy as well as of vitamin D, they cannot strictly be considered foods, according to our dietary habits. The flesh of such oily fishes as sardines, herring, tuna, and salmon, fresh or canned, contain considerable, but variable amounts of the vitamin. Less generally used fishes such as menhaden, lamprey, and eel, and doubtless many other fishes, contain it. Fish roe contains the vitamin in fair amounts, as do turtle's eggs and hen's eggs (Daniel and Munsell, '37). In the latter it has been shown that the season of the year and the system of feeding affect the vitamin D content. DeVaney, Munsell and Titus ('33) reported for February and June eggs, 140 I.U. and 390 I.U.

respectively, per 100 gm. All the vitamin is in the yolk. Drake (cited by Park, '38) estimated that approximately five eggs supplied the vitamin D equivalent of one teaspoonful of cod liver oil. When calciferol (viosterol), fish liver oils, or irradiated foods are fed to the hen, vitamin D is transferred to some extent to the eggs. Cod liver oil is much less effective in this respect than calciferol. Irradiation of the hen by the ultraviolet rays of sunlight increases the vitamin D content of her eggs.

Ordinary milk contains but little vitamin D. Campion and coworkers ('37) reported that milk from cows kept out of doors in June, in England, contained 17 to 26 I. U. per quart, whereas milk from cows kept indoors contained 5 to 8 I. U. per quart. Bechtel and Hoppert ('36) found 5 I. U. per quart of winter milk, and 40 I. U. per quart in summer milk. Pasture feeding has almost no influence on the content of vitamin D in milk, but exposure of the cows to sunlight increases it. The vitamin D content of milk may be increased by feeding irradiated yeast to the cow. Average values obtained by assay of vitamin D milks produced in the following ways are:

Yeast-fed cows	432 I. U. per quart
Cod liver oil concentrate-fed cows	405 I. U. " "
Irradiated milk	135 I. U. " "

When the milk is irradiated, or cod liver oil concentrate is fed, the form of the vitamin is probably activated 7-dehydrocholesterol.

The vitamin D of milk is in the fat moiety, and hence passes into butter. Average butter contains about 80 I. U. per 100 gm., or about 1 per cent of the value of ordinary cod liver oil.

Mammalian liver contains 10 to 45 I. U. of vitamin D per 100 gm. Other glandular organs of land animals contain very little of the vitamin, but it probably occurs in significant amounts in the flesh of birds and animals which subsist largely upon fish. Seal flesh is high in vitamin D. The most abundant natural sources of vitamin D are cod liver oil and percomorph oil.

Plant foods and vegetable oils are markedly deficient in vitamin D.

Vitamin D Milk.—As a food of general and regular consumption, milk is an appropriate carrier of vitamin D especially for infants and growing children. However, since vitamin D milks usually provide about one-third the usual therapeutic dose of D, they are chiefly preventive agents. Health commissioners generally approve the fortification of milk with vitamin D. This is a sound policy and it has been endorsed by the Council on Foods of the American Medical Association ('37): "Of all the common foods available, milk is most suitable as a carrier of added vitamin D. Vitamin D is concerned with the utilization of calcium and phosphorus, of which milk is an excellent

source. The Council has recently made the decision that for the present milk is the only common food which will be considered for acceptance when fortified with vitamin D." The Council recommends from 400 to 500 I. U. daily as a reasonable addition to the diet during childhood and later years.

Several processes are in commercial use for increasing the antiricketic potency of whole or evaporated cow milk. They are the feeding of activated yeast to cows, a small fraction of the vitamin D (calciferol) being transmitted to the milk; ultraviolet irradiation of the milk itself; and fortification of the milk with irradiated ergosterol or fish liver oil concentrates; a fourth method involves the addition to milk of ergosterol activated by exposure to cathode rays from the Coolidge tube. Direct irradiation produces milk containing 135 I. U. per quart as a maximum and therefore infants taking this milk need additional vitamin D. Irradiated yeast, if fed in controlled amounts, produces milk of satisfactory antiricketic potency. If fortified milk is standardized to contain 400 I. U. per quart of whole milk it is an adequate antiricketic agent. Milk of such activity will prevent rickets if ingested in amounts of only one pint daily although at this level the margin of safety is probably small.

Determination of Vitamin D Potency.—There is no chemical method for the assay of vitamin D potency of any product. Hence some type of animal assay, or bio-assay, must be employed. All procedures depend upon the effect of a given dose of vitamin D on calcification of the bones of severely ricketic rats. The first diet suitable for producing rickets for the purpose of bioassay of vitamin D was Diet 3143 of McCollum and his associates. This consisted of:

	Per Cent
Whole wheat, ground	33.0
Yellow maize, ground	33.0
Gelatin	15.0
Wheat gluten	15.0
Calcium carbonate	3.0
Sodium chloride	1.0

One hundred gm. of this ration contains 1.22 gm. of calcium and 0.30 gm. of phosphorus. Weight ratio Ca:P, 4.04:1.

Steenbock and Black proposed a ricketogenic diet (No. 2965) which fulfills the dietary requirements above mentioned for the production of rickets. It consists of the following substances:

	Per Cent
Yellow maize, whole seed, ground finely	76.0
Wheat gluten	20.0
Calcium carbonate	3.0
Sodium chloride	1.0

Observations of the deposition of bone as revealed by visual examination of bone sections stained with silver nitrate, radiographs of the bones of the living rat, and determination of the ash content of the bones, after a suitable interval following the administration of vitamin D to test animals, have been the criteria on which most bio-assays have been based. Then there is the possibility of both a preventive and a curative procedure. Many methods have been advanced, which consist of modifications of one or another of these procedures. Any of several methods may be reliable in the hands of an experienced experimenter provided control experiments are made simultaneously with a standard product of known value. The technic of vitamin D assays has been thoroughly discussed by Coward ('38), who also provides a select bibliography of original literature.

The "Line Test" for Antiricketic Substances: In the ricketic bone, growth is proceeding in the sense that cartilage cells are proliferating and undergoing metaplasia into osteoid tissue. But calcium phosphate is not deposited as in the normal bone. The initial ossification in the normal growing bone is in the intercellular matrix of cartilage cells, on the diaphyseal (shaft) border of the metaphysis. Schmorl ('09) showed that the first sign of healing, in the bones of ricketic children, was the deposition of calcium salts in this matrix, which he termed the "provisional zone of calcification."

The Baltimore group in its studies of experimental rickets sought to make use of the observation of Schmorl as a test for the antiricketic substance. Since rickets in infants or animals may be of various degrees of severity, and in its milder forms the bones may show some degree of calcification, it was essential for the development of the desired biological test to discover a diet which would produce without fail in young rats florid rickets in which the bones would be free from areas showing ossification. The provision of vitamin D would then initiate the normal process of calcification and the amount of such calcification in a given period of time could be used as a basis of estimating the amount of the vitamin administered. Examination of the bones of rats restricted to many dietary formulae showed that these conditions were met by Diet 3143, the composition of which has been given.

When the ricketic diet (No. 3143) was fed to young rats they developed acute rickets in 19-21 days. When a source of vitamin D was provided during 6 to 8 days there appeared in the ricketic metaphysis, at right angles to the axis of the shaft of the bones, a honey comb-like deposit of calcium salts in the intercellular matrix of the cartilage cells. Treatment of a split section of the bone with silver nitrate and exposure to light caused a blackening of the newly formed calcified area

which could be easily seen in sufficient detail under low magnification. This test is still the basis of most bioassays for vitamin D.

Composition of Blood: Howland and Kramer ('21) had pointed out that during active rickets in children the inorganic phosphorus of the blood serum is reduced. Since then investigators of this deficiency disease have been interested in the use of this criterion for diagnosing rickets. The most extended experimental work with animals has given results in most respects similar to those reported for children. Ricketic rats, especially those on the usual high-calcium, low-phosphorus diets, like ricketic children, have a normal or slightly low blood calcium, and a decidedly low content of inorganic phosphate. In case of experiments with rats, this method has the disadvantage that such animals being small have so little blood in their bodies that even with micro methods for the determination of calcium and phosphorus, the changes in blood composition must be followed not on a single rat, but on groups, each group being maintained under the same conditions of experiment.

The X-ray Method: Bourdillon, Bruce, Fischmann and Webster ('31) worked out the details of this procedure and used this method extensively in their work. The bones of the ricketic rats or children are examined before administration of vitamin D and after 14 days of vitamin therapy. The X-ray photograph gives a picture of healing bone similar to that given by the line test, except that the latter shows a section of the layer of healing, whereas the X-ray picture shows the whole thickness of the zone of healing.

Serum Phosphatase as an Index of Rickets: As has already been stated, rickets can be diagnosed with certainty only by a histological examination of the bones which, while very valuable in experimental studies on animals, is of no use in clinical work. There is general agreement that X-ray signs frequently fail to parallel clinical signs. Barnes and Carpenter ('37) have pointed out that one worker may be able to check his own X-ray reading, but that there is much variation when different individuals read the plates, even though all are experienced.

It is relatively safe to assume that an infant with a serum inorganic phosphorus value of only 4 mg. per 100 cc. of serum, or less, has active rickets. The converse of this, namely, the conclusion that the finding of a normal serum phosphorus content indicates that the infant does not have rickets, is often false. It is equally misleading to assume that because the serum phosphorus of an infant has risen from 4 mg. per cent the disease has been cured. The serum phosphorus responds with great rapidity to antiricketic treatment.

A more recently developed diagnostic measure is the blood serum level of the enzyme phosphatase. Kay ('30) reported that this enzyme,

which hydrolyses certain phosphoric acid esters, is abnormally high in the serum of ricketic infants. Bodansky and Jaffe ('34) concluded that the level of serum phosphatase is an excellent index to the activity of rickets, and is also a measure of the rate of healing. Barnes and Carpenter (l.c.), using Bodansky and Jaffe's technic, determined this enzyme in the sera of 187 infants in Detroit. According to X-ray diagnosis, only 50, or 26.7 per cent, had active rickets, and only 37, or 19.8 per cent, showed a serum inorganic phosphorus level below 4 mg. per cent. By the criterion of the phosphatase of the serum, however, 123 of the 187 infants, or 65.8 per cent of the group, had active rickets. Thus the serum phosphatase level is in much closer agreement with the clinical findings than the results obtained by X-ray or blood serum inorganic phosphorus determinations.

The Bone Ash Method: In this method the percentage of ash in the fat-extracted dry bone is determined. This indicates the absolute calcification of the bone. This is one of the most accurate means for quantitatively determining the vitamin D content of a substance. It is a laborious and time consuming method.

Determination of Vitamin D by Means of Chicks. The X-ray technic and the "line test" as used on rats cannot be used on chickens, for healing of chicks does not show itself as a line of calcification across the metaphysis in the cut bone. In this species healing is diaphyseal. Consequently the criterion used in measuring calcification in chicks is the ash content of the bones.

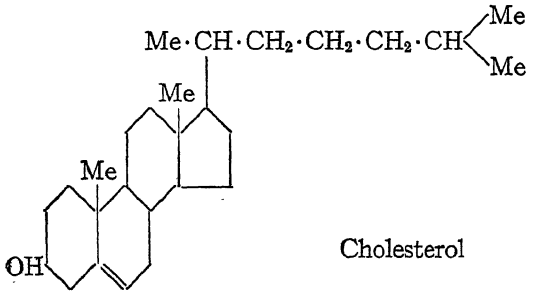
The Unit of Vitamin D.—In the earlier application of bio-assays, several investigators proposed units for vitamin D, and the result was great confusion. In 1934 the International Unit, defined by a Committee of the League of Nations, was adopted for the U. S. Pharmacopoeia, and also for the New and Non-official Remedies of The American Medical Association. This unit has now replaced all of the older units. A list of equivalents used by the earlier investigators is presented, in comparison with the International Unit, for the benefit of those who may wish to interpret earlier data. This list expresses the potency of average cod liver oil of medicinal grade.

International Units	100 per gram
U.S.P.10th Ed. Units	100 " "
British, of Medical Research Council Units	100 " "
Steenbock Units	37 " "
American Drug Manufacturer's Units	350 " "
Oslo, or Poulsson (1928) Units	110 " "
Oslo, or Poulsson (1933) Units	160 " "
German Units (rat)	15 per cc.
German Units (clinical)	0.15 per cc.
Cod Liver Oil Coefficient (New and Non-official Remedies)	2.8 D
Cod Liver Oil Coefficient, true	1.0 X

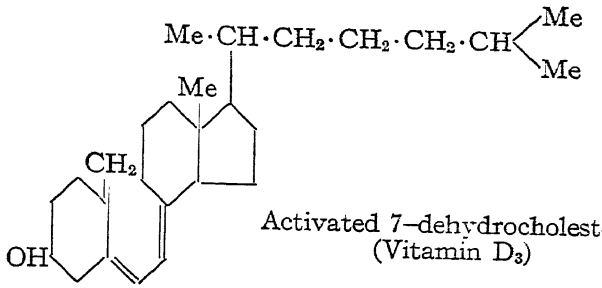
On the basis of the above values, a solution containing 1,000,000 I. U. per gram of irradiated ergosterol (calciferol) is 10,000 times as potent as average cod liver oil. This would formerly have been labeled 10,000 X or 28,000 D, and would have been equivalent to 370,000 Steenbock Units per gram.

Biochemical Relation to Vitamin D, Sex Hormones, and Cancerigenic Substances.—The relation of the sterols, especially vitamin D, the sex hormones, and cancerigenic substances is noteworthy at this point. The investigations on the structure of the various forms of vitamin D and the sex hormones have stimulated the production, characterization, and degradation of the sterols so that the structural relationships between certain of these groups are now well established. The carbon skeleton common to these compounds consists of a condensed isocyclic system composed of 3 six-membered and 1 five-membered rings. The sex hormones are products with the identical tetracyclic carbon skeleton, but with modified side chains. The only difference in constitution between the sex hormones and the vitamins D is in the long side chain common to the latter which is attached to the five-membered ring. Cholesterol is believed to be the single parent substance from which all of the sex hormones may be derived. The stages of the transformation consist of the total or partial degradation of the side chain resulting in the formation of the unsaturated ketones and then the oxidation of the hydroxyl group.

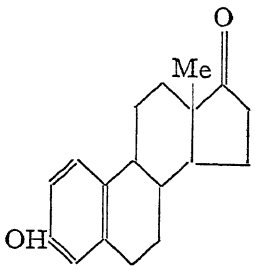
Another group of hydrocarbons whose molecular structure bears a certain resemblance to the sterols is the cancerigenic compounds which also exert a noticeable estrogenic action. It is thought that the mode of formation of these substances is as follows: dehydrogenation of sterols forming the estrogenic hormones and the further dehydrogenation gives rise to cancerigenic compounds having the phenanthrene nucleus, as for example, methyl cholanthrene.



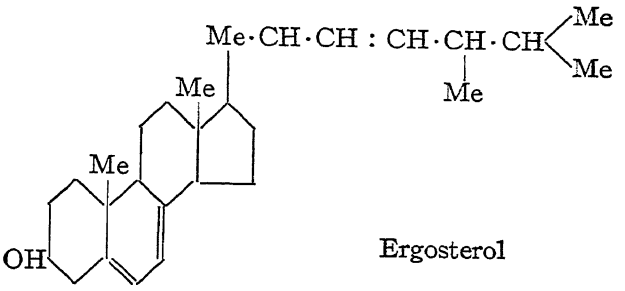
Cholesterol



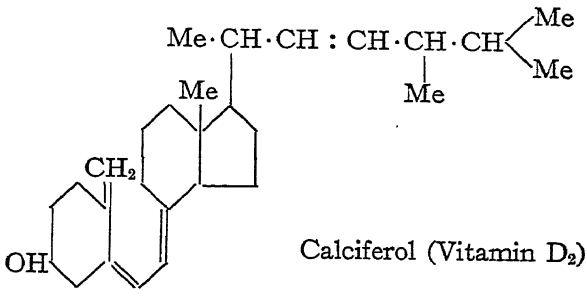
Activated 7–dehydrocholesterol
(Vitamin D_3)



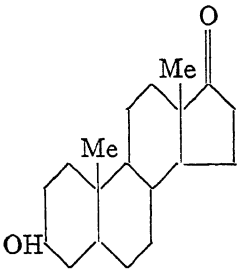
Estrone



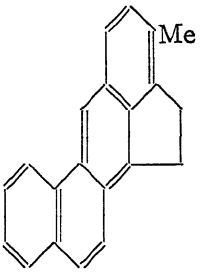
Ergosterol



Calciferol (Vitamin D_2)



Androsterone



Methylcholanthrene

The relation between the sterols, vitamins D, sex hormones, and cancerigenic substances is emphasized by the discovery that ergosterol, calciferol, and other sterols, as well as some of the cancerigenic hydrocarbons, 1:2 benzpyrene and 5:6 cyclopenteno-1:2-benzanthracene, for example, were found to have definite estrogenic activity. Still more important is the newly found correlation between estrogenic and cancerigenic properties. Thus a single molecule may have two entirely different effects, such as the cancerigenic and estrogenic properties, or the antiricketic and estrogenic properties. The question arises, therefore, as to whether metabolic substances originating from the vitamins D or sex hormones may not be concerned in the production of malignant tumors.

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CHAPTER

XV

Significance of Vitamin D in Rickets and Related Diseases

AS RESEARCH accumulates it becomes increasingly evident that the antiricketic substances are concerned with physiological processes other than calcification of growing bone. This chapter will be devoted to the discussion of various aspects of vitamin D in nutrition, which include the present concepts regarding its mode of action in rickets, its relationship to the state of calcium and phosphorus in the blood and to the dietary content of these minerals, storage, absorption and excretion, species differences in utilization of the various forms of vitamin D, normal requirement and distribution of this factor, the role of vitamin D in diseases related to rickets, and the therapeutic uses of this substance.

Structure of Normal Bone.—The growth of normal bone at the junction of the epiphysis, or head, with the diaphysis, or shaft, is preceded by the following changes in the cartilage of the epiphysis:

The cells of the indifferent or resting cartilage become enlarged and arrange themselves into columns of four to six cells, and become indented by mutual pressure so as to present, in cross section, a roughly rectangular shape. This forms what is known as the proliferative zone of cartilage. Near the shaftward end of this proliferative zone the calcium is deposited in the intercellular substance of the cartilage, forming the so-called zone of provisional calcification which abuts on the end of the growing shaft in a curved or straight, but always clear-cut regular line. Each cartilage cell is enclosed in a capsule of calcified intercellular matrix. The shaft grows at the expense of the cartilaginous epiphysis. Each calcified cell capsule is perforated by a single advancing capillary loop from the marrow cavity. The presence of the blood supply thus brought to the cartilage cell causes it to undergo dissolution. The capillary loop advances to the next inter-

cellular matrix wall and again liberates and destroys the neighboring cartilage cell. During this process of destruction of cartilage on the shaft end of the cartilage disc, new swollen cartilage cells are arising on the epiphyseal end, so the number of cells in this state remain more or less constant at four to six in a column. The calcified intercellular substance which separates the cells in the direction parallel to the long axis of the bone is not attacked by the blood vessels, and remains imbedded in the advancing shaft as fine lines thickened at the points where the intercellular walls running at right angles to the long axis of the shaft were destroyed. Osteoblasts which have advanced from the marrow along with the blood vessels attach themselves to these spicules of calcified intercellular substance and surround themselves with osteoid tissue (bone tissue without a deposit of calcium salts); this is speedily converted into bone by the deposition of calcium salts, chiefly calcium phosphate. New osteoblasts continue to settle on the growing bone trabeculae and the process described is repeated, resulting in their enlargement.

Structure of Bone in Rickets.—In ricketic bones a provisional zone of calcification is not formed, since lime salts fail to be deposited in the intercellular substance of the cartilage. The proliferative cartilage persists in some places, and in others is destroyed in an irregular way by aberrant blood capillaries which sprout in a disorderly fashion from the parent vessels in the shaft and invade the cartilage in all directions. It is for this reason that the line of junction of the epiphysis and the shaft becomes very irregular and long tongues and islands of cartilage are left isolated. Either the cartilage cells undergo metaplasia into osteoblasts or osteoblasts follow the blood vessels as they invade the cartilage and form osteoid tissue which, however, does not receive a deposit of lime salts. Lack of calcification results in lack of rigidity of the bones, and the body apparently attempts, unsuccessfully, to compensate for this weakness by producing osteoid tissue in excessive amounts, hence the enlargement of the ends of the bones in rickets. Because of the abnormal growth at the end of the shaft, a zone known as the ricketic metaphysis is formed, which consists of connective tissue, osteoid tissue, marrow elements, blood vessels, and cartilage cells in all stages of degeneration and metaplasia into osteoid substance. The directive influence exercised by calcification of the intercellular substance in cartilage being absent, all other growth phenomena in the growing bone become aberrant, and the anatomical elements become disarranged.

Immediately upon the administration of vitamin D in sufficient amount, the content of ionized calcium and of inorganic phosphate in the blood, which may fall below the normal level in subjects in the ricketic state, tends to return toward normal values. There is at pres-

ent no way to decide whether the solubility product of calcium and phosphate ions ($Ca^{++} \times PO_4^{\equiv}$), expressed in milligrams of calcium and of inorganic phosphate per 100 cc. of blood, alone determines the deposition of lime salts in the intercellular substance (provisional calcification), or whether, in addition, there is a conditioning of the cells of the cartilage which brings about this deposition, or whether both effects are involved. The fact is that provisional calcification immediately begins and with this the directive processes leading to normal bone growth are established. Those tissue elements in the ricketic metaphysis which are in disarray are quickly cleared away, and the histological appearance of sections of the bone show that normal bone growth has been resumed.

The Etiology of Rickets.—Rickets was established as a definite disease in 1650. Little was known about the exact nature of the disease until Pommer in 1885 made his extensive studies of ricketic bones. In 1890 Palm reported the results of a geographic investigation of this disease and pointed out the beneficial effect of sunlight in its treatment. Buchholz ('04) first called attention to the value of treatment of rickets with artificial light; his observations, however, attracted but little notice. In 1919 the etiology of rickets was still a mystery. At this time Huldschinsky ('19-20) reported on the curative action of ultraviolet rays from a mercury vapor quartz lamp, and Hess and Unger ('20-21) confirmed his findings.

Contemporaneously with the studies on sunlight in relation to rickets, another group of investigators followed the theory that rickets was the result of a faulty diet. The work of Mellanby ('19) gave great impetus to the study of experimental rickets. Mellanby ('21) studied rickets in puppies about two months of age. Having formulated a diet consisting of skim milk, bread, linseed oil, yeast, orange juice, and sodium chloride, which regularly produced rickets in pups, he supplemented it with various kinds of fats and noted their effects on the skeletons. His pups were in most cases kept indoors, but some were closely confined, some were kept out-of-doors, or allowed to run in an enclosure. As criteria of rickets he employed the appearance, X-ray findings, content of calcium in the bones, and histological appearance of the bones. He found cod liver oil to be very effective in preventing abnormalities of the bones. Suet and butter fat had considerable antiricketic value, while lard had none. Peanut oil was better than coconut oil; rapeseed, cottonseed, palm kernel, olive, and linseed oils had little or no value, and babassu oil was considered the "worst."

Hopkins (1920) was the first to show that if oxygen passed through heated butter fat, vitamin A is readily destroyed. In consequence, Mellanby ('21, l.c.) compared the antiricketic effect of heated and unheated

butter and found that the oxidized fat did not prevent rickets in pups. However, oxidized cod liver oil retained the antiricketic effect. He was undecided "Whether this difference . . . can be simply explained by the fact that cod-liver oil contains a much greater quantity of anti-rachitic vitamine than butter, so that the destructive change takes a longer time, or whether some other explanation must be sought." He found that lean meat had an antiricketic action sufficient to prevent or limit the severity of rickets under favorable circumstances, but under unfavorable conditions it did not prevent the disease. The higher the content of bread in the diet, the more severe was the rickets. Casein containing some calcium prevented rickets, but casein prepared by precipitation with hydrochloric acid and free from calcium seemed to intensify the rickets in pups. The addition of tricalcium phosphate to the diet did not prevent the disease. Mellanby employed the term "antirachitic vitamine" and expressed the belief that when the diet is well constituted a small amount of the vitamin would suffice for protection; if badly constituted, in a ricketic sense, a larger amount would be necessary. He found that older animals were less susceptible to rickets, and had less need for the antiricketic vitamin. Also, it was his belief that "osteporosis" in dogs may give rise to deformities somewhat identical with those seen in rickets. He stressed environmental conditions and confinement as etiological factors in rickets. The composition of the inorganic moiety of the diet was not taken into consideration. More extensive histological studies would have made these experiments more convincing, since it is generally agreed that gross lesions of deformity resembling closely those seen in rickets may result from other causes. The ricketic bone has the following characteristics: (1) calcification of the provisional zone of cartilage (intercellular substance) does not occur; (2) cartilage cells persist in the shaft below the normal epiphyseal line; (3) there is irregular invasion of the cartilage by blood vessels and connective tissues; (4) newly formed preosseous tissue remains uncalcified and is produced in abnormal amounts—the so-called osteoid tissue; (5) there is developed the ricketic metaphysis.

The investigation of the etiology of rickets by McCollum, Simmonds, Parsons, Shipley and Park ('21) was undertaken with the criteria just mentioned as the basis of judgment concerning the effects of numerous diets on the skeletal development of young rats. Park and Shipley were skilled in the histology of the bones. An examination of the bones of rats kept during several weeks on each of several hundred modifications of diet, in which all the known factors were systematically varied, the animals being kept indoors, showed that two dietary characteristics were of outstanding significance in the etiology of rickets, resembling in all details that seen in infants. These were the

ratio between calcium and phosphorus and the presence or absence of certain fats in the diet.

The observation of Mellanby concerning the antiricketic value of cod liver oil, and the much smaller value of butter fat, was confirmed. It was found that when a diet, devoid of an antiricketic fat, provided about 0.20 gm. of phosphorus and 1.2 gm. of calcium per 100 gm. of food, acute and severe rickets developed in young rats in 19 to 21 days. Diets containing two to three times this amount of phosphorus, and half as much calcium, were not effective in producing rickets in the rat, an animal which is rather resistant to the disease. In the latter case the bones were not entirely normal in histological structure, but it was evident that a wide ratio of these elements was of fundamental importance and inimical to the normal development of the animals. With the wide ratio of P/Ca of 0.20:1.2, the regulatory mechanism in bone development failed entirely; but the provision of an antiricketic fat furnished the necessary regulatory effect, and the bones were normal in structure.

When the diet provided 0.043 per cent of calcium and 1.2 per cent of phosphorus, rickets also developed, but it was complicated by tetany. Analysis of the blood plasma of ricketic rats on the high-calcium low-phosphorus diet showed that the calcium content was normal and the phosphorus content low. With the low-calcium high-phosphorus diets both the calcium and phosphate values were low.

It was further shown (McCollum, et al., '22) that the feeding of oxidized cod liver oil, which had been prepared by blowing hot air through it to destroy vitamin A, afforded protection against rickets in experimental rats. The cod liver oil was tested and found to be incapable of protecting rats against the xerophthalmia characteristic of vitamin A deficiency. Hence it was concluded that another fat-soluble vitamin existed, and that it was abundant in cod liver oil, and much less abundant in butter fat, and all but absent in various vegetable fats. This new factor was designated vitamin D.

In 1921 Sherman and Pappenheimer published an important paper which described the production of rickets by means of a diet low in phosphorus, and its prevention by the addition to the diet of alkaline potassium phosphate. Their diet was composed of white flour 95 per cent, calcium lactate 3 per cent, sodium chloride 2 per cent, with or without the addition of a trace of ferric citrate. When 0.4 per cent of the potassium phosphate was added and an equivalent amount of calcium lactate withdrawn, the animals were completely protected against rickets. These investigators, therefore, demonstrated independently of McCollum and associates the profound importance of a proper balance of calcium and phosphorus in the diet for the development of normal bones. They did not study the influence of fats on the

etiology of rickets. Their diet was lacking in vitamin A and all the water-soluble vitamins, as well as in vitamin D, but rickets developed so promptly that the other deficiencies did not have time to vitiate their experiment. Soon afterwards Pappenheimer, McCann, Zucker and Hess ('21-22) showed that addition of small amounts of yeast, orange juice, butter fat, and potassium, did not exert a protective influence on the animals.

It is clear that when the nutritional state is adequate as respects all other factors, the normal calcification processes involving the osseous tissues are chiefly dependent upon the calcium and phosphorus ratios in the food and upon an adequate supply of vitamin D. The mechanism is by no means so simple as this, however. Shohl and associates ('32) have demonstrated that when the content in the diet of both calcium and phosphorus is greatly increased, the ratio being as wide as in the rickets-producing diets, the development of rickets is prevented. When the total intake of these elements is lowered, the rickets-producing quality of the diet is increased when the Ca:P ratio is unfavorable. Thus, at each level of calcium a different ratio is necessary to produce rickets, as measured by X-ray, histology, blood serum calcium and phosphorus, and bone ash. There is, therefore, a degree of protection afforded against rickets by the feeding of favorable ratios of calcium and phosphorus and of both elements in amounts which provide them in abundance. Excellent discussions of this subject have been written by Park ('23) and Hess ('29).

Gross Anatomical Changes in Rickets.—In the long bones the main deformities are due to bending. When recovery occurs, the deformities are fixed, with consequent impairment of bodily mechanics. The ribs are enlarged at the junction of the rib and sternal cartilage, forming the ricketic rosary. Deformity of the thorax may result, in severe cases, in the so-called "chicken-breast," or the "funnel-shaped chest." Changes in the shape of the thorax together with softness of the ribs impairs breathing, and the enlarged costo-chondral junctions may press from the underlying portions of the lungs causing collapse in these areas, and in turn, compensatory emphysematous areas may form along the anterior borders. Pneumonia is not infrequently a complication of moderate or severe rickets, which causes a marked susceptibility to respiratory infection.

The spinal column and the vertebrae are usually not deformed, except in severe cases, when the column may be curved as in scoliosis or kyphosis or both. The vertebrae are abnormally soft, the intervertebral discs varying in width, and the ligaments are flabby. The cranium shows characteristic changes, the fontanel tending to be abnormally wide, and deformities of the skull result from softness of the bones along the suture lines. The frontal bones may be greatly hypertrophied,

giving rise to the "Olympian front" which is characteristic of rickets.

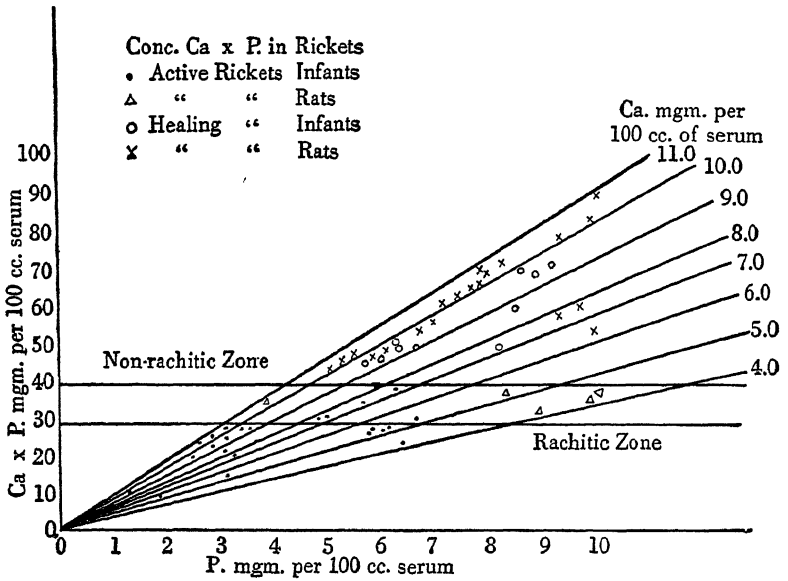
Cretinism, chondrodystrophy, osteogenesis imperfecta, and scurvy, are also characterized by bone deformities. However, they are entirely distinct from those seen in rickets.

The Chemical Composition of the Blood in Rickets.—Howland and Kramer ('21), from their studies of the inorganic phosphorus of the blood serum of both normal and ricketic children, concluded that inorganic phosphate represents a definite chemical entity in the blood, since it is present in nearly constant amounts in normal individuals of the same age. In rickets, there was a marked decrease in inorganic phosphorus of the serum, and to this deficiency they ascribed the failure of deposition of calcium salts in the bones. The blood serum of non-ricketic infants and young children contained between 10 and 11 mg. of calcium and about 5 mg. of inorganic phosphorus per 100 gm. All children under two and a half years with 3 or less than 3 mg. of phosphorus had active rickets. When cod liver oil was administered the phosphorus content of the serum returned to normal, and the rise in the phosphate of the serum was accompanied by healing of the ricketic lesions. Kramer and associates ('21) found the serum calcium of cases of rickets associated with tetany to be about 5.4 mg., or roughly half the normal. They found the inorganic phosphate of the sera of these infants who had rickets associated with tetany to be within the normal limits, or slightly above normal.

In rats, Kramer and Howland ('22) found the concentration of serum calcium to be 9.5 to 10.5 mg. and of phosphorus from 7 to 8.5 mg. per 100 cc. These values are not increased by changes in the diet or by ultraviolet light. In animals fed diets somewhat low in calcium or phosphorus the content of these elements in the serum could be diminished by as much as 50 per cent. On the same diets the provision of cod liver oil brought the values back to normal. They found that irradiation of the animals with ultraviolet rays likewise raised the phosphorus content of the serum, as did also a few days of starvation. Brown and coworkers ('22) reported a study of two breast-fed infants with rickets accompanied by tetany. The mothers' diets were of very poor quality. The milks of both women were abnormal in their ash contents, one being low in calcium and high in phosphorus, the other was low in both calcium and phosphorus. The blood sera of both infants were low in calcium and phosphorus.

Howland and Kramer ('22, '23) showed that in uncomplicated rickets the concentration of calcium in the serum was normal or nearly so, whereas that of the phosphorus was regularly low, resulting in a product of the two concentrations, expressed in milligrams per 100 cc. at or below 30. In tetany the product was also low on account of the

striking reduction of the calcium content. After giving cod liver oil, or ultraviolet therapy, there was such a great rise in inorganic phosphorus as to make the product two or three times the one before treatment. The following graph taken from their paper was constructed from data obtained from the analysis of sera of both children and rats. These experimenters interpreted the results shown in this graph to indicate that when the Ca x P product is 30 or below, rickets is to be expected; and when the product is 40, it is possible to have rickets. When it is above 40, either healing is taking place or rickets is absent.



Graph taken from paper by Howland and Kramer, Monatschr. f. Kinderheilkunde, 1923, xxv, 279.

Since these findings were reported, numerous experiments have demonstrated the complexity of the calcium and phosphorus in the blood, consequently this criterion of diagnosing rickets cannot be regarded as representing a simple chemical state conforming to the law of solubility product (Hess et al., '30; McGowan, '33).

The blood phosphatase and bone phosphatase are markedly increased in rickets. Phosphatase is an enzyme which hydrolyzes certain organic compounds of phosphorus and liberates the element as inorganic phosphate. Recovery from rickets is indicated by a slow decrease in serum phosphatase. In normal children the phosphatase content of blood ranges from 5 to 14 Bodansky units per 100 cc. The measure-

ment of this enzyme serves as an aid in diagnosis and treatment of avitaminosis D.

The Effect of the Ratio of Calcium to Phosphorus in the Diet on the Content of These Elements in the Blood Serum.—Kramer and Howland ('32) analyzed the blood serum of rats prepared by McCollum and Simmonds for the purpose of showing the influence of the calcium and phosphorus content and of vitamin D on the concentration of these elements in the blood serum. The diet employed consisted of rolled oats 40, gelatin 10, wheat gluten 7, NaCl 1, KCl 1, and dextrin 41 gm., respectively. The proportion of calcium to phosphorus was varied by adding $CaCO_3$ or KH_2PO_4, or both. The animals were restricted to the diets for about 6 weeks. The calcium of the diet was varied from 0.14 to 1.24, and the phosphorus from 0.2 to 1.2 gm. per 100 gm. of food. Vitamin D was provided by 1 per cent and 10 per cent of butter fat, and by 2 per cent of cod liver oil. These amounts of the fats provided very low, low, and liberal amounts of the vitamin. Typical results are shown in the following table:

EFFECT OF VARIABLE VITAMIN D IN DIET

VITAMIN D	SERUM Ca	SERUM P	WEIGHT GAIN, GM.
Diet Ca/P=0.4	P=1.0	Ca=0.44	
B.F. 1	6.3	10.4	7.0
B.F. 10	10.7	9.9	43
C.L.O. 2	9.8	7.6	70
Diet Ca/P=1.0	P=1.0	Ca=1.04	
B.F. 1	8.0	8.0	20
B.F. 10	10.7	8.8	55
C.L.O. 2	10.3	8.3	68
Diet Ca/P=1.5	P=0.8	Ca=1.24	
B.F. 1	10.5	8.7	23
B.F. 10	10.5	8.8	55
C.L.O. 2	10.2	8.5	82
Diet Ca/P=6.0	P=0.2	Ca=1.24	
B.F. 1	10.2	3.0	20
B.F. 10	11.2	3.9	12
C.L.O. 2	13.6	7.5	10

The maintenance of a normal concentration of calcium and inorganic phosphorus in the serum is an example of biological regulation. Other examples are the maintenance of a nearly constant body temperature in warm-blooded animals, of a fixed hydrogen ion concentration in the blood, and of a constant osmolar concentration in the serum. Various factors such as the concentration of calcium and phosphorus in the diet, vitamin D, protein, fat, carbohydrates, various salts other than calcium and phosphates, and the balance between acid and basic ions, all play a part in determining the absorption of bone-forming salts.

The type of phosphorus in the diet is also of great importance. In the case of the phosphorus of cereals, for example, it was found that their "anticalcifying" effect was due to the presence of phosphorus in the form of inositolphosphoric acid which is poorly assimilable. The biological availability of the phosphorus from different sources has been discussed in detail in Chapter VII. Endocrine factors may influence the ebb and flow of calcium salts from and into the tissues and their excretion from the body.

If the intake of calcium is low and that of phosphorus is high, the excess of phosphorus which is absorbed is excreted, and it appears that, notwithstanding the needs of the body for calcium, the phosphate ion carries with it some calcium, thus depleting the blood of its ionized calcium. When the situation is reversed, phosphate ions are carried away with the excess of calcium excreted, even though this be to the disadvantage of the body. When young rats are caused to develop acute rickets by restricting them to a high-phosphorus low-calcium diet, the blood calcium is lowered to a level which produces tetany as an accompaniment of rickets. Tetany never complicates rickets produced by a high-calcium low-phosphorus diet.

Role of Vitamin D in Mineral Metabolism.—There is a striking regulatory power of vitamin D on the calcium and phosphorus metabolism of the body. The action of the antiricketic vitamin in the body is directly concerned in the preservation of the mineral balance. Vitamin D not only acts as a regulator of the metabolism of these elements; it permits the body to operate with greatly increased economy. When it is present in optimum amount, the metabolism of these minerals is maintained in a nearly normal manner even when one or both of them is provided at almost starvation levels. The vitamin brings about maximal utilization of calcium and phosphorus under these conditions, both by reducing excretion and by increasing absorption.

In active rickets there is a diminished retention of calcium and phosphorus. Infants (Orr, Holt, Wilkins and Boone, '23) with active rickets fail to retain calcium and phosphorus although adequate

amounts are present in the diet. On irradiation with ultraviolet light these elements are readily retained. It is generally assumed that vitamin D, either from direct administration or from light acting on the skin, increases the absorption of calcium and phosphorus by the intestine. Thus the retention of these minerals returns to normal.

McGowan ('33, l.c.) observed a marked shift in phosphate excretion from feces to urine after administration of large doses of the vitamin. He observed a definite and consistent rise of the serum phosphorus; the serum calcium was unchanged. McGowan believes that calcium plays a secondary role to phosphorus and is affected only inasmuch as it acts to neutralize the excretion of phosphate ion.

Stolzberg and Meyer ('32) noted that in rats on ricketogenic diets, early in the deficiency the blood phosphorus decreased more rapidly than the calcium. Rominger, Meyer and Bomskov ('32) made similar observations in human rickets and stated that "rickets is a primary disturbance in phosphorus metabolism." Kay and Guyatt ('33) conclude from their experimental studies that rickets is a phosphorus deficiency disease. On the basis of their evidence, rickets appears to be primarily due to phosphorus deficiency, and lack of vitamin D produces rickets by decreasing the retention of phosphorus which secondarily depletes the organism of calcium; and it is this depletion which is the cause of rickets.

The experiments of Shohl and Bennett ('27, '28), Karelitz and Shohl ('27), Williams ('28), and Warkany ('30), as well as that of Shipley, Kramer and Howland ('26), suggest a close relation between rickets and a low phosphorus retention. In contrast to these observations, another group of investigators (Murdoch, '27; Courtney et al. '28; Koch and Cahan, '27) express the belief that the abnormal excretion of phosphorus in rickets may be a result of a change in the calcium metabolism rather than an impaired power of phosphorus absorption. Daniels and collaborators ('29) also concluded that the imbalance in the amount of calcium and phosphorus retention in rickets is due more frequently to a deficiency of calcium. Skaar ('27) made similar observations; however, he considers that there are two ricketic processes, one where calcium metabolism is affected, the other where phosphorus metabolism is first affected. Cod liver oil therapy causes the blood calcium and phosphorus to return to the normal and the balance of these mineral elements becomes positive. If phosphorus in the form of Na_2HPO_4 is added to the ricketic diet, the ricketic condition is greatly aggravated and the calcium-phosphorus balances rapidly decrease. The serum phosphorus is markedly lowered. Skaar and Haupl ('29) conclude that in some individuals the disturbance is concerned with the calcium metabolism and in others with the phosphorus.

Vitamin D in Relation to the Parathyroid Glands.—Wilder and Howell ('36) reviewing the literature relating to hyperparathyroidism, have discussed the relation of vitamin D deficiency to parathyroid enlargement. Deficiency of this vitamin results, among other effects, in a fall of the ionized calcium of the blood serum. Blood calcium concentration is controlled, in part, by the parathyroid hormone, and in avitaminosis D there is a compensatory enlargement of the parathyroid glands to offset the deficiency of this nutrient. They state that the compensation for deficiency of this vitamin that is effected by hyperplasia of the parathyroids is usually not overdone, in that it does not exceed the increased need for it. In certain cases, however, adenoma formation occurs. The rarity of this condition in man, they suggest, is due to the fact that it occurs only in those cases where there are embryonic cells, or cell nests, in the parathyroids. In such cases deprivation of vitamin D, which causes hypertrophy in the gland, leads to the proliferation of the embryonic cells, causing parathyroid tumors. The resulting tumor frequently possesses the power of synthesizing the parathyroid hormone. If it does, its function will be without the restraint that regulates the normal parathyroid gland, and thus it will provide a supply of the hormone unrelated to the requirements of the body. In this condition an excessive amount of calcium salts will be mobilized, the content of calcium in the blood will rise, and excessive amounts will be excreted. On this hypothesis, the etiology of osteomalacia, or bone softening, may be tentatively explained. They suggest that in most human subjects there are no embryonic cells in the parathyroids, and that in such subjects, in vitamin D deficiency, the parathyroids are capable of increasing their function without hypertrophy. Hamilton and Schwartz ('32) have emphasized the increased function of the parathyroids in children insufficiently provided with vitamin D.

Wilder and Howell ('36, l.c.) point out that hyperparathyroidism is exceedingly rare in sunny climates, and occurs with increasing frequency at higher latitudes. They correlate this incidence with the amount of sunlight available. In the North Atlantic States 113 cases have been reported per 100,000 of population, as against 9.5 cases in Italy. Hyperparathyroidism may result in osteitis fibrosa. The evidence seems to be conclusive that deficiency of vitamin D demands compensatory hormonal production by the parathyroids.

The Antiricketic Activity of Skin, Hair, Wool, and Feathers.—Since vitamin D_3 is formed in the skin through the action of ultraviolet light, the effects of irradiating the skin are in all respects comparable with those of the administration of D_2 or D_3 in fish liver oils. Eckstein and Wile ('26) found 19 per cent of the total lipids of human skin to be cholesterol, which constitutes about 1 per cent of the total

weight of the skin. Hess and Weinstock ('25) showed by feeding tests that unirradiated skin has no antiricketic value whereas on irradiation it becomes antiricketic. Hair and feathers also are activated by light.

Wool fat contains a provitamin D and is activated on irradiation. Rekling ('27) reported that ultraviolet rays did not protect rats from rickets when they were prevented from licking their fur, indicating that such vitamin D as is formed in their skins is not transferred to the blood. But when the hairs are swallowed, the provitamin D in the lipid dressing derived from the sebaceous glands having been activated, the vitamin is absorbed. Hou and Tso ('28, '29, '30) state that the skin of normal rabbits was slightly antiricketic, the dorsal skin more than the ventral; but that the skin of ricketic rabbits, or of rabbits reared indoors was without protective action. Hou ('30) noted that after the skin and hair had been washed with ether, ultraviolet irradiation was but little effective in preventing rickets. Rowan ('28) found that birds of prey on a meat diet developed rickets, but that they were protected when fed feathers. Hou ('31) also observed that birds differ from mammals in having but one gland of a sebaceous nature, viz., the preen gland, which supplies a waxy secretion that the bird distributes upon its feathers for their protection against wetting. Preen gland oil becomes activated on irradiation. Thus vitamin D is furnished by ingesting feathers, or by absorption from the skin which gets it from the feathers. The feathers and skin of ricketic birds or birds from which the preen gland had been removed, possessed little or no antiricketic property. Removal of the preen gland made birds susceptible to rickets, and birds without this gland were not benefited by exposure to sunlight or to ultraviolet rays. Nocturnal birds and carnivorous animals may derive their vitamin D from the fur or feathers which they ingest. In line with this view one may explain, in part, the nutritive effect observed from the feeding of rabbits or of small birds, including fur or feathers, respectively, to young Carnivora. Young Carnivora have been frequently fed too much muscle meat and too little bone and glandular organs to supply sufficient calcium, phosphorus, and vitamins; consequently, their feeding on small animals which may be eaten in their entirety, corrects this difficulty, for the fur or feathers exert a beneficial effect, being sources of vitamin D. Horses are said not to thrive if they are frequently scrubbed with soap and water, which may perhaps be explained on the basis of the importance of the sebaceous secretion upon and in the skin, for absorption after exposure to sunlight. Helmer and Jansen ('37) found that washing removes substances from the human skin which ultraviolet rays can convert into an antiricketic agent. Water removes this fraction much less effectively than does soap and water. It may be, there-

fore, that persons who are addicted to frequent bathing with the use of soap may remove so thoroughly the provitamin and vitamin D of the skin that they would derive much less benefit from exposure to sunlight than would be the case were the washing less thorough. This may have a bearing on the question of the need of a dietary source of vitamin D by certain persons during the summer as well as during the winter months.

Hume, Lucas and Smith ('27) found that by anointing the skin of ricketic rats with an oil solution of irradiated impure cholesterol, cure of rickets resulted. Similar conclusions have been reached by Astrowe and Morgan ('35), Amrhein ('34), and others. This is not surprising since it has long been known that fatty substances are absorbed through the skin. It is not yet decided whether, when the skin is irradiated, vitamin D is formed in the skin, or whether, as Hess, Weinstock and Helman ('25) suggested, the antiricketic activity of sunlight may come about through activation of the provitamin which is excreted by the sebaceous glands, and the vitamin then is reabsorbed. Fodor ('34) concluded that ten times as much of D must be supplied by way of the skin as by mouth in order to initiate the healing of rickets in rats. However, it appears that the skin plays a definite role in the absorption of antiricketic substances formed by ultraviolet irradiation.

Storage.—The animal body maintains a reserve of vitamin D, the concentration varying with the dietary supply and the length of exposure to sunlight or other sources of ultraviolet radiation. The depletion of this store when the body is not receiving a supply of the vitamin is influenced by the calcium and phosphorus content of the diet.

Individual differences in susceptibility to rickets in both experimental animals and infants have been traced to the diet of the mother during pregnancy and lactation (Hess and Weinstock, '24a; Toverud and Toverud, '31). The antiricketic potency of the mother's milk varies with the D content of her diet. A transfer of vitamin D from the maternal organism to the fetus seems to occur. In cases where the diets are deficient in vitamin D the bodily storage of D is so low that the young become extremely susceptible to rickets.

From studies with different species it has been shown that D is normally present in blood. Heymann ('37) has investigated the fate of this factor within the animal organism and its storage in various tissues. He demonstrated that upon administration of a single large dose of viosterol (200,000 U. S. P. units of vitamin D) to male rabbits, some of the vitamin remained in the different tissues for various lengths of time. In the brain it was stored for but 1 to 2 weeks, in the red blood corpuscles 5 to 6 weeks, the skin retained it for 6 to 8 weeks, kidneys 6 to 9 weeks, and it remained in the liver and blood

as long as 12 weeks. Heymann believes that the time of storage in the liver is of significance and agrees with Gerstenberger ('33) that this organ may play a decisive role in the functioning of vitamin D. He also concludes that conditions other than the purely chemical composition of the tissues influence the retention of vitamin D.

Mode of Absorption and Excretion of Vitamin D.—Greaves and Schmidt ('33), studying the role of bile in the intestinal absorption of vitamin D, concluded that the vitamin was not absorbed in the absence of bile. Administration of vitamin D failed to cure rickets in rats with bile fistulas.

Heymann ('37a) pointed out that liver injury caused by biliary obstruction might also impair the effectiveness of vitamin D. Upon further investigation ('37b and c) he confirmed Greaves' and Schmidt's findings. Heymann suggests that the non-absorption of the vitamin under these circumstances indicates that bile appears to act on the vitamin D molecule itself. These results substantiate the hypothesis advanced by Gerstenberger ('33, l.c.) that the action of vitamin D is concerned with some function of the liver. Heymann's general conclusion is that impaired liver function is responsible for the diminished effect which vitamin D has upon ricketic rats in which liver damage had been induced.

In studying excretion of the antiricketic factor, Heymann ('37b and c, l.c.) found that when bile was excluded from the intestine of dogs, vitamin D was present in their feces for but 10 to 16 days after the administration of large single doses, whereas the normal animal upon similar treatment retained the vitamin in the feces from 6 to 8 months. These findings indicate that in case of the normal dogs, after about the first two weeks following the ingestion of a large single dose, any vitamin D found in the feces must have been absorbed and then slowly excreted.

Transmission of Vitamin D to Milk by Lactating Women.—A number of studies have been made to determine whether taking vitamin D by the human mother suffices to protect her breast-fed infant against rickets. Hess and Weinstock ('24b) found that 15 of 28 babies developed rickets although their mothers took cod liver oil during the last two months of pregnancy. Gerstenberger, Hartman and Smith ('27) observed that breast milk from women receiving one tablespoonful of cod liver oil daily, failed to cure rickets in rats. McCollum, Simmonds, Becker and Shipley ('27) found that when cod liver oil was given to rats before mating, during pregnancy, and during the first two weeks of lactation, considerable protection against rickets was afforded their young. As has already been noted, when irradiated yeast is fed to cows in sufficient amount, the vitamin D contained therein is passed on to a considerable extent by the cow,

so that milk containing as much as 400 I. U. per quart may be obtained by this procedure. On the other hand, feeding cod liver oil in liberal amounts to cows results in contributing little vitamin D to their milk. Bunker, Harris and Eustis ('33) found that feeding 20 cc. daily of mixed breast milk from women whose diets varied widely, caused no healing of rickets in rats. On the other hand, a similiar amount of breast milk from women who took daily 40 ounces of milk, 24 ounces of which was from yeast-fed cows ("metabolized" vitamin D milk) caused a definite antiricketic effect when fed for 8 days. This result indicates but a low antiricketic value in the milk. A number of other studies, which space does not permit to mention, are in accord with these results.

Administration of Vitamin D during the Summer Season.— The question whether administration of cod liver oil or other source of vitamin D should be continued during the summer months is often raised. Park points out that although theoretically this is not necessary, practically it is better to continue feeding vitamin D throughout the year. The sunlight in cities often contains little or no ultraviolet rays, and is, therefore, not effective in preventing rickets. Also, interruptions of feeding the vitamin often breaks down the habit of taking it, on the part of both mother and child, and makes resumption difficult or impossible. When there is a suspicion that cod liver oil is causing digestive disturbances, or when it is distasteful, a concentrate or vitamin D milk should be used. Since the dose of vitamin D is likely to prove insufficient when vitaminized milk is fed, the physician should be consulted as to the source and dosage of the vitamin to be given, particularly in case of premature infants. It is sometimes necessary to increase the dose even to 10,000 I. U. or more daily for such infants in order to protect them. Park ('38) has discussed in detail the problems arising in vitamin D therapy in infants suffering from various ailments.

Relation of the Distribution of Ultraviolet Light to the Geographic Distribution of Rickets.—Palm (1890, l.c.) was the first to express the view, based upon an extensive correspondence with medical missionaries in China, India, and elsewhere, that rickets is principally caused by deficiency of sunlight, since its occurrence is comparatively rare where sunlight is abundant, and of frequent occurrence where there is but little sunlight. Modern studies have brought to light the explanation of this relationship between climate and the distribution of the disease. In Northern countries, however, the paucity of sunshine during much of the year is compensated for by the character of the diet of the people, which consists largely of fish and fish-eating marine animals, bird skins, and at times, eggs. There are areas in the tropics where, because of excessive cloudiness, rickets

occurs among children. Lack of sunlight, and of a dietary source of vitamin D, also accounts for the prevalence of rickets in the infants of mothers who are secluded, as under Purdah, in India. It is in temperate regions, where lack of vitamin D in the food, together with paucity of sunshine during winter, and the wearing of heavy clothing, deficiency of outdoor life, and in cities the absorption of ultraviolet rays of the sunlight by the smokepall, that rickets has been most prevalent. After the discovery of vitamin D in 1922, the tendency has been increasing throughout the world to provide vitamin D in some form for most infants and children. The result, viz., the reduction of the incidence and severity of rickets in the young, has been one of the greatest achievements in preventive medicine.

Air Pollution in Relation to Ultraviolet Light in Sunlight.—The loss of solar ultraviolet due to a sooty atmosphere is of importance from a public health standpoint. The extent of this loss has been pointed out by a number of investigations measuring the intensity of sunlight in rural and urban regions. Shrader, Coblenz and Korff ('29) found that in the open country outside of Baltimore the amount of ultraviolet light was about 50 per cent greater than in that city. A survey made by the U. S. Public Health Service ('34) of 14 of the largest cities in the United States, showed that there was 50 per cent more ultraviolet rays in the sunshine on clear days, in the least smoky group, than in the most smoky group. On cloudy days there was 100 per cent more. The maximum atmospheric pollution in all these cities occurred during the winter months when the ultraviolet light is at a minimum because of the low altitude of the sun, cloudiness, smoke, and the shortness of the days. Even in clear weather, and in the country, sunlight contains no appreciable ultraviolet light except when the sun is above 30 degrees from the horizon. The extent to which air pollution may occur is shown by the study of Pincus and Stern ('37) who found that during a recent typical year, 300,000 tons of soot, tar, cinders, and ash were discharged into the air over New York City. In certain areas of the city, soot and dust were found to settle at the rate of 1800 tons per square mile per year. Figures as high or higher have been reported for other cities. Even in small cities, pollution may be as bad when soft coal is burned as fuel in homes and factories. Such statistics make it clear why the administration of some form of vitamin D is necessary for infants and children, and probably also for adults, throughout the year, except where sunshine is especially abundant.

Tisdall and Brown ('28) found the average antiricketic value of the sunshine in Toronto during April to August, inclusive, to be about eight times that during November to January, inclusive. They use the term "skyshine" to designate the sun's rays which are reflected from the

sky and clouds in contradistinction to the direct rays of the sun itself. They found the antiricketic value of skyshine to be approximately one-half to two-thirds that of direct sunlight. They suggest that rickets will not occur or will be very mild in regions where the minimum noonday altitude of the sun is 30 to 35 degrees, or less, for only a few weeks in the year. Places with a minimum altitude of 35 degrees lie along latitude 31° 30′, which runs a little south of Savannah, Ga. At the latitude of Glasgow, Scotland, (55°) the solar altitude is below 35° during six months of the year. In the latitude of Chicago the noonday altitude of the sun is below 35° for four months of the year. Clouds and fog cut off much of the ultraviolet light from the sun in certain places. P. A. Day ('32) states that the average daily amount of sunshine necessary to afford the same partial protection in rats receiving a rickets-producing diet, varied from 5 minutes in May, June, and July, to 168 minutes in December, with intermediate values for other months. Knudson ('32), using rats, found that in Albany, 90 to 120 minutes' daily exposure to sunshine during June to August inclusive, afforded complete protection, whereas exposures of 240 to 270 minutes daily during December and January caused little or no healing. Blunt and Cowan ('30) and Laurens ('33) have published extensive reviews on light in its relation to rickets.

General Metabolism.—Among the metabolic processes other than those involved in the prevention of rickets, vitamin D is concerned with energy metabolism.

A number of investigations have suggested a relation between antiricketic substances and the body's rate of gaseous exchange. Nitschke ('36) has pointed out that the basal metabolic rate may be considerably below normal in ricketic infants and rats, and that the rate is increased by vitamin D therapy. Administration of the vitamin to hedge-hogs prevented hibernation, a condition in which there is a depression of metabolism. Recently, Presnall ('37) demonstrated that vitamin D influences the rate of oxygen uptake by the skins of rats. The oxygen uptake of pieces of shaved skin from the back of young albino rats on an unsupplemented ricketogenic diet was only 35-84 per cent of that of skin from rats on the same diet but receiving 2.5 I.U. of vitamin D daily in the form of viosterol. When vitamin D was added to the diet of ricketic rats, the skin respiration increased as the rickets became healed. Further observations of a similar nature are those of Deutsch, Reed and Struck ('36), Siel ('29), and Landelius Ljungqvist ('34) who found that the lowered rate of oxygen consumption in rickets returns to normal with vitamin D intake. A finding of particular interest is that of Gelfan ('35), who demonstrated a response to vitamin D by a cold-blooded form of animal life when he showed that isolated muscles of frogs

treated with the antiricketic substance utilize more oxygen than do those from normal frogs. Rappaport and collaborators ('33, '34) Reed ('34) and Dreyer and Reed ('35) have shown that massive doses of vitamin D considerably increase the metabolic rate of normal dogs and rats.

The muscular weakness which occurs in rickets may be regarded as being the result of the disturbance in mineral balance. Since the blood phosphorus is low, faulty muscle metabolism must be attributed to depletion of body phosphorus. The phosphate ion plays a significant role in carbohydrate metabolism and hexose phosphate is an active intermediary in the cellular metabolism. This intimate metabolic associate of glucose and phosphate suggests that the depletion of phosphate in rickets may interfere with carbohydrate metabolism. Evidence that such is the case is indicated by Hentschel and Zöller ('26) who found that the inorganic and organic phosphate content of the muscles was considerably reduced in rickets, and that in active rickets the ability to synthesize hexosephosphate in the muscles is greatly impaired. Landsberger and Silber ('27) found in florid rickets impaired carbohydrate tolerance and a hyperglycemic curve that was not counteracted by insulin.

More recently Sure and Smith ('31) reported no demonstrable changes in the concentration of blood sugar, alkaline reserve, or the glycogen content of the liver in moderate or severe rickets. Freudenberg and György ('20), Freudenberg and Welcker ('26), Adam ('27), and Konig and Lenart ('27) observed a disturbance of the carbohydrate metabolism in rickets as indicated by an increase in the diastase in the urine and feces and a decrease in the glycolytic power and the lactic acid level of the blood serum. Konig and Lenart also found an increased alimentary glycemia in children with rickets. DeToni ('30) confirmed this observation.

Baldwin, Nelson and McDonald ('28) believe that the antiricketic factor is essential to normal carbohydrate metabolism in the chick. They obtained changes in the respiratory quotient suggesting a rapid loss in ability to utilize carbohydrates. During the period that leg weakness of the chicks was marked, the respiratory quotient indicated the utilization of fats only, in spite of the fact that 47 per cent of the diet consisted of carbohydrates. Administration of cod liver oil or irradiation brought the respiratory quotient to normal.

Hutchison ('26) noted no disturbance of the fat metabolism in rickets. Telfer, however, ('26) reports that although the concentration of fat in the feces tends to be normal, combined fatty acids are relatively lowered.

Niemann ('17) and Landsberger ('25) reported that ricketic children are especially subject to ketosis. Hottinger ('28) found an

increase in the excretion of ketone acids in the urine. A hypocholesteremia appears to be characteristic in rickets. The cholesterol concentration rises with the healing of rickets. (Dorlencourt and Seiszoff, '29; Lesné, Sylvestre and Zizine, ('29). This however, may be secondary to impaired carbohydrate metabolism.

Since the phosphate ion plays an important role in muscle metabolism not only through its association with glucose, but by its combination with creatine, it may be that loss of phosphate may also impair creatine metabolism. Further investigation on the effect of loss of this ion on fat metabolism might also yield significant results.

Vitamin D depletion does not affect absorption of protein (St. Julian and Heller, '30).

Vitamin D deficiency, apparently, does not directly affect hematopoietic function (Sure and Kik, '31). However, H. Day and Stein ('38) have noted that a deficiency of Vitamin D in diets containing an excess of phosphorus, relative or absolute, causes a greater degree of anemia than occurs with similar diets containing vitamin D.

Role of Vitamin D in Development of the Embryo.—From experiments with both animals and humans, evidence is available showing that a relation exists between vitamin D and embryonic development. Hess and coworkers ('28a), investigating the production of hens' eggs, observed that the concentration of vitamin D varies according to the diet and exposure to ultraviolet rays. Hens fed a vitamin D-deficient diet laid comparatively few eggs which contained little of the antiricketic factor. Fertile eggs from these hens failed to hatch. The hens' livers were also low in antiricketic potency. Experiments on young chicks tended to show that the lack of hatchability was due to lack of development of the embryo when the hens' diet was deficient in vitamin D, and was to be attributed to defective absorption of calcium from the shell into the yolk. Irradiated milk-fat preparations have been shown to increase the weight and hatchability of hens' eggs.

Hess and coworkers made similar studies on fish ('28b). They showed that the roe contained as much or even more of the antiricketic factor as the liver, whereas the newly hatched fish were practically devoid of it. They believe that this factor had been used up in the course of development of the larvae.

The number of studies on the effect of vitamin D on the development of the human embryo is small. Abels ('27) reported a case where cod liver oil administration during the second pregnancy resulted in the second child weighing 60 per cent more than the first. Similar results were observed by Vogt ('28).

Relation of Vitamin D to Linear Growth.—Stearns, Jeans and Vandecar ('36) and Slyker and associates ('37) have demonstrated

that the rate of linear growth is influenced by the intake of vitamin D. Generous amounts of vitamin D increase the skeletal growth. These workers present evidence that the greater rate of growth was not due to any other factor, and the conclusion drawn from these experiments is that vitamin D is the chief factor affecting the rate of linear growth, since growth in length is dependent primarily on skeletal growth.

The Relative Effectiveness of Vitamins D_2 and D_3 from Different Sources for the Human Infant.—The question arises as to whether the human reaction to the different antiricketic substances is like that of the rat or like that of the chicken. The evidence (Kramer and Gittleman, '33; Drake et al., '34; Jeans and Stearns, '34; Wyman et al., '35; Report of the Secretary of Agriculture, '35) at present available, while not conclusive, is at least indicative that, rat unit for rat unit, the various forms of vitamin D have equal value for humans.

Hume ('37) has reviewed the literature relating to the relative potency of vitamin D from different sources for the rat, chick, and human subject. She concludes that it "seems fairly certain that for the human infant, vitamin D from fish liver oils, irradiated ergosterol, irradiated cholesterol, and vitamin D milks of various kinds, has the same relative potency as for rats." She further points out that sources of vitamin D for use with poultry should be standardized by means of tests with chicks only, and the standard must be some source other than irradiated ergosterol. For practical purposes, any form of vitamin D may safely be assayed by using the rat as a test animal.

The importance of these observations at once attracted the attention of pediatricians and stimulated much research. Park ('38, l.c.) lists references to 39 such studies designed to show the comparative potencies of these different sources of vitamin D. He states: "Out of the conflicting and most confusing results only generalizations of a tentative nature are possible. For practical purposes calciferol, the vitamin D in viosterol, may be regarded as being equal to the vitamin D of cod liver oil. If viosterol is inferior to cod liver oil, rat unit for rat unit the difference cannot be great, or it would have come out more clearly." Dr. Park concludes from the data available that vitamin D, dispersed in milk, is more effective than in the usual oil menstrua. This may possibly be explained as due to its dissemination in the minute fat droplets, which could afford more effective absorption, or perhaps on the assumption that it is advantageous to divide the dosage as occurs in milk feedings. It is generally accepted that milk enriched with vitamin D in the three ways mentioned, may, from the practical point of view, be regarded as of equal value when administered in quantities affording equal potencies as judged by the

rat assay method. He states that the evidence suggests that the vitamin D in irradiated milk may be slightly superior to that in milk of cows fed irradiated yeast, which is generally called "metabolized" vitamin D." This term is misleading because there is no change wrought by the metabolic activities of the cow upon the calciferol administered to her in the form of irradiated yeast. The cow absorbs the calciferol and passes it on into her milk. The only apparent superiority which such milk might possess over the other sources of the vitamin would be due to better absorption and spacing of dosage due to milk feedings to the infant. In the absence of conclusive evidence to the contrary, the present indications are that the rat unit is a satisfactory measure of the value of an antiricketic substance for humans, and that the question of source or form of vitamin D is of significance only for chickens; the reaction of humans parallels that of rats and not that of chickens. The relative effect of different sources of the vitamin on humans is directly proportional to the number of rat units which each provides.

Requirements for Vitamin D.—Vitamin D is needed throughout life, the requirement being greatest during infancy and during pregnancy and lactation. Rickets prevention alone can no longer be regarded as a criterion of adequacy of vitamin intake (Jeans, '36). The consensus of medical opinion is that feeding vitamin D in amounts well above the minimum levels necessary for prevention or cure of rickets has no deleterious effect, and that the rule should be to give it in ample quantity to cover any possible unrecognized requirement.

Since the periods of greatest susceptibility to rickets are the early months of life, it is important that vitamin D be given at the beginning of the third week, with a dose of one-half teaspoonful of cod liver oil, or of its equivalent in other preparations. This provides about 200 I.U. daily. After a few days, the dose should be increased to one teaspoonful of the oil, or a dose of 400 I.U. per day. During the fourth week, the infant should receive 800 I.U. daily, and if the oil is well tolerated, it may be increased to three teaspoonfuls during the second month. Twelve hundred to 1500 I.U. per day will do no harm. Eight hundred units per day should be given throughout the second year and thereafter. It should be pointed out that if any oil is allowed to run down the larynx into the lungs, it may cause pneumonia. Hence, the form in which the vitamin is given to sick infants should be determined by the physician, who will, in many cases, advise using a concentrate or vitamin D milk when vomiting occurs frequently and there is danger of the infant aspirating cod liver oil into the lungs.

For the prevention of rickets the regular administration of 400

I.U. per day is regarded adequate. Jeans ('36, l.c.) and Jeans and Stearns ('38) have discussed the subject in detail, and have also presented the views of the Committee on Foods of the American Medical Association ('36). Park ('38, l.c.) suggests that for "working purposes," in the case of fish liver oils or viosterol, 800 to 1000 I.U. per day be regarded as the lowest levels which it is wise to give to the infant. Since irradiation of milk does not produce vitamin D in excess of about 135 I.U. per quart, such milk without a supplement of the vitamin cannot be considered entirely satisfactory as a source of the vitamin for infants or children.

There is a paucity of data on the vitamin D need of adults. In the case of pregnant and lactating women, the League of Nations Health Committee ('36) recommends a minimum daily intake of 340 I.U. However, there is reason to believe that the optimum for any age is several times greater than the minimal level necessary for protection against hypovitaminosis D.

In rickets, the rapidity of cure depends on the dosage. The therapeutic dose of vitamin D has generally been regarded as 1000 to 1500 I.U. per day for the human infant. Twelve hundred units, provided by cod liver oil preparations, are considered adequate by clinicians for rapid correction of rickets. When the vitamin is administered in the form of viosterol (calciferol) or irradiated ergosterol, it should be given in larger doses than cod liver oil. A double unit dose is recommended. The vitamin D of irradiated milk has given excellent clinical results with less than the usual dosage. Whether the quality of the vitamin, the vehicle, or extent of absorption of any other factor is the reason for these differences is not as yet known.

Breast-feeding not a Specific Protective Factor Against Rickets. —Hess ('29, l.c.) emphasized the fact that rickets has a lesser incidence among nursing infants than among those fed cow's milk. He pointed out the tendency to credit breast milk with greater protective value than it actually possesses, and stated that "woman's milk is far from being a specific." He cited observations by himself and Weinstock ('27) as well as those of Macy and her associates ('28) which show that there is little, if any, vitamin D in certain samples of human milk. Macy and coworkers ('28) found that 25, 30, or 40 cc. daily of human milk failed to cause healing of rickets in experimental rats. Cow's milk was effective when 30 cc. per day was fed to rats.

Coons and Coons ('32) found a much better retention of calcium during pregnancy of women in Oklahoma than was noted by Coons and Blunt ('30) who studied women in Chicago. They attributed the difference to the superior quality of the sunlight to which the southern women were exposed. McBeath and McMahon ('33) studied two groups of breast-fed babies selected at the age of two months

from a pediatric clinic. The mothers of one of these groups were given daily one quart of vitamin D milk containing 432 I.U. per quart; while the mothers of the other group received a like amount of ordinary milk. Clinical and X-ray observations during six months showed great differences in the two groups of babies. Of the 16 babies whose mothers received vitamin D, 2 had severe rickets and 4 slight rickets, while 20 out of 26 in the control group had florid rickets requiring direct therapeutic treatment.

The Effects of Excessive Dosage of Vitamin D.—There are in the medical literature a number of statements to the effect that irradiated ergosterol (calciferol) is an extremely toxic substance. This view arose through the use of an early German preparation, *vigantol,* which was observed to cause pathological calcification in various parts of the body. It is now clear that this preparation was toxic because (a) the ergosterol was irradiated in alcohol, which is not a suitable solvent in this connection, and (b) because the ergosterol was greatly over-irradiated, with the formation of toxic products. The present-day method of irradiating ergosterol dissolved in ether, with proper time regulation, avoids the formation of toxic products.

The margin of tolerance of the vitamin for man is not known. Numerous experiments suggest that there must be large individual variation. Excessive dosages of vitamin D have been given to adult patients as a treatment for chronic arthritis and psoriasis. Some of the patients apparently were able to tolerate such doses fairly well. An excellent review on this subject by Bills ('35) is available. When Wyatt, Hicks and Thompson ('36-37) administered 300,000 I.U. daily, they observed violent persistent nausea, intense headache, and profuse sweating. One patient suffered severe diarrhea, loss of appetite, and headache. These symptoms, together with nocturia and frequent micturation, have also been described by others. In over-dosage there are high concentrations of calcium and phosphate in the blood, and calcium casts have been observed in the urine, such as have been described by Albright and Bloomberg ('34) in cases of hyperparathyroidism. Elevation of the calcium content of the blood serum above 12 mg. per cent is an indicator of toxic effects of vitamin D.

Numerous investigators have demonstrated that the minimum toxic dose of vitamin D or of properly irradiated ergosterol is much greater than the therapeutic dose—as stated by Bills ('35, l.c.): "It is characteristic of vitamin D that the range between its ordinary therapeutic dose and the dose which produces adverse symptoms is extraordinarily wide; wider, perhaps, than is the case with any other potent drug."

Harris and Innes ('31) have reviewed the literature relating to hypervitaminosis D. Harris and Stewart ('29) showed that the ad-

ministration of excess of vitamin D brings about hyperphosphatemia or hypercalcemia, or both, in experimental animals. There is a high net absorption of calcium and phosphate from the gut, and the severity of the hypervitaminosis D is regulated by the intake of these elements. In other words, the situation in hypervitaminosis D is the reverse of that seen in deficiency of the vitamin.

A characteristic lesion in hypervitaminosis D is excessive calcification of the bone ending. The above mentioned investigators in their experiments employed daily doses of irradiated ergosterol as high as 2 mg. per rat. An increase in the calcium content of the diet (or in the Ca/P ratio) intensifies the hypervitaminosis and gives rise to increased formation of calcareous deposits in the kidneys, arteries, non-striated muscle, and other sites at a given level of vitamin D excess. When the diet was virtually devoid of calcium and phosphorus, calcareous deposits were not produced by excess of vitamin D, but there was a greatly increased resorption of bone substance. Large doses of the vitamin stimulate osteogenesis, and in contrast with rickets, a densely calcified overgrowth appears at the growing end of the bone. Still greater excess causes extensive resorption, and the cortex of the shaft and other compact bone becomes spongy. The blood calcium and phosphate content are raised. It is not definitely established to what extent increased absorption from the gut and increased withdrawal from the bones take place, but it is clear that vitamin D in excess exerts a distributive action, resulting in deposition of calcareous deposits at abnormal sites. Withdrawal of the vitamin excess causes greatly increased excretion of fecal calcium and phosphorus. In some respects the mode of action of vitamin D appears to be the reverse of that of the parathyroid hormone.

Therapeutic Effects of Vitamin D Other than in Rickets.—The use of vitamin D has been found beneficial in diseases other than rickets. Some of these will be presented here.

Infantile Tetany: Tetany is associated with a hypocalcemia as contrasted with the hypophosphetemia of rickets (Miraglia, '26; György, '29). Ultraviolet rays are capable of raising the calcium level of the blood to normal. Of the group of substances which exert a curative effect on tetany, irradiated ergosterol is the best (Gleich and Goodman, '28; Rohmer et al., '28; Bakwin et al., '29; Wilkes, '29). Shelling and Hopper ('36) found that tetany did not develop in any of their cases receiving vitamin D.

Osteomalacia: Osteomalacia represents the manifestations of rickets as they appear in the adult, where the element of growth is absent, resorptive processes are in the ascendancy and bone softening with deformity are the outstanding features of the disease. This disease affects mainly pregnant and lactating women. It occurs in

many parts of the world, notably in India, China and Japan. Numerous workers have pointed out the close relation between vitamin D and osteomalacia. Pommer (1885), in his studies upon the histological changes in osteomalacia and rickets, concluded that the essential abnormality in each disease was a cessation of calcification. Schmorl ('06) also found evidence of deficiency of provisional calcification and irregularity of endochondral ossification in young subjects of osteomalacia, changes similar to the disturbances of endochondral ossification in rickets. Hess ('29, l.c.) believed that the differences in the pathological lesions in rickets are quantitative rather than qualitative. The underlying pathological process is the same. Maxwell ('30) claimed that true osteomalacia is the manifestation of rickets in bones that have reached maturity and is connected with a shortage of vitamin D. When a source of vitamin D is provided, and the diet is improved in quality generally, striking improvement in cases of osteomalacia takes place. (Maxwell, '30, l.c.; Wilson and Surie, '30; Hottinger, '27; Lasch, '28; Green-Armytage, '28; Mathez, '29).

Celiac Disease: This disease is often followed by development of rickets, osteoporosis, and tetany. One marked feature of this condition is the loss of large amounts of undigested fat which is excreted in the feces. Since vitamin D is associated with dietary fats, it is believed that absorption of this nutrient is faulty in celiac disease even though the quality and quantity of the diet is adequate for protection against rickets. It is also thought that absorption of calcium is impaired due to the formation of calcium soaps. Dietotherapy is used in these cases and the omission from the diet of fat-containing foods is customary. Vitamin D is administered by mouth or frequently the individual is irradiated. Neale, Smallwood and Shippam ('36) and Shelling and Hopper ('36, l.c.) reported recovery and normal development of children with celiac disease upon treatment with vitamin D.

Treatment of Arthritis with Massive Doses of Vitamin D: Rappaport, Reed and associates ('33 l.c., '34 l.c., '35 l.c.) reported abatement of the symptoms of arthritis (atrophic, both rheumatoid and infectious; hypertrophic, both degenerative and menopausal, and mixed cases) when 200,000 I.U. per day of vitamin D were administered as viosterol, the signs of improvement appearing after periods of 1 week to 6 months. In some cases, they increased the dosage to 1,000,000 I.U. per day. There was a fairly high percentage of failures. Livingston ('36), Wyatt, Hicks and Thompson ('36, l.c.), and Farley ('37) have also used this therapy successfully. The mechanism of the clinical improvement, when it occurs, is not yet understood.

Treatment of Hay Fever with Massive Doses of Vitamin D: Rappaport and Reed and coworkers ('33, l.c., '34 l.c.) studied the effect of viosterol on allergic conditions. The viosterol employed was specially prepared, about 100 times stronger than commercial 250 D. It contained 920,000 U.S.P. (I.U.) units per cc. This treatment gave moderate to complete relief to 82.4 per cent of the cases. Analysis showed that the relief was due not to an increase in the total calcium or to any consistent alteration in the calcium partition of the blood. However, the K-Ca ratio became lower and less fluctuating.

Treatment of Psoriasis with Vitamin D: Psoriasis is a disorder of the skin common among peoples in northern regions. It is more severe in the winter than in summer.

While studying the effect of large doses of vitamin D in arthritis, Ceder and Zon ('37) observed complete disappearance of the psoriasis in a patient who had both arthritis and psoriasis. Vitamin D was then administered to a group of individuals who had chronic, widespread psoriasis of several years' duration. No local or dietary treatment was employed. Direct sunlight was excluded as much as possible during the summer months. After 6 to 12 weeks of vitamin D therapy the disease was checked. There was recurrence in some cases, but it was gradual and less severe. These investigators suggest, therefore, that vitamin D is effective in the treatment of psoriasis.

Trichinosis and Vitamin D Therapy: The possible relationship between trichinosis and vitamin D therapy has been investigated. In this disease when the protective mechanism begins to function, cysts begin to form about the larvae in the muscular tissues. These cysts become calcified and as the calcification progresses the disease abates. In an attempt to determine whether or not this protective calcification might be hastened by the administration of vitamin D, Wantland ('34) conducted an experiment on rabbits suffering with trichinosis. Calcium lactate and varying amounts of irradiated ergosterol were administered. The rate of calcification of the cysts was found to vary directly with the amount of vitamin D and calcium lactate given. Marked calcification of the cysts was obtained after four to six weeks. Control animals showed no calcification of cysts after periods of from 6 weeks to 3 months. Whether or not similar results would follow vitamin D therapy in cases of human trichinosis has not yet been demonstrated.

Eye Changes in Vitamin D Deficiency: Blackberg and Knapp ('34) state that in pups restricted to a diet deficient in vitamin D and low in calcium, there was a weakening of the cornea and sclera, which they regard as apparently identical with changes in the eyes in man, the cause of which was unknown. Since these changes are concerned with the refractive properties of the eye, they suggest that

human myopia may be dependent in part upon a deficiency of calcium, phosphorus, and vitamin D. They have also reported ('37) favorable results in such cases by rectifying these dietary deficiencies.

Effect of Vitamin D on Coagulation Time: In surgical procedures of gall bladder disorders, slow clotting of the blood is a serious problem. McNealy, Shapiro and Melnick ('35) reported preoperative administration of viosterol, with bile salts to aid in its absorption, to be an effective means of shortening the time of coagulation of the blood. Johnston ('37) also has employed this method with success. In three patients before treatment the coagulation time was 480, 275, and 480 seconds, respectively. The normal time is about 240 seconds. After treatment the times were reduced to 180, 225, and 240 seconds respectively. He also administered glucose and calcium intravenously. Such results as are available indicate that the calcium level of the blood is not generally reduced in this condition.

Vitamin D and Calcium Therapy in Pregnancy Toxemia: Theobald ('37) states that the daily administration of vitamins A and D, and calcium, reduced the occurrence of albuminuria, vomiting, high blood pressure, headaches, edema, cramps, and insomnia of pregnant women. He observed 100 healthy women, not more than 24 weeks pregnant, who were allocated in two groups at random. One group was directed to take daily 20 gm. of calcium lactate, 11,000 I.U. of vitamin A, and 450 I.U. of vitamin D. The other group did not take any supplements. All were observed until the termination of pregnancy. The above named symptoms occurred 23 times among the women receiving the supplements, whereas in the non-supplemented group they occurred 49 times. In the treated group, 8 developed albuminuria and/or hypertension, and 6 had other symptoms of toxemia. In the untreated group the corresponding figures were 17 and 11. He regards the results as statistically significant in favor of the nutritional therapy. This view is supported by the fact that those of the cases which were admitted to the hospital after development of symptoms, lost their symptoms on dietetic treatment. Obviously, further observations will be necessary to establish the value of this treatment.

Milk Fever: Milk fever is generally believed to be caused by deficiency of calcium in the blood. Since the subject is discussed in Chapter VII, it will suffice to note here that the evidence available supports the view that administration of vitamin D before the young are born will tend to prevent the disease.

Vitamin D in Resistance to Infantile Paralysis: Since the question of the portal of entry of the virus of poliomyelitis is still being strongly debated, no appraisal is attempted here of the evidence upon which divergent views of investigators rest. It is appropriate, however,

to call attention to the view of Toomey ('37) which he supports by weighty evidence, that infection with the virus may be by way of the gastrointestinal tract. He found normal monkeys difficult to infect by means of this path, but monkeys suffering from rickets are easily infected by this route. He expresses the belief that the spread of the virus is usually stopped in normal animals by some factor in the white and not in the grey fibers. The former contain 3.5 per cent of cholesterol, the latter but 0.7 per cent. Cholesterol absorbs and destroys the poliomyelitis virus. When rickets is produced in rats by vitamin D deficiency, the nerve myelin becomes involved, characterized by a foamy appearance attributed to edema of the neurokeratin framework. When rickets is produced in monkeys, it is assumed that the nerve myelin and its cholesterol become involved; the virus is not absorbed or destroyed, thus accounting for the ease of infection of such monkeys. On the other hand, when monkeys have had plenty of vitamin D, and inferentially normal myelin sheaths, the disease cannot be produced *via* the gastrointestinal tract.

It should be pointed out that vitamin D increases the permeability of the intestinal mucosa to calcium and phosphate (or decreases excretion). It is conceivable that altered permeability, in the opposite sense, to the virus may occur.

Role of Vitamin D in Wound Healing: Bond ('28), as well as others, has described the clinical application of vitamin D as cod liver oil or irradiated ergosterol. Bond observed that irradiation of ergosterol produced a fat-soluble substance containing oxygen. He also noted that ozonization of an ergosterol film converted ergosterol into an oxidizing substance, probably a peroxide or ozonide of ergosterol. Whether this substance is identical with vitamin D, or whether the formation of the peroxide is a subsidiary reaction during the process of vitamin D formation is not clear, but Bond suggests that the action of irradiated ergosterol on wounds and granulating surfaces may be due to the peroxide rather than to the vitamin.

Fractures and Vitamin D: Vitamin D has been observed to exert a healing effect in the delayed or imperfect union of fractures in old people and children and in all conditions which are accompanied by decalcification of the epiphyses (Bors, '27; Roi, '30; Collazo et al., '30; Knoflach, '28; Morelle, '29; Roederer, '29; Cuthbertson, '30).

Vitamin D in Relation to Dental Caries.—A separate chapter is devoted to the relation of the diet to tooth development and to tooth preservation, therefore only a few observations will be recorded here which bear upon this subject.

Since both the enamel and dentin of teeth consist of mineral deposits made up principally of calcium phosphate, with some carbonate, their formation is obviously a biological process involving

a calcifying mechanism. Any calcifying process involves cellular activity, and a proper nutrient medium in which the cells may function. The state of nutrition, as respects those factors which influence either of these tissues will influence, for good or bad, the structure of the teeth.

The organ which applies the enamel to the developing tooth is the ameloblast. Each cell builds a single enamel rod or prism of calcified substance. These are fitted together as a mosaic to form the tooth enamel. The ameloblasts are specialized cells derived from epithelial tissue, and in accord with the special sensitivity of epithelial tissues generally, to deficiency of vitamin A, it is shown that ameloblasts share this sensitivity. Hence if the diet is optimum in other respects, but deficient in vitamin A, the ameloblasts will be abnormal histologically and functionally, and defective enamel will be formed. If the diet is deficient in ascorbic acid, the odontoblasts will be unable to lay down normal dentin. If both vitamins A and C are provided in abundance, but the calcium and phosphate content of the blood is too low, as occurs in deficiency of vitamin D, and to a lesser extent in deficiencies of calcium or phosphorus, or both, both the ameloblasts and odontoblasts will be unable to form their normal products, enamel and dentin.

Due to the fact that in temperate regions almost all foodstuffs are deficient or lacking in vitamin D, and until the past fifteen years adequate supplements of this vitamin were generally not provided to infants, children, and pregnant and lactating women, and the prevalence of rickets was widespread, it is evident that deficiency of vitamin D must have had an unfavorable effect upon the structure of the teeth. Because defective enamel predisposes teeth to decay, this deficiency must have contributed to the high incidence of dental caries of persons who passed through infancy in the pre-vitamin D days.

Since sunlight containing adequate amounts of ultraviolet rays, acting upon the skin, is a source of vitamin D, dental caries would be expected to occur more frequently in children in the northern states than farther south. Of interest in this connection is a survey begun in 1933 under the auspices of the American Dental Association, in which 8,000 dentists in 26 states collaborated, and in which over 1,400,000 children were examined, representing 11.6 per cent of the estimated number of children 6 to 14 years of age in the areas studied. The results were published in 1936 by the U. S. Public Health Service. In the Southern States 60 per cent of the children had caries of the permanent teeth, whereas in the Northern States the percentage was 76. In the South the number of cavities per 100 children was 202; in the Northern States the figure was 293. In searching for the

cause or causes of this difference in incidence of dental caries, one may postulate that the diet, in one or more respects, is inferior in the Northern States to that in the South. In the absence of more detailed data this factor cannot be evaluated. It seems logical to attribute the difference in incidence of tooth decay, in some measure at least, to deficiency of effective sunshine in the North. In 12 Northern cities (between latitudes 37° and 43°) in February at 9 a. m., the ultraviolet light was only about one-ninth as intense as it was in a similar number of Southern cities (between latitudes 29° and 35°). For the month of November at the same hour the intensity of ultraviolet light was about one-fourth as great in the Northern cities. At noon the Northern cities received about one-half as much ultraviolet light. The six states showing the highest incidence of dental caries were Maine (3.97 per child), Pennsylvania (3.74), Massachusetts (3.65), Wisconsin (3.63), Ohio (3.31), and New Jersey (3.02).

M. Mellanby ('29-'36), Boyd, Drain and Stearns ('37) and others have reported partial control of dental caries by the provision of an adequate diet, and have clearly achieved the arrest of caries in some cases, through the administration of vitamin D. The role of this factor will be further considered in the chapter on the diet in relation to teeth.

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CHAPTER

XVI

Chemical Nature of Ascorbic Acid (Vitamin C)

IN NO DEPARTMENT of nutritional research has there been more dramatic progress than in that relating to the nature of scurvy and its prevention through the isolation and characterization of the specific factor, ascorbic acid, the deficiency of which causes scurvy. In conformity with the vitamin hypothesis there was for years a prevailing belief that scurvy had its origin in the deficiency of a specific dietary factor. The isolation of this substance eluded workers for more than a decade and after the task had been accomplished by Szent-Györgyi in 1927 it was not until five years later that anyone, including Szent-Györgyi, knew that it was vitamin C. The chemical nature, methods of determination, and distribution of this interesting substance will constitute the principal content of this chapter.

Nomenclature.—There has been some confusion regarding the nomenclature of ascorbic acid. Originally the factor which prevents scurvy was designated as the *antiscorbutic vitamin.* Gradually this was more or less replaced by the term *vitamin C* during the early part of the nineteen twenties. In 1933 Szent-Györgyi and Haworth named the substance *ascorbic acid.* This designation met with general approval among chemists, both here and abroad, but owing to its therapeutic suggestiveness the American Medical Association sought a name without that objectionable connotation. The Council on Pharmacy and Chemistry of the American Medical Association adopted a new designation, *cevitamic acid,* and attempted to gain its general acceptance but the effort failed. Recently the Council abandoned the new name. For the sake of simplification in vitamin designation it is probable that the term, *ascorbic acid,* will gradually supersede all others, since it has been generally employed by chemists from the time it was originated in 1933.

Isolation and Identification of Ascorbic Acid.—King ('36) has briefly reviewed the major facts concerning this subject and, as pointed out in his review, the principal workers were Zilva et al. (Zilva, '28), Bezssonoff and associates (Bezssonoff, '31), and King and coworkers (Smith and King, '31). Also, Cox ('37) has outlined some of the important contributions in the isolation and identification of this nutrient. In connection with his studies of reducing substances in various tissues, Szent-Györgyi ('27) isolated a compound which ". . . was named by its protocol number 'C_{xii}' being the twelfth substance prepared and examined in my work on tissue oxidation and the function of the adrenal cortex." The use of the letter "C" was fortuitous and without implication that the substance was related to vitamin C. Since it appeared five years later that this compound was vitamin C it is of interest to discuss briefly some facts regarding its behavior in animal and plant tissues.

Food chemists had for many years relied upon the guaiacum reaction as a test to determine whether a substance, *e.g.* milk, or fruit juice, had been heated. If to a small amount of a solution of gum guaiac in water a few drops of hydrogen peroxide is added, the guaiaconic acid present is not oxidized. If now a few drops of raw potato juice, or other unheated vegetable juice, or raw milk is added, the hydrogen peroxide is decomposed, with the formation of active oxygen, which oxidizes the guaiaconic acid, and the solution turns blue-green. The enzyme decomposing the hydrogen peroxide is called peroxidase. If the juice has been heated above 78° C. the peroxidase is destroyed. Szent-Györgyi was the first to possess the acumen to observe that when this reaction is carried out using a solution of purified peroxidase plus a few drops of an alcoholic solution of gum guaiac and a few drops of 0.01 N hydrogen peroxide, the development of the blue-green color is immediate. When the same reagents are applied to the press juice of a plant which contains peroxidase, the reaction remains negative and no color appears. If, however, the quantity of hydrogen peroxide is gradually increased, after a certain amount has been added, the color suddenly appears and grows deeper with the further addition of peroxide. He demonstrated that the effect of natural juices was not due to inhibiting substances, as had been supposed, but to the consumption of the first active oxygen made available by another reaction. He was able to separate in crystalline form the substance which, in this reaction, takes precedence to the guaiaconic acid in using up active oxygen, the latter becoming oxidized only after the former has been used up in the oxidation process. The new substance had the empirical composition $C_6H_8O_6$. Since it was isomeric with the lactone of glycuronic acid it was believed to belong to the uronic acid group and was referred to as

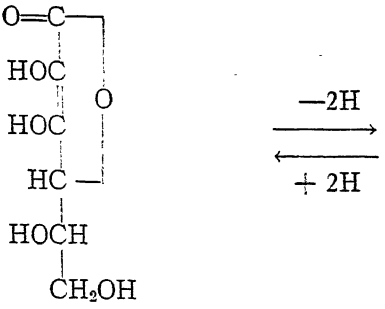
hexuronic acid (Szent-Györgyi, '28). At that time, 1928, Szent-Györgyi believed the substance to be associated with vitamin C but that it was not vitamin C itself. This view was shared by Zilva ('32).

In this country King and associates (McKinnis and King, '30; Smith and King, '31, l. c.) were actively engaged in investigations of relationships between vitamin C and "hexuronic acid." Finally on April 1, 1932, King and Waugh ('32) announced the isolation of vitamin C in crystalline form and stated that, "The recrystallized substance corresponds in chemical and physical properties to a hexuronic acid, and is apparently identical with the hexuronic acid described by Szent-Györgyi. . . ." On April 16, 1932, Svirbely (who had been recently associated with King in work on vitamin C concentration) and Szent-Györgyi ('32a) announced that they had, by means of 1 mg. of hexuronic acid daily, protected guinea pigs from scurvy during 56 days. But it was not until May 7, 1932, that they (id. '32b) claimed definitely the identity of hexuronic acid with vitamin C. These fundamental studies of Szent-Györgyi and of King et al. have been amply confirmed by other workers. It is of particular interest that Tillmans and associates ('32) pointed out the marked correlation between the antiscorbutic value of foods and their quantitative reduction of 2,6-dichlorophenolindophenol, the oxidation-reduction indicator widely used at present in the chemical determination of ascorbic acid.

Following identification of the important material, isolated independently by King and associates and Szent-Györgyi, attention was focused on the improvement of methods of concentration. Waugh, Bessey and King ('33) were able to secure 100 to 150 mg. of ascorbic acid per liter of lemon juice. Szent-Györgyi's discovery of paprika as a convenient source of the natural vitamin made it possible for him to prepare several hundred grams for the international cooperative study of its chemical nature.

Structural Formula of Ascorbic Acid.—The structural formula of ascorbic acid was determined by a series of brilliant investigations by Hirst, Haworth, and their coworkers (Cox, Hirst and Reynolds, '32), Karrer and associates (Karrer, Salomon, Morf and Schöpp, '33; Karrer, Schwarzenbach and Schöpp, '33; Karrer, Schöpp and Zehender, '33), von Euler and Klussmann ('33), and Micheel and Kraft ('33). Other investigators also contributed to this important work.

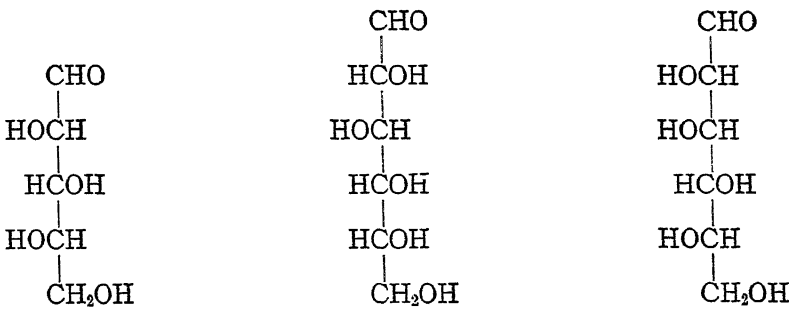
Among several formulae proposed, the following, along with its reversibly oxidized form, is now generally accepted as best accounting for all of the vitamin's properties:



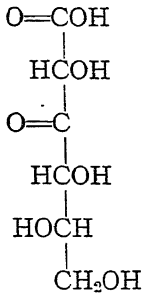
l-ascorbic acid (vitamin C) dehydroascorbic acid

Important evidence concerning the molecular structure was contributed by Herbert, Hirst, Percival, Reynolds and Smith ('33), who studied the behavior with oxidizing agents. The data indicated that the compound is related to l-gulose. The first oxidation product was 2,3-diketo-l-gulonic acid. Thus it was deduced that ascorbic acid is a reduced derivative of this lactone or 3-keto-l-gulonolactone. In order to determine whether the ring system, thus revealed, had a furanose or pyranose structure, methylated derivatives of ascorbic acid were studied. It was finally concluded that in solution the compound is the enolic form of 3-keto-l-lfurano-lactone.

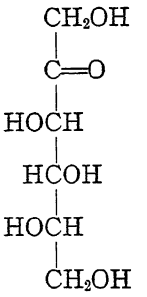
The sugars closely related to ascorbic acid, including l-glucose, are:



l-xylose d-glucose l-gulose

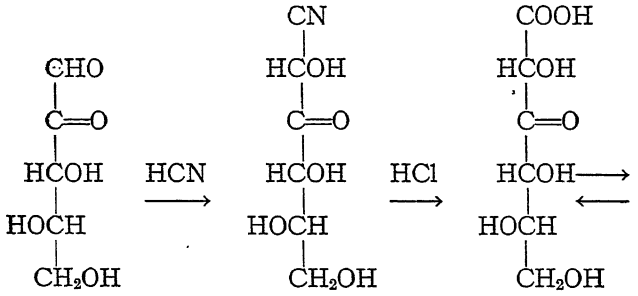


3-keto-l-gulonic acid

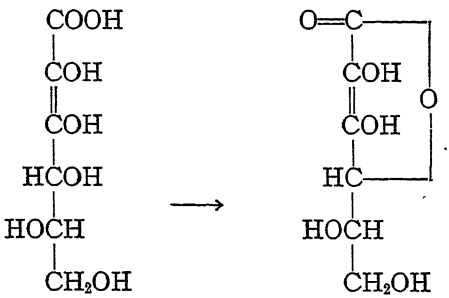


l-sorbose

Synthesis of Ascorbic Acid.—Before the structural formula as represented above had been fully established, Reichstein and coworkers ('33) announced the synthesis of ascorbic acid based on the following series of reactions:



l-xylosone — β-keto-nitrile — keto-acid



keto-acid (enol form) — l-ascorbic acid

Both the d- and l- forms of ascorbic acid were synthesized, starting from d- and l-xylosone respectively. Almost simultaneously Haworth and Hirst and their collaborators (Ault et al., '33), by a procedure similar to that employed by Reichstein et al., also synthesized both the d- and l- forms of ascorbic acid.

The English workers reported that the reaction involving the addition of hydrogen cyanide goes to completion in the course of a few minutes and almost simultaneously the keto-acid is formed by hydrolysis in the acid solution. This intermediate product is readily changed into the vitamin in the presence of dilute HCl at 40 to 50° C. Micheel and Kraft ('34) described a third method for the synthesis. The principal reactions involved are:

$$\text{d-glucose} \xrightarrow{H_2} \text{sorbitol} \xrightarrow{\text{B. } xylinum} \text{l-sorbose} \xrightarrow{\text{phenylhydrazine}}$$

$$\text{l-sorbosone} \xrightarrow{Br_2} \text{2-keto-l-gulonic acid} \xrightarrow{HCl} \text{l-ascorbic acid.}$$

The 2-keto-l-gulonic acid is readily converted into l-ascorbic acid by formation of the ethyl ester which is then hydrolyzed into the vitamin through the action of weak alkali, or better, salts of weak acids or weak tertiary bases such as pyridine.

A synthesis by Reichstein and Grüssner ('35) which, according to King ('36, l.c.), has been used in the commercial synthesis of ascorbic acid, is:

$$\text{d-glucose} \xrightarrow{H_2 \text{ and Pd}} \text{sorbitol} \xrightarrow{\text{B. } xylinum} \text{sorbose} \xrightarrow{\text{acetone}}$$

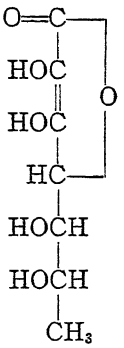
$$\text{diacetone sorbose} \xrightarrow{KMnO_4} \text{diacetone sorburonic acid} \xrightarrow{HCl}$$

$$\text{2-keto-l-gulonic acid} \xrightarrow{HCl} \text{l-ascorbic acid.}$$

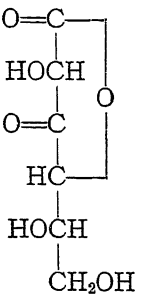
It is stated that the yield is 20 to 30 per cent from sorbose. Thus in the space of two or three years brilliant chemical work reduced vitamin C from the realm of recondite "little things" in nutrition to that of a relatively cheap sugar of known chemical structure and general properties.

Compounds Physiologically Related to Ascorbic Acid.—According to Reichstein, Schwarz and Grüssner ('35), the essential condition for antiscorbutic activity is the d- configuration of the fourth carbon

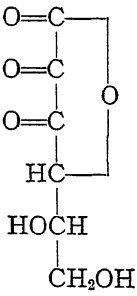
atom in ascorbic acid. Thus it was found that 6-methyl-l-arabo-3-keto-hexonic acid is active, having about one-fifth the activity of ascorbic acid. d-erythro-3-keto-hexonic acid (isoascorbic acid), which differs from ascorbic acid only in the steric arrangement of the groups on the fifth carbon atom, has about one-twentieth to one-fiftieth the activity of the natural vitamin (Dalmer and Moll, '33). Dehydro-ascorbic acid has approximately one-fourth the antiscorbutic potency of ascorbic acid in the reduced form. Structural relationships between these compounds are as indicated:



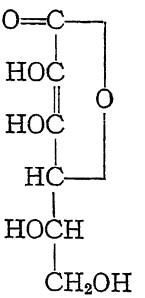
6-methyl-l-arabo-
3-keto-hexonic acid



d-erythro-
3-keto-hexonic acid



dehydro-ascorbic
acid



l-ascorbic acid
(vitamin C)

Some of the compounds which are inactive are: d-ascorbic acid, d-gluco-ascorbic acid, and d-galacto-ascorbic acid.

The physiological significance of these relationships has not been determined, but in the case of dehydroascorbic acid it appears that the activity is due to the presence in blood of an enzyme capable of converting the oxidized vitamin into reduced ascorbic acid, which is the form in which the vitamin is believed to exist in the blood (Roe and Barnum, 36).

Chemical and Physical Properties of Ascorbic Acid.—In the relatively few years that ascorbic acid has been available in pure form, a considerable amount of information has accumulated concerning its chemical and physical properties. It is freely soluble in water and somewhat soluble in acetone and methyl, ethyl, and propyl alcohols. It is insoluble in ethyl ether and petroleum ether. Salts are formed with the various cations. For example, basic lead acetate was extensively used several years ago as a means of concentrating the vitamin. At present the sodium salt is frequently used for parenteral injections.

The compound forms colorless crystals whose melting point is 192° C. the rotation is $+24°$ in water and $+48°$ in alcohol. There is no mutarotation as is characteristic of somewhat related sugars. The absorption maximum is 260 mμ in water and 263-265 mμ in alcohol.

The first dissociation constant has been determined at various ionic strengths. According to Ball ('37) the true dissociation constant is pK 4.21. pK_2 is said to be 11.57.

Outstanding in properties is the oxidation-reduction of ascorbic acid. It is the reductant of a thermodynamically reversible system and sluggish in electro-motive activity. As stated by Ball, this sluggishness may account for the diversity of opinion with regard to its oxidation-reduction behavior. Borsook and associates ('37) claim that only the first oxidation, ascorbic acid $\rightleftarrows$ dehydroascorbic acid, is physiologically reversible and significant in its antiscorbutic action. It is interesting at least that ascorbic acid, in non-aqueous media, is almost completely without reducing action.

At pH 7.0 and below the vitamin is not autoxidizable, but this occurs in alkaline solutions and increasingly as the pH is raised (Barron, DeMeio and Klemperer, '36). This form of lability is of much physiological significance and it is plausible to expect that various factors, both *in vivo* and *in vitro,* greatly influence the oxidation-reduction of this substance. Among the metallic salts tested (Mn, Ni, Fe, Co, Ca, and Cu) copper is the only catalyst for the oxidation of ascorbic acid, its action being detectable in concentrations as small as 46 micrograms of copper per liter. The catalytic effect is completely inhibited by 0.001 M KCN, and to the extent of 80 per cent by mixtures of $CO:O_2$ of 95.5. This marked effect of copper

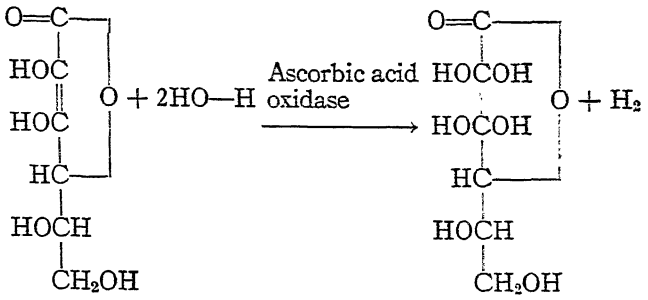
can be controlled by means of certain compounds such as metaphosphoric acid, diethyldithiocarbamate, 8-hydroxyquinoline, glutathione, and other substances which form copper complexes (Lyman, Schultze and King, '37).

The oxidation product obtained with aqueous iodine undergoes further oxidation with alkaline sodium hypoiodite to yield, quantitatively, oxalic and trihydroxybutyric acids. Another name for the latter substance is l-threonic acid. Ascorbic acid reacts with ozone, two atoms of oxygen being added, with the formation of an alpha-oxalyl derivative of 3,4-dimethyl l-threonate.

In both plant and animal tissues, enzymes play a prominent role in the oxidation-reduction functions of ascorbic acid. The compound, in its dehydrogenated state, is reduced in minced muscle, liver, or kidney which has been properly buffered. Szent-Györgyi ('28, l.c.) was the first to demonstrate the presence in plant tissue of an enzyme which specifically catalyzes the oxidation of ascorbic acid. He pointed out that this enzyme, in the absence of ascorbic acid, is without action on glutathione. However when the former is present the latter is readily oxidized. Hopkins and Morgan ('36) amplified this observation and found that when ascorbic acid and glutathione are together in the presence of Szent-Györgyi's "hexoxidase," the latter wholly protects the former from oxidation to dehydroascorbic acid. In this system the glutathione is oxidized at a rate which, with the same concentration of enzyme, is exactly similar to that when ascorbic acid is oxidized alone. Only when the reduced glutathione has practically disappeared from the system does the oxidation of ascorbic acid begin.

When ascorbic acid has been reversibly oxidized to dehydroascorbic acid, its reduction by pure glutathione is a very slow process; but in the presence of the enzyme, under proper conditions, the reduction may be five times as fast as the oxidation induced by the same concentration of the enzyme without glutathione. Glutathione also completely protects ascorbic acid from oxidation by copper catalysis. From these data it appears that both ascorbic acid and glutathione, the two most conspicuous and, so far as is known, the most active reducing substances in living tissues, play important roles in biological oxidation-reductions.

Tauber, Kleiner and Mishkind ('35) have isolated an extremely powerful enzyme from the pericarp of the Hubbard squash, which differs considerably from the one described by Szent-Györgyi. It oxidizes ascorbic acid instantaneously and completely without the interaction of other catalytic agents. Molecular oxygen of the atmosphere serves as hydrogen acceptor, and the oxidation process is visualized as an introduction of two OH groups at the double bond:



Reduced form of ascorbic acid

Oxidized form of ascorbic acid

It is of much interest that the oxidase appears to be absent from mammalian tissues (Tauber, '36-37), while at least the blood cells and plasma of humans and rats contain an enzyme that reduces dehydroascorbic acid. The latter enzyme, it will be observed, has the opposite effect from the ascorbic acid oxidase of plant tissues. Cucumbers contain the oxidase, but it is absent from the juice of orange, lemon, tangerine, and grapefruit. It is in part due to this absence of ascorbic acid oxidase that the antiscorbutic value is preserved on storage, but there is also a stabilizing effect of the relatively low pH of these fruits. Tauber takes issue with Sharp ('36) who attributes the rapid destruction of ascorbic acid in milk to the presence of the oxidase. Tauber could not detect appreciable oxidase action in milk. He attributes the ascorbic acid destruction to the presence of traces of copper.

Visible light is said to have a markedly destructive action on the vitamin. Even light which passes through a milk bottle causes a rapid destruction. Whether this effect is related to the presence of copper or other catalysts is not clear.

In view of the above it is understandable that ascorbic acid is readily destroyed during the ordinary cooking of foods. However in an inert atmosphere it is quite stable to moderate heat, although the reaction might be alkaline. But in the presence of oxygen, destruction is rapid and complete when the pH is above 7 and heat is used. The vitamin is more stable to oxygen in acid media, hence it undergoes less destruction in foods containing free acid such as citrus fruits, tomatoes, etc., than in less acid foods. Modern commercial processes of canning foods prevent marked ascorbic acid destruction by removing much of the air from food before it is sterilized in the cans by heat. The process of drying foods tends to destroy much of the vitamin.

Chemical Methods of Estimating Ascorbic Acid.—Until 1932 biological assays were the only methods of estimating the vitamin C content of materials. In that year Tillmans and associates ('32, l.c.) developed a chemical procedure based on the quantitative reduction of an oxidation-reduction indicator, 2, 6-dichlorophenolindophenol.

Dye Indicator Method: The essential features involve a preliminary extraction of the material to be assayed with trichloracetic acid and titration of the acid extract with the indicator. But as pointed out by King ('36, l.c.) three hazards are particularly involved in titrations of this type. These are: "(a) other substances may be present which reduce the titration reagent; (b) a portion of the vitamin may be present in the reversibly oxidized form, and (c) substances may be present which interfere with the reaction of either the oxidizing or reducing agent."

Each year since the discovery of this method has seen the publication of numerous attempts to improve its usefulness, as well as to supplant it by other procedures. Van Eckelen and Emmerie ('36), in numerous papers, claim that preliminary precipitation with mercuric acetate is necessary for specificity in titration. They also insist that preliminary treatment with hydrogen sulfide is necessary to reduce any oxidized vitamin present to the reduced form. Many workers, however, feel that these procedures are not advantageous. Recently metaphosphoric acid has been used successfully for the extraction of ascorbic acid. By this means ascorbic acid oxidase activity is inhibited and the interference of copper is prevented. In brief, useful information can be obtained by this method and in spite of various criticisms the procedure has not been superseded by anything more satisfactory for most purposes.

Furfural Colorimetric Method: This procedure, devised by Roe ('36), is based on the determination of furfural formed by boiling an acid extract of a tissue in which the ascorbic acid has been oxidized by passage through norite, with HCl alone and with HCl containing $SbCl_3$. It is claimed that the method is applicable to both plant and animal tissues.

Microchemical Method: Glick ('35) has improved a microchemical procedure described by Birch, Harris and Ray in 1933 which makes possible the extraction and titration of ascorbic acid with considerable precision in amounts as small as 0.001 mg. of the substance. By this method he and his associates have been able to determine quantitatively the ascorbic acid content of histological sections of liver, adrenal, hypophysis, and other tissues. Moreover they have been able to estimate the concentration of ascorbic acid in various zones of the tissues, the relative number of different cells in these zones, and the ascorbic acid content per cell.

Ascorbic Acid Estimation in Living Tissues: It has been claimed by Rotter ('37) that the rate of decoloration of 2, 6-dichlorophenolindophenol, injected into the sole of guinea pigs, is dependent upon the amount of ascorbic acid in the tissues. From his experiments on human subjects and guinea pigs Rotter concludes that a decoloration time of about 5 minutes indicates saturation of the tissues with ascorbic acid and that more than 10 minutes indicates deficiency.

Methylene Blue Method: Lund and Lieck ('36) found that if a small amount of ascorbic acid is added to a solution of methylene blue and the solution is exposed to strong light the color will completely disappear in 30 seconds. The decoloration is promoted by low pH and the presence of sodium chloride. Estimation of serum ascorbic acid is conducted with a stock solution of KH_2PO_4, 9 gm.; NaCl, 2 gm.; and 0.004 per cent of methylene blue, in 100 cc. of water. It is stated that reaction to light is not brought about by glutathione, ergothioneine, creatine, creatinine, urea, adenine, guanine, hypoxanthine, xanthine, uric acid, cystine, phenol, and hemoglobin; nor do these inhibit the reaction.

Enzymatic Method of Estimating Ascorbic Acid.—It appears that the ascorbic acid oxidase method of Tauber, Kleiner and Mishkind ('35, l.c.) is more specific than any of the chemical procedures. The method is based on the specific oxidizing property of their oxidase for ascorbic acid. The technic consists in determining, by suitable procedure, the reducing power of a tissue extract before and after the action of the enzyme. The difference is a measure of the amount of ascorbic acid present. The enzyme, obtained from the Hubbard squash, does not affect phenols, glutathione, cysteine, or adrenalin. This would seem to be a promising method.

Biological Methods of Estimating Ascorbic Acid.—Owing to the marked progress in chemical and enzymatic methods of estimation, the biological procedures have passed their peak of usefulness with respect to ascorbic acid. There are needs, however, for the latter technics, particularly as checks on the reliability of the former methods. Coward ('38) has discussed in detail the various useful biological procedures.

In general, the biological method is based on a determination of the minimum dose of a foodstuff which will just afford complete protection of guinea pigs against symptoms of scurvy. Some of these signs are tenderness and swelling of the joints, a tendency to sit crouched up, and the assumption of a "face-ache" position of the head, cessation of growth, and loss of weight. A special application of the method is that devised by Höjer. It is based on the histological examination of the teeth of guinea pigs restricted to appropriate test diets. Since ascorbic acid deficiency causes marked and characteristic

changes in the teeth (see the subsequent chapter) the method has played a useful part in studies of ascorbic acid.

International Units.—Several years ago (1931) the International Vitamin Conference defined a unit of "the antiscorbutic Vitamin C" as "the Vitamin C activity of 0.1 cc. of fresh juice of the lemon." Harris and Ray ('33) found considerable variability in the potency of lemon juice and stated that the average amount of ascorbic acid was such that 1 mg. of ascorbic acid contained 21 I. U. of vitamin C. The mean concentration of ascorbic acid in lemon juice is about 0.64 mg. per cc., or 0.064 mg. per 0.1 cc. At present one International Unit is 0.05 mg. of l-ascorbic acid. Hence there is some discrepancy between the present standard and the old.

State and Function of Ascorbic Acid in Plant Tissues.—During the past few years considerable discussion has occurred over the state of ascorbic acid in plant tissues. Several workers, including Guha and Pal ('37) and Levy ('36), hold to the view that some of the vitamin is present in the combined state in certain plants. The principal basis of this conclusion is the fact that an increase in the amount of titratable ascorbic acid occurs on controlled heating of certain food materials. However, Stone ('37), Mack ('36), and others have convincingly shown that the effect is entirely attributable to the action of heat on ascorbic acid oxidase in such foods.

When plant tissues containing the oxidase are heated, under controlled conditions, the process inactivates the enzyme, thus preventing a conversion of some of the vitamin to dehydroascorbic acid. Since this latter product causes no reduction of 2,6-dichlorophenolindophenol it is evident that food materials containing the enzyme would cause less reduction of the dye if the oxidase were allowed to act on the ascorbic acid, as happens in the processes of extraction which do not employ heat or other procedures liable to inactivate the enzyme.

Considerable significance has been attached to the close correlation between photosynthetic functions of plants and the content of ascorbic acid in structures containing chlorophyll, on which photosynthesis is dependent. Randoin, Giroud and Leblond ('35) observed that guinea pigs became scorbutic when their only possible source of ascorbic acid was the colorless portions of plants, but animals receiving the chlorophyll-containing portions remained normal. Thus there seems to be a parallelism between the distribution of ascorbic acid and that of chlorophyll. Giroud et al. have shown also that in the growth of plants, ascorbic acid precedes the appearance of chlorophyll. Hence it would seem that synthesis of the vitamin is not dependent upon the action of chlorophyll. It is possible, however, that ascorbic acid is essential in photosynthesis.

It has been known for many years that sprouted grains are antiscorbutic. The ascorbic acid is formed almost simultaneously with the initiation of sprouting and quickly reaches a high concentration in all rapidly growing parts of plants. It seems probable, therefore, that the vitamin is essential in the growth processes and before these can occur it may be necessary for ascorbic acid to be synthesized.

Practically all of the ascorbic acid in plants appears to be present in reduced form.

Species-Differences in the Ability to Synthesize Ascorbic Acid.— A source of confusion in the early experimental studies was the failure of rats to become scorbutic when restricted to a diet which caused death from scurvy in guinea pigs. Finally it became evident that a marked species-difference existed with respect to the need for this vitamin. It was shown that rats are able to synthesize the substance in considerable amounts, since scorbutic guinea pigs could be cured by feeding them small amounts of liver from rats restricted for many weeks to a diet free from the vitamin (Parsons, '20). Of the various vitamins known at present none are characterized by such large species-differences in requirements as in the case of ascorbic acid. In fact it appears that a majority of the animal kingdom is readily capable of synthesizing this factor. The only species known definitely to require a dietary source are man, guinea pigs, and monkeys. Rats, dogs, prairie dogs, pigeons, chickens, ducks, geese, turkeys, guinea fowl, and pheasants do not require the vitamin in their diet. The status of mice, rabbits, swine, and cattle in this regard is not clear. Kleiner and Tauber ('36) have observed that mice grow at a subnormal rate and show abnormal behavior when the diet is deficient in ascorbic acid. However, these latter symptoms eventually disappear. It appears that rabbits on an ascorbic acid-deficient diet fail to remain in good health but they do not develop typical scurvy (Findlay, '21). The data are confusing regarding swine. Hughes and associates ('28) claim that pigs thrive without any obvious source of the vitamin, but Zilva and coworkers ('24) state that they have produced scurvy in such animals. According to Thurston, Eccles and Palmer ('26), calves also apparently do not require ascorbic acid in the diet. It would be of interest to study the question of species-differences in other animals, particularly in the various sub-human primates. On the basis of present data it may be tentatively assumed that mice, rabbits, swine, and cattle need some ascorbic acid in the diet. It is possible that they are able to synthesize the vitamin to a limited extent.

Apparently there is no sharp distinction between different species as respects their needs for a dietary source of ascorbic acid. On the basis of body weight, guinea pigs seem to require proportionately

much more ascorbic acid than monkeys or children. It is evident that the phylogenetic aspects of ascorbic acid synthesis and bodily requirement are in need of being further clarified.

Biosynthesis and Origin of Ascorbic Acid in Nature.—Various attempts to determine the mechanism of ascorbic acid formation in plants and certain animals have resulted in scarcely any clarification of the question. Ray ('34) studied ascorbic acid formation at various stages in the germination of seeds. Various hexoses were used in the culture medium and of these mannose seemed to serve as the most effective precursor, since the largest amount of ascorbic acid seemed to be formed when it was employed. The significance of mannose has been further indicated by the various studies of Guha and Ghosh ('36) who have investigated ascorbic acid synthesis by *in vitro* and *in vivo* experiments. They claim that ascorbic acid is formed when embryonic guinea pig tissue and rat liver, kidney, or spleen are incubated with mannose. Solutions of other hexoses were not effective. Moreover they claim that parenteral injection of mannose causes an increase of the ascorbic acid content of rats. But neither von Euler, Gartz and Malmberg ('35) nor Kleiner and Tauber ('36, l.c.) were able to confirm these findings. Until the studies of Ray, and Guha and Ghosh are strengthened by more convincing evidence the conclusion cannot be changed that neither the site of synthesis nor the precursors of ascorbic acid are known.

Studies have been made of the effect of various dietary imbalances and deficiencies on the concentration of ascorbic acid in tissues of the rat, it being assumed that such dietary manipulations might interfere with the formation of this vitamin and thus suggest the nature of its precursors and the site of its synthesis. Hopkins and Slater ('35) found that during fasting, or on a diet very low in carbohydrate, the content of ascorbic acid in the liver falls, while there is an increase in that of the intestines. They cautiously suggested that the vitamin is synthesized in the intestinal epithelium and that in the absence of carbohydrate, protein or fat may serve as sources of the precursors. The hypothesis was flatly denied by Zilva ('36) on the grounds that it was not supported by adequate evidence. Svirbely's ('36) conclusions tend to support those of Hopkins and Slater, but it would seem that Zilva's opinion also applied to his interpretations. Zilva reasons that the increased concentration of ascorbic acid in the intestinal wall ". . . could just as well be assumed to be due to the transfer of the vitamin from the other parts of the body to the organ in question. . . ." On the basis of these studies, as well as those which cannot be mentioned, owing to lack of space, it must be stated that scarcely anything is known concerning the precursors of ascorbic acid and the site of its synthesis.

Distribution of Ascorbic Acid in Tissues.—Ascorbic acid appears to be present in all living tissues. Animals which do not require a dietary source of the vitamin synthesize it in relative abundance, thus indicating that this substance is of fundamental significance in the metabolism of all living cells. The concentration tends to be highest in glandular tissues and lowest in muscular and fatty tissues. The order of concentration is somewhat as follows: adrenals, pituitary, corpus luteum, thymus, pancreas, liver, spleen, testes, ovaries, brain, thyroid, submaxillaries, intestines, heart, kidneys, lungs, muscles, and adipose tissue.

The approximate ascorbic acid content of some human tissues, as determined by Yavorsky, Almaden and King ('34), is given in the following tables:

ASCORBIC ACID CONTENT IN DIFFERENT AGE GROUPS

(Mg. per Gm.)

AGE GROUP . . .	1–30 days	1–12 mos.	1–10 yrs.	11–45 yrs.	46–77 yrs.
No. OF CASES . .	11	9	11	17	19
Adrenal	0.581	0.525	0.550	0.393	0.230
Brain	0.460*	0.189*	0.433*	. . .	0.110*
Pancreas	0.365	0.304	0.225	0.152	0.095
Liver	0.149	0.148	0.163	0.135	0.064
Spleen	0.153	0.112	0.157	0.127	0.081
Kidney	0.153	0.122	0.098	0.098	0.037
Lung	0.126	0.057**	0.058	0.065**	0.045**
Heart	0.076	0.049	0.042	0.042	0.021
Thymus	0.304	0.319	0.190	. . .	0.046*

* Average of two specimens only
**Average of six specimens only

TYPICAL INDIVIDUAL VARIATIONS IN ASCORBIC ACID CONTENT OF HUMAN TISSUES

(Mg. per Gm.)

AGE	ADRENAL	PANCREAS	LIVER	SPLEEN	KIDNEY	HEART
14 days . . .	0.913	0.237	0.244	0.126	0.200	0.076
Still-born . .	0.745	0.343	0.110	0.184	0.099	0.042
24 days . . .	0.059	0.056	0.078	0.018	0.047	0.020
3 mos. . . .	1.300	1.000	0.238	0.188	0.305	0.117
11 mos. . . .	0.084	0.080	0.068	0.048	0.034	0.017
5 yrs. . . .	1.030	0.274	0.234	0.161	0.145	0.078
5 yrs. . . .	0.191	0.087	0.114	0.124	0.067	0.049
64 yrs. . . .	0.076	0.177	0.276	0.272	0.040	0.053
55 yrs. . . .	0.033	0.022	0.029	0.023	0.022	0.027
70 yrs. . . .	0.027	0.022	0.018	0.012	0.011	0.017

DEPLETION OF ASCORBIC ACID FROM GUINEA PIG TISSUES

DIET	ADRENAL	LIVER	KIDNEY	NO. OF ANIMALS
	mg. per gm.	*mg. per gm.*	*mg. per gm.*	
Basal diet + spinach	0.70	0.10	0.08	8
Basal diet, ascorbic acid-free, 15 days (beginning to lose weight)	0.08	0.03	0.03	6
Basal diet, ascorbic acid-free, 28 days (severe scurvy)	0.03	0.01	0.01	6

It will be noted that marked age differences appear to exist. Whether these are physiologically significant or only referable to differences in nutritional status is not known. Yavorsky et al. noted that patients with a history of inadequate dietary ascorbic acid had correspondingly low amounts of the substance in their tissues.

Biskind and Glick ('36), in an important series of histochemical investigations, have estimated the distribution of ascorbic acid within certain organs, using a method sensitive to ± 0.001 mg. of the vitamin. Their technic permits a study of various portions of an organ. According to their findings the pars intermedia of the pituitary contains the highest concentration of any tissues on record, being about 1.5 times that of the adrenal cortex. Cow's corpus luteum contained about 1.4 mg. of ascorbic acid per gm. of tissue when the organ was most fully developed and the value fell to 0.3 mg. per gm. with regression. Such studies are useful as means of indicating the functions of cells and their interrelationships. The general technic appears to be considerably more reliable than the silver nitrate stain for ascorbic acid in tissues.

Vitamin P.—The evidence concerning vitamin P does not warrant much discussion. This principle was first described by Szent-Györgyi and associates in 1936 (Bentsáth, Rusznyák and Szent-Györgyi, '36). According to these workers pure ascorbic acid is not effective in decreasing the permeability of capillaries in scurvy. But, they state, extracts of Hungarian red pepper or lemon juice are beneficial. On fractionating lemon juice they were able to isolate a crystalline flavone, "citrin," which was responsible for the decrease in capillary permeability. Hence the name "vitamin P" was chosen for this factor. Studies of the substance in several different clinical conditions, as well as in scorbutic guinea pigs, led Szent-Györgyi and coworkers to conclude that experimental scurvy is due to a combined deficiency of ascorbic acid and vitamin P.

However it is not generally conceded that the indispensability of the principle, vitamin P, is established. Zilva ('37) was unable to demonstrate any beneficial effect of this factor in delaying the onset of scurvy or the fatal termination of that disease in guinea pigs. Several observations in this country of the maintenance of apparently good health over long periods of time in guinea pigs receiving pure ascorbic acid and no source of vitamin P suggest further that the evidence is still in question regarding the necessity of a "vitamin P" for the maintenance of proper capillary permeability.

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CHAPTER

XVII

Nutritional Significance of Ascorbic Acid (Vitamin C)

IN THIS DECADE of synthetic ascorbic acid and rapidly expanding knowledge of its physiological significance, it is somewhat difficult to understand how our ancestors were so slow in recognizing the preventives of scurvy—fresh fruits and vegetables. The fault was not utter lack of knowledge since Bachstrom in 1734, according to James Lind (1753), positively asserted that fresh fruits and vegetables are "alone effectual to preserve the body from this malady." But the erroneous theories of scurvy were intrenched and not easily changed, since the concept of specific dietary deficiencies as causative factors in certain diseases did not exist except in a very vague fashion.

Perhaps the most readily available history of the disease is the admirable book by Hess ('20). But the symptomatology of scurvy and the great waste of life and health occasioned by that "calamity of sailors" were clearly set forth by Lind in 1753. Lind deserves the credit for having established, by many observations, that scurvy can be prevented or cured by the provision of fresh fruits or vegetables in the diet. Although his dramatic experiments were made in 1747 it was not until 1795 that the British Navy began the regular administration of lime juice to the sailors and it was as late as 1865 that the British Board of Trade adopted this life-saving regulation.

Expeditions and armies, as well as sea-farers, have suffered great losses from scurvy. A notable instance was the voyage of Jacques Cartier in 1536 (Bigger, '24). While exploring the St. Lawrence River he lost 26 of his party from scurvy, but saved the remainder by means of a pine needle infusion recommended by the Indians of that region, as a specific remedy against scurvy.

But it was not until the present century that scurvy was studied in animals. In 1907 Holst and Frölich found that guinea pigs become

scorbutic on a diet of oats and bran. The immediate advances made by this important discovery will not be recounted here since they are accessible in various reviews (Sherman and Smith, '31; Waugh, '34). The synthesis of ascorbic acid, which has made it available in pure form and at low cost, and the development of chemical methods of estimation, have contributed to the accumulation of many studies on the physiological significance of this nutrient. A discussion of various aspects of this subject will be the purpose of this chapter.

Effects of Ascorbic Acid Deficiency.—In humans a deficiency of ascorbic acid scarcely ever occurs unaccompanied by other inadequacies or dietary imbalance. These tend to produce variability in the symptoms referable to scurvy but in general it may be stated that scurvy has the following characteristics: The adult loses weight, is anemic, weak, and short of breath. The gums become swollen, bleed easily, and frequently ulcerate. The teeth loosen and may drop out. Necrotic areas in the jaw bones may occur. Hemorrhages into the mucous membrane and skin are characteristic. Blue-black spots develop in the skin after trivial injury, or they may occur spontaneously. The ankles become edematous, and in severe cases there develops a hard boardlike condition of the skin and subcutaneous tissues. Nervous symptoms of various types may appear. Children and infants are fretful, anemic, and without appetite. They fail to grow satisfactorily and exhibit vague evidences of illness.

These general symptoms, alone, are only vaguely suggestive of the role of ascorbic acid in bodily processes. Since the histopathologic effects of the deficiency are more important for this purpose, greater attention will be given to them here. The present information has been critically discussed by Wolbach ('37) who has contributed much of the data on this subject. In brief, ascorbic acid functioning is particularly concerned with cells of mesenchymal origin. This nutrient is necessary for the formation of all intercellular substances having collagen, or collagen-like substance, as their basis. Thus the characteristic hemorrhages of scurvy are attributable to the inability of the body to produce intercellular material. The latter is necessary to prevent separation of the single layer of endothelial cells which form the walls of blood capillaries. It is the cementing substance which holds these cells together. This intercellular material constitutes the foundation of all fibrous structures, the matrices of bone, dentin, cartilage, and all non-epithelial cement substance, including that of the vascular endothelium.

This general concept of ascorbic acid in relation to specific types of cells was reached through studies of human scurvy, which may have been accompanied by other dietary defects, and experimental scurvy in guinea pigs. The latter probably was unassociated with any

other dietary defects. It will be profitable, therefore, to describe the principal pathologic findings in such structures as are notably affected by ascorbic acid deficiency. These are teeth, bones, and blood vessels.

Teeth: As early as 1919 it was shown experimentally by Zilva and Wells that radical changes in tooth structure are associated with deficiency of the antiscorbutic nutrient. Howe (1919) also investigated dental changes in this deficiency, being primarily concerned with studies of the cause of dental caries. However, it was the extensive and classic work of Höjer ('24) which revealed in detail the pathologic effects of ascorbic acid deficiency on the teeth. A summary of the changes he observed, stated in his own words, is as follows:

1. The gradual change of the odontoblast layer. This seems to be a sure sign of scurvy and one of the earliest changes to set in. The odontoblasts assume another shape, grow shorter, more rounded, and show another arrangement, the normal regular, creeper-like formation makes way to a layer, which in some places curves in towards the center of the pulp and is soon split through the cells secreting a hard tissue between themselves, so that the syncytial character of the cell layer does not plainly appear. The odontoblasts have been transformed into osteoblasts placed in bone canals. . . .

2. The amorphous calcification of the predentine.

3. Widening of Tomes' canals in the dentine before the onset of scurvy.

4. New formation of bone instead of dentine. This bone is first lying as a thin layer inside the calcified predentine, but soon extends reticularly towards the center of the pulp. This bone has a spongy, porous character.

5. Dilation of vessels and in early stages hyperemia; sometimes hemorrhages in the pulp.

6. Atrophy and resorption of pulpa tissue, pulpa cells, the newly formed bone and the old dentine. This resorption which, as everywhere in bone formation, will surely be found also with scurvy in all stages, appears more distinctly after all the new bone formation in the final stage has stopped, and may proceed so far, that in place of pulpa tissue there is seen nothing but some large hollows filled with fluid.

7. In the healing of the scurvy—at an earlier stage—reorganization of the pulpa bone into irregular dentine, osteodentine, with bone canals and dentinal canals.

8. In a scurvy which is latent all through or very much mitigated, when at least half the amount of antiscorbutic needful to an individual is provided—forms which are the most common in man—the progress is similar, though not so pronounced and presents pictures that differ considerably less than the normal. If the antiscorbutic dose provided is 0.5-0.7 of the minimum protective dose, an irregular dentine is formed with Tomes' canals in most places, but in the lingual part of the pulp there are found in some symmetric places ridges of a hard tissue, with the character of bone and lacking dentinal canals. In this pulpa bone as

well as in the newly formed irregular dentine, there are canals of Havers' type and isolated bone corpuscles which may be considered to consist of transformed odontoblasts.

With an antiscorbutic dose of 0.8 or more of the minimum protective dose, the picture here described is changed so far that there is no pulpa bone, but all the newly formed hard tissue in the pulpa consists of osteodentine, or even dentine (the first formed layer).

Thus Höjer believed that the characteristic and basic effects of the deficiency was a development of active osteoblasts from odontoblasts, resulting therefore in the production of bone instead of dentin. This view was not questioned by Wolbach and Howe ('26) but they were unable to confirm the dental changes except by alternate feeding of deficiency and complete diets. When their diets were very deficient, formation of "osteodentin" or pulp bone, as reported by Höjer, did not occur.

Wolbach and Howe's diet consisted of autoclaved soy beans 50, rolled oats 29, dried milk powder (Klim) 10, brewer's yeast 4, butter fat 5, calcium carbonate 1, and sodium chloride 1, parts respectively. Filter paper was included as roughage. Guinea pigs were reared from weaning to 3 or 4 years of age when they were given this diet supplemented with 8 cc. daily of orange juice. Teeth of guinea pigs restricted to the deficient ration underwent dramatic changes. It was noted that after 7 days the odontoblasts had begun to separate from the dentin; also, their form had begun to change. After 12 days extensive separation had occurred and vacuoles were to be seen between the dentin and odontoblastic layer. This separation was practically complete at the end of 14 days. In addition, the Tomes fibrils, processes which extend from the odontoblasts and permeate the Tomes canals of the dentin, had been ruptured. At this stage, if the animal is given orange juice (ascorbic acid), and is killed after 72 hours, newly formed dentin is present. After 7 days of recovery the newly formed dentin is more extensive, and is irregularly traversed by Tomes fibrils, which arise through regeneration and are not continued into the old dentin. As stated by Wolbach ('37, l.c.), "In guinea pigs, dentin formation is resumed in volume and rapidity suggestive of the jelling of a liquid material between pulp and old dentin."

A theory to account for the action of ascorbic acid on dental structures has been advanced by Fish and Harris ('34). It considers that the nutrient is primarily needed to promote the functional activity of the formative cells, i. e., odontoblasts, ameloblasts, osteoblasts, etc. The concept of Höjer is rejected that odontoblasts change their function and revert to osteoblasts. They emphasize that in severe degrees of ascorbic acid deficiency during tooth development the

ameloblasts (enamel formers) may actually disappear or become badly impaired. This causes serious defects in the enamel formed at the time of the deficiency. Since the defects are irreparable the tooth remains in constant danger of decay at the site of these weak areas.

Bones: Gross and histological effects of deficiency observed in bones are basically similar to those in other structures and tissues, namely, a failure in the proper functioning of intercellular material. Rarification of the long bones occurs and this, naturally, is frequently accompanied by fractures, especially at the epidiaphyseal junctions in growing bones. In young guinea pigs deprived of ascorbic acid the first demonstrable bone change is seen at the epidiaphyseal junctions and it is characterized by an increase in the number of osteoblasts applied to the cartilage columns. As Wolbach ('37, l.c.) has written, "Formation of cartilage and bone matrices ceases, and the osteoblasts become elongated, assume the shapes of fibroblasts and migrate toward the diaphysis. Here these cells become surrounded by liquid, presumably a deficient product of continued activity toward matrix formation, and give rise to an apparent region of edematous connective tissue at the ends of the diaphysis, the Gerüst Mark (framework marrow) of German authors."

As the deficiency progresses resorption of bone matrix occurs. This causes profound changes in the epiphyseal cartilage. Since periosteal bone formation also ceases, trabeculae of the spongy bone fail to maintain a sufficient degree of attachment with the epiphyseal cartilage. Consequently fractures occur between the epiphysis and diaphysis. Movement occurs between epiphysis and diaphysis, resulting in a "Trümmerfeldzone." The periosteum becomes separated from bone cortex owing to the continued proliferation of osteoblasts of the periosteum. Hemorrhages occur in these tissues, forming, in addition to the cellular changes, a characteristic pattern. These changes are most striking at the costochondral junctions. Here, as in other bony structures, the osteoblasts increase in number but no intercellular substance is formed. During this period there is resorption of bone salts, thus accounting for the rarification.

Dramatic reparative processes quickly take place when ascorbic acid is restored to the diet. Newly formed intercellular material appears within 24 hours as the osteoblasts resume their role in the formation of this material. Capillary formation, which is essential to the deposition of bone salts, is resumed and other restorative processes continue.

These histopathologic evidences of bone changes are substantiated by X-ray examination and gross inspection. Park and associates ('35) have made important contributions to our information on the clinical aspects of ascorbic acid deficiency, particularly with reference to its

recognition by means of X-ray changes. These studies indicate that insufficient attention has been given to the effects of scurvy on bones. In view of the apparently widespread lack of adequate ascorbic acid in the dietaries of both children and adults it seems probable that careful study of the bones of such persons would reveal definite abnormalities attributable to a deficiency of this vitamin.

Blood vessels: It has been emphasized that the characteristic hemorrhages in various parts of the body are referable to mechanical weakness of the blood vessels and their supporting structures. This, in turn, is related to the reduced cohesion of the vascular endothelium. In ascorbic acid deficiency the "cement substance" is less effective in producing cohesion between the endothelial cells. Hence bleeding from the gums, and extravasations of blood into the skin and other tissues are attributable to the loss of this intercellular material necessary for the maintenance of tissue integrity. Since changes in the fragility of capillaries are somewhat amenable to objective measurement, various tests of "capillary fragility" have been employed to detect subacute degrees of ascorbic acid deficiency. These will be briefly discussed subsequently.

Other Tissues and Structures: Bessey, Menten and King ('33) have extended our knowledge of the pathologic changes in the organs of scorbutic guinea pigs. In the adrenals there occurs, in severe scurvy, a translocation of fat which, in the normal gland, is present only in the cytoplasm and is distributed as a film on the periphery of granules. This fatty material is unsaturated. It is transferred in droplets to the vessels which transport it to the medulla. The capillaries may be loaded with freed fat and if the animal survives, the fat may disappear from the cortical area. The cholesterol likewise disappears from this area. Coincident with the extreme lipid alteration in the adrenals, extensive fatty degeneration occurs in foci irregularly distributed in the myocardium. This begins by a shifting of the fat from the dark or anisotrophic band into the light band where it increases until droplets are formed. These become fused with loss of morphology of the myocardial cell. This, they infer, constitutes an irreversible change and forms the beginning of the typical patches of fatty degeneration like those seen in diphtheria. Similar fatty changes are observed in the skeletal muscles. There is also fatty infiltration of the liver in varying degrees of scurvy, and it is so marked that in the terminal stages the cells of an entire lobule are almost entirely composed of fat. The infiltration is associated with fatty degeneration which they regard as probably the primary lesion.

In severe scurvy they observed extreme degenerative changes in the testes, with death of the germinal epithelium and spermatozoa. They also described an edematous condition of the periportal connec-

tive tissues of the splenic septa and the media of the small arterioles of the lungs. In the lung, such vessels occasionally presented the appearance of obliterative endarteritis. They interpret these changes as primarily alterations in the colloidal state of the collagen, as postulated by Wolbach.

Effects of Ascorbic Acid in Massive Doses.—Although pure ascorbic acid has been available for about 4 years at reasonably low prices, the effects of this substance in massive doses have not been adequately studied. The present evidence indicates, however, that no harm is done although the quantities ingested may be many times the approximate daily requirement. Demole ('34) found that up to 5 gm. of the pure vitamin per kilogram in one dose were tolerated without symptoms by animals. Also, no histological changes were observed in the kidneys, liver, heart, and lungs. Likewise no changes or symptoms were noted in animals given 12 gm. per kilogram over a period of several days.

After the tissues become saturated with the substance excesses appear to be readily and quickly excreted unchanged in the urine. As shown by Everson and Daniels ('36) working with children of preschool age, retentions of ascorbic acid parallel the ingestion level only up to approximately 7.5 mg. per kilogram of body weight. Ingestions of 10 to 12 mg. per kilogram did not appear to augment the level of retention.

It is probably true that the body can excrete excesses of ascorbic acid without difficulty over long periods of time. However, information is needed regarding the effects of continuous high dosage of this substance. Is there any similarity between renal clearance of ascorbic acid and that of creatinine, xylose, urea, and other substances which have been investigated? Are there any species-differences in ascorbic acid tolerance, especially as related to animals which can synthesize the substance as compared with those which are unable to synthesize it? Does the continuous ingestion of ascorbic acid in large amounts cause any degree of structural injury in the kidneys, liver, or other organs and tissues of the body? These are some of the questions which future investigations should attempt to answer.

Factors Affecting the Retention and Excretion of Ascorbic Acid.—Various data indicate that retention of ascorbic acid will not occur when the body is "saturated" with this substance. "Unsaturated" tissues, however, have remarkable capacities for retention of the vitamin. Schultzer ('36) reports the interesting cases of three scorbutic patients who required 7.0, 9.5, and 14.4 gm. respectively of ascorbic acid to "fill" the tissues before additional ingestions of the substance resulted in significant renal excretion. By comparison, the daily adult requirement appears to be about 40 mg. All the evidence suggests that

the kidneys have a rather definite ascorbic acid threshold and that levels of ingestion beyond a certain limit, determined by various factors, result in rapid urinary excretion of the excess. Probably the blood level of ascorbic acid is the determining factor in its excretion since the value is usually below 0.4 mg. per cent in cases of ascorbic acid deficiency (Taylor, Chase and Faulkner, '36) and when liberal amounts of the vitamin are being ingested the level is 2 to 3 times that amount. According to van Eekelen ('36) saturation of the organism coincides with a certain level in the blood which is about 1.3 mg. per 100 ml. When this level is exceeded excretion in the urine results. Thus the retention seems to be somewhat similar to that of certain common freely diffusible blood constituents.

The ability of guinea pig and human tissues to retain ascorbic acid depends in some measure on factors other than the content of this substance already in the body. For example, Hawley and associates ('36) found that normal persons saturated with ascorbic acid excreted almost 100 per cent of daily test doses of the pure vitamin when ammonium chloride was administered with it. However the same dosage of vitamin, accompanied by sodium bicarbonate, was followed by an excretion of only approximately 50 per cent of ascorbic acid in the urine. When guinea pigs were given sufficient amounts of sodium bicarbonate to produce a highly alkaline urine, the ascorbic acid, fed in doses of 10 mg. per animal per day, was retained in significantly greater amount in the adrenals and liver than in the case of guinea pigs given the vitamin alone or with ammonium chloride. It may be significant also that Jacobsen ('35) noted a better retention of ascorbic acid in guinea pig tissues when cabbage was used instead of the pure vitamin. For example, the adrenals contained 18 mg. per cent of ascorbic acid when each animal was given 20 mg. of ascorbic acid per day for 3 months. When cabbage was fed, the value was 78 mg. per cent. We have no explanation for this apparent superiority of natural foods over synthetic material.

Acetylsalicylic acid (aspirin) (Daniels and Everson, '36) and ether (Bowman and Muntwyler, '37) also cause increased excretion of ascorbic acid. Although the effect appears to be of short duration there is justification for assuming that regular use of aspirin might necessitate an increased intake of ascorbic acid to balance the heightened urinary excretions.

Finally, the body's ability to subsist without injury during periods of deficient ascorbic acid intake is greatly limited. As shown by O'Hara and Hauck ('36), normal adult women restricted to an experimental diet furnishing approximately 5 mg. of the vitamin per subject per day soon showed evidence of ascorbic acid depletion. After only one or two days on such a diet the daily urinary output of the

vitamin fell to about 15 mg. After 4 weeks on the diet one subject developed swollen gums and other evidences of scurvy. These disappeared promptly when ascorbic acid, furnished by orange juice, was given. Approximately 2 to 3 gm. of the vitamin were required to resaturate the subjects. Thus the evidence accumulates that even temporary dietary restrictions of ascorbic acid, such as may occur in the treatment of certain diseases, *viz.*, gastric ulcers, may lead to dangerous consequences.

Relationships between Ascorbic Acid Intake and its Content in Milk.—Selleg and King ('36) supplemented the diets of lactating women with ascorbic acid and determined the effect on the ascorbic acid content of their milk, using the dye indicator titration method. The following table shows typical results:

Average Daily Ascorbic Acid Supplement	Number of Cases	Ascorbic Acid (mg. per cc. of milk)						
		Initial Values	Values Following Supplementation					
		Days Post Partum						
		3 to 6	5	6	7	8	9	10
None . . .	17	0.053	0.056	0.058	0.060	0.066	0.064	0.064
210 mg. . .	19	0.056	0.067	0.070	0.068	0.071	0.072	0.073
432 mg. . .	17	0.055	0.065	0.070	0.076	0.077	0.078	0.081

It was found that supplementing the diet with ascorbic acid causes a slow and limited rise in the antiscorbutic value of the milk. This reached a maximum value which is not increased by excessive dietary intake of the vitamin. The excess is rapidly excreted in the urine without disturbing the lactation level.

Selleg and King, reviewing the data of other investigators, conclude that the normal level of ascorbic acid in human milk is much higher than in cow's milk, the latter having a content of approximately 0.02 to 0.025 mg. per cc. Milk rapidly loses its antiscorbutic value. The average value for raw (unpasteurized) market milk is approximately 0.01 mg. per cc.

Riddell and coworkers ('35) have studied the effect of ascorbic acid supplements on the ascorbic acid content of cow's milk and have reviewed the literature on the subject. By means of both chemical and biological methods the milk of 74 cows fed a dry ration plus

silage contained 25.8 mg. of ascorbic acid per liter. The value for milk from cows on green pasture was 26.5 mg. One would infer from these data that the cow is able to synthesize ascorbic acid, since the dry ration would have provided very little of this substance. It also appears that the content found represents the maximum level of mammary secretion and that an excess of ascorbic acid in the ration is destroyed or excreted in the urine. It is generally accepted that pasteurization destroys practically all of the ascorbic acid in milk.

If the normal ascorbic acid requirement of a human infant may be estimated from the concentration in human milk, this may be considered, on the basis of an intake of 21 ounces daily, to be about 40 mg. per day. But, as shown by Selleg and King, the milk of certain women may contain as little as 0.019 mg. of ascorbic acid per cc. Infants dependent upon such milk for all their ascorbic acid probably would not receive enough. The significance of optimal ascorbic acid intake in lactating women is indicated by the findings of Yavorsky and coworkers, already cited, that the tissues of certain infants are very deficient in ascorbic acid. There is no evidence that human tissues, in the embryonic or infantile state, can synthesize ascorbic acid.

Functions of Ascorbic Acid in the Body.—In the previous chapter it was shown that ascorbic acid may be essential in photosynthesis although it is formed in sprouting seeds before chlorophyll can be detected. The present opinion is that the substance is an essential participant in cellular respiration and that a deficiency of this nutrient may disturb various enzyme systems. The fact that protozoa, bacteria, moulds, lichens, and algae give a positive ascorbic acid reaction, when tested with acetic acid-silver nitrate, suggests that vitamin C is distributed throughout all forms of life (Bourne and Allen, '35). If this is true, the very existence of living protoplasm may be dependent upon the presence of ascorbic acid.

The studies of Barron and associates (Barron and Barron, '36) are suggestive of an important role of ascorbic acid in blood formation and the maintenance of a physiologic level of erythrocytes in the circulating blood. When ascorbic acid was injected intravenously into rabbits simultaneously with cobalt sulfate, polycythemia failed to appear. But when the ascorbic acid was withheld it appeared in 6 or 7 days. Moreover, ascorbic acid tended to reduce polycythemia. Cobalt seems to produce polycythemia through the inhibition of the respiratory function of immature cells, thus resulting in their extrusion into the general circulation as mature non-respiring cells. Hence, a normal function of ascorbic acid may be the regulation of the level of red cells in the circulating blood even though cobalt is not present in abnormal amounts.

On the basis of evidence cited thus far ascorbic acid appears to

have essential roles in (a) cellular respiration and in (b) the formation and regulation of intercellular matrix. Whether these functions are mutually exclusive is not known but it seems plausible to assume that they are not. It may be that intercellular matrix formation and regulation is dependent upon the oxidation-reduction role of ascorbic acid. As summarized by Stotz and associates ('37) ascorbic acid can function as a hydrogen transfer agent in oxidation-reduction reactions because of: "(a) its great sensitivity to reversible oxidation, (b) its occurrence in practically all actively respiring tissues of plants and animals . . . , and (c) a certain amount of evidence that the vitamin can act directly upon recognized metabolites and enzymes *in vitro*."

As pointed out elsewhere (Chapter XVI), glutathione protects ascorbic acid against oxidation by oxygen and ascorbic acid oxidase. The mechanism of this reaction is that the oxidized ascorbic acid reacts with glutathione, thus causing the reformation of ascorbic acid (reduced) and the production of oxidized glutathione, this reaction being much faster than the oxidation of ascorbic acid. Ascorbic acid is regarded, therefore, as a coenzyme for the glutathione oxidation.

The available *in vitro* and *in vivo* studies do not clarify the role of ascorbic acid in oxidation-reduction much further than is stated here. Phillips, Stare and Elvehjem ('34) found that ascorbic acid deficiency in guinea pigs produced relatively little effect on the rate of oxygen uptake of liver. Stolz and associates reported the contrary, stating that the oxygen uptake of liver was very noticeably augmented with the onset of scurvy. The problem is one for further study.

Requirements for Ascorbic Acid.—Adequate evaluations of ascorbic acid requirements are dependent upon objective methods of determining the health status in relation to ascorbic acid ingestion. Defects in the determination of either the health status or the amount of ascorbic acid ingested make it impossible to evaluate the requirement. Although this simple truth is obvious it will bear emphasis.

Chemical methods of assaying foods for ascorbic acid have been made fairly reliable in recent years, and it appears that some of the tests of subacute ascorbic acid deficiency may be used to give indications of ascorbic acid requirements, such as capillary resistance, simple blood analysis, and "saturation" tests, the latter of which involve titration of urinary ascorbic acid before and after the ingestion of a standard quantity of ascorbic acid. But these are based on plausible assumptions. Since the amount of this vitamin in the blood of non-scorbutic persons is higher than in those with scurvy, it has been suggested that the content of ascorbic acid in the blood may be used as a measure of the health status of a person with respect to

this nutrient (Farmer and Abt, '35; and others). The saturation tests, originated by Harris and Ray ('35), serve a useful purpose in the diagnosis of subacute ascorbic acid deficiency, but their use in estimating requirements for the vitamin, other than for the prevention of scurvy, are not sufficiently refined for unequivocal acceptance. It is therefore impossible to discuss ascorbic acid requirements except in terms of broad generalities.

On the basis of capillary resistance methods, Göthlin ('34) and others have concluded that the average adult needs about 25 mg. of ascorbic acid daily to prevent the slightest objectively determined prescorbutic ill effects. On the basis of this figure Harris and associates ('35) have presumed that adults are not receiving even the minimal requirements if the urinary excretion of ascorbic acid, by titration, falls below 10 to 15 mg. per day. Van Eekelen ('36, l.c.), by means of a saturation test procedure which may not be very reliable, has concluded that an adult weighing approximately 70 kg. requires about 60 mg. of ascorbic acid per day in order to remain in good health. This value has been confirmed by Heinemann ('36) by means of a similar procedure. Since it appears that the dietaries of many adults who appear to be in good health furnish not more than 30 mg. of ascorbic acid per person per day it seems probable that 60 mg. is an amply safe figure for the adult requirement, since it is twice the approximately minimum requirement.

Harris and associates ('35, l.c.) tentatively concluded that the requirements of infants and children, calculated per unit of body weight, are greater than those of an adult. This conclusion is supported by Everson and Daniels ('36, l.c.) but there are not yet any data which unmistakably indicate the requirements for this age group. Everson and Daniels found that ascorbic acid retentions in children 3 to 5 years of age paralleled the ingestions only up to 7.5 mg. or thereabout per kilogram of body weight, but higher levels of ingestion caused no increase in the retention. Certainly it would seem that infants should receive at least one-third as much ascorbic acid as adults. Thus the minimum level for infants should not be less than 10 mg. Children over 9 years of age probably should receive as much ascorbic acid as adults.

Without doubt pregnant and lactating women need considerably more ascorbic acid than normal adults. Neuweiler ('35) has described ascorbic acid excretion studies on non-pregnant, pregnant, and lactating women which he conducted in order to estimate the requirements during pregnancy and lactation. As would be expected, it was necessary for the pregnant and lactating women to ingest considerably larger amounts of the vitamin in order to maintain an excretion level regarded as indicative of adequate vitamin intake. The data do not

offer sufficient evidence to warrant definite commitments regarding the requirements in absolute terms of ascorbic acid. However it was fully demonstrated that the needs during lactation are even greater than in pregnancy. On the basis of these data and others in the literature, it is probably safe to assume that pregnant women should ingest 75 to 125 mg. of ascorbic acid per day and that lactating women should take 100 to 150 mg. per day.

Data on the ascorbic acid requirements of guinea pigs may be of some value in considerations of human needs. According to Dann and Cowgill ('35), ". . . the vitamin C requirement of the guinea pig is directly proportional to the body weight, and is almost exactly 1 cc. of lemon juice per 100 gm." They conclude from their data that young, rapidly growing guinea pigs do not require a proportionately greater amount of ascorbic acid than the adult. This is at variance with the opinions cited above concerning human needs. Without doubt the usual conclusion applies here that further investigation is needed.

Estimation of Sub-acute Degrees of Ascorbic Acid Deficiency. —The discussion of dietary requirements has emphasized the need for reliable and useful means of estimating mild degrees of ascorbic acid deficiency. Various procedures have been proposed but none are fully satisfactory. The methods include measurement of capillary fragility, X-ray examination of bones, concentration of ascorbic acid in blood, urinary excretion of ascorbic acid, and "tissue saturation" or ascorbic acid "tolerance." The latter method is considerably more promising than the others although the blood level of this substance is undoubtedly a useful criterion of mild deficiency.

The simplest tests are those involving capillary fragility. They are based on the well-known fact that blood vessels are weakened by the lack of ascorbic acid and the degree of fragility seems to correspond somewhat to the degree of deficiency. Among the more recent improvements of the test is that described by Wright and Lilienfeld ('36). Göthlin ('33) and Dalldorf ('33) have contributed valuable information on capillary fragility. The general procedure used by Wright and Lilienfeld, and Göthlin, is the compression of arm veins by means of a rubber cuff around the arm in order to constrict the capillaries. The number of petechiae formed with a given pressure is regarded as a measure of the degree of ascorbic acid deficiency. Dalldorf's method is based on the production of negative pressure outside the capillaries by means of a suction cup pressed against the skin. Weld ('36) and others have criticized capillary resistance tests stating that they obtain great variations in response which in many cases certainly are not related to ascorbic acid deficiency.

X-ray examination of bones is not promising, although the bone

changes in scurvy are characteristic. The method is more expensive and probably less sensitive to slight degrees of deficiency.

Abt, Farmer and Epstein ('36) believe the concentration of ascorbic acid in blood appears to be closely related to the state of nutrition as respects ascorbic acid.

The urinary excretion of ascorbic acid alone is regarded by Abbasy, Harris, Ray and Marrack ('35, l.c.), and others, as a good indication of the ascorbic acid nutritional status, but they believe the use of test doses of ascorbic acid gives a much more objective and reliable indication of the nutritional status as respects this vitamin. It is generally agreed that the basis of this test is sound and that the results do agree with such other criteria as can be used. In the method of Harris and associates the ascorbic acid content of 24-hour samples is determined by titration. The subject is restricted to his habitual diet and given a test dose orally of pure ascorbic acid. For adults of average size 600 mg. are recommended. The amount for children is less, depending on their approximate body weight. Following this the urinary excretion is again determined and from the results a curve is constructed which indicates the rate and amount of ascorbic acid excretion. In subjects well nourished with respect to ascorbic acid the curve rises rapidly to a high level and quickly decreases to the basal level. In persons whose bodies are deficient in the substance the curve rises slowly to a low level and gradually recedes to a level which tends to be a little higher than the basal, depending upon the degree of ascorbic acid deficiency.

The data of Ralli and associates ('37) are instructive in this regard. Normal adult subjects given 100 mg. of pure ascorbic acid by intravenous injection excreted more than 40 per cent within 3 hours. Persons restricted to ascorbic acid-low diets excreted 11 per cent under similar conditions. Clinical subjects with scurvy excreted less than 3 per cent of the test dose.

Ascorbic Acid in Immunological Reactions.—Numerous investigations of the role of different dietary essentials in bodily resistance to disease have shown that various types of nutritional deficiencies are accompanied by a lowering of the immunological defenses. However, there has been scarcely any agreement on the question of direct relationships between dietary essentials and immunological processes. Certainly in the case of vitamin A there is no convincing evidence that the formation of antigens or antibodies, for example, is directly affected by a deficiency of this nutrient. The role of this vitamin in resistance to infection is related to the maintenance of healthy mucous surfaces, as pointed out elsewhere (Chapter XIII). Hence its function is indirect.

But in recent years it has been indicated that ascorbic acid may

actually play a direct role in bodily resistance to disease. For instance, Harde and Thompson ('35) suggest that complement may contain ascorbic acid as a component since the thermolability of both substances is similar. After histamine shock the content of complement and ascorbic acid in blood was reduced. Injection of ascorbic acid led to a significant rise in complement. Marsh ('36) has reported that the "complement" complex in the blood of the guinea pig disappears or suffers reduction in titer when ascorbic acid is withdrawn, completely or partially, from the diet. Moreover, he states that the complement titer can be restored to normal by means of a diet rich in ascorbic acid. Horgan's ('36) observations are in complete agreement with those of Marsh. In addition, Horgan noted that guinea pigs rarely died from the effects of intra-cardiac puncture when the diet contained generous amounts of ascorbic acid.

These claims are at variance with some of the older data. Zilva ('19) did not detect any difference in the content of natural antibodies in normal guinea pigs and those fed a scorbutic diet. Hamburger and Goldschmidt ('22) observed no appreciable difference in complement titer between normal and scorbutic guinea pigs and children. But the studies of Koch and Smith ('24) suggested that there is a connection, although seemingly obscure, between ascorbic acid and complement.

Recently the role of ascorbic acid in the function of complement has been greatly clarified by the studies of Ecker and associates ('38) who, using improved methods of titrating complement, have demonstrated a marked decrease in complement parallel with the reduction in ascorbic acid of the serum. Administration of ascorbic acid to scorbutic guinea pigs increased the complement titer. Moreover, the addition of ascorbic acid to scorbutic serum increased the titer to normal. An increase of the ascorbic acid content of the serum beyond 1 mg. per 100 cc. did not increase the titer. Apparently the vitamin functions, in relation to the complement titer, through its reducing properties. Presumably information will be available soon on the relation of ascorbic acid to complement in humans.

As pointed out by Perla and Marmorston ('37), in a review on the role of ascorbic acid in resistance, skin reactivity to drugs, such as arsphenamine, is greatly diminished in guinea pigs depleted of ascorbic acid. This is true also in the case of skin hypersensitiveness to poison ivy. Sulzberger and Oser ('34) found that large doses of ascorbic acid reduced and inhibited the susceptibility of the guinea pig's skin to experimental sensitization with neoarsphenamine. The amount required was larger than that necessary to protect against scurvy.

These evidences of a direct role of ascorbic acid in the immunologic

found to be almost uniformly lowered (Rinehart, et al., '36). This alone is not convincing evidence that ascorbic acid plays a unique role in rheumatic fever, since infectious diseases in general seem to increase the requirement for this nutrient, thus lowering the blood level when the intake is not adequate. Sendroy and Schultz ('36), as well as others, have criticized the views of Rinehart and associates. They have pointed out that a significant percentage of rheumatic fever cases appear to be unaccompanied by ascorbic acid deficiency. Moreover, administration of this vitamin in doses approximately twice the probable normal requirement has not resulted in cure of the disease. But, as Rinehart has pointed out, an adequate study of the effect of high intake of ascorbic acid in rheumatic fever has not been made. We are without sufficient information concerning variability in ability to utilize ascorbic acid to conclude that present evidence proves the absence of a causal relation between systemic ascorbic acid insufficiency and rheumatic fever.

As recently shown by Taylor ('37), scurvy in guinea pigs leads to valvulitis and myocarditis with acute and subacute inflammatory foci. No organisms were found in many of the severest lesions. However, gram-positive cocci or bacilli were present in about one-half of the diseased hearts. This study certainly suggests that opponents of Rinehart's views have not established their claims that disturbances in rheumatic fever, as respects ascorbic acid, are of no greater significance than those of similar character observed in other infections. Further investigation may show that ascorbic acid insufficiency is not a specific factor, but it seems very improbable that this nutrient is without some effect in the prevention of rheumatic fever.

Pulmonary Tuberculosis: In recent years it has been indicated that tuberculous persons require a larger intake of ascorbic acid in order to maintain a normal ascorbic acid balance. The studies of Heise and Martin ('36) are important in this respect. They estimated the urinary ascorbic acid excretion of 54 patients with mild, moderately advanced, and far advanced tuberculosis, using the Tillmans titration method. The diets of all were presumably adequate. The data were classified in 4 arbitrary groups in the order of their magnitude. The number of patients with active tuberculosis was then determined for each group, and with striking results. Six patients excreted only 0 to 5 mg. daily. Five of these had active tuberculosis and it was believed that the sixth had some degree of activity. Fourteen excreted 5 to 8 mg. daily. Active infection was present in 9 of the 14. Twenty-one excreted 8 to 14 mg. daily and only 7 of the 21 showed activity. Finally, in the group excreting over 14 mg. daily only 1 of the 13 showed activity.

The significance of these data was amplified by estimations of ascorbic acid "tolerance." Heise and Martin administered 4 ounces

daily of orange juice, containing about 55 mg. of ascorbic acid, to the same patients. This amount was sufficient to cause a significant increase in the amount excreted by normal persons ingesting an ordinary diet presumably containing an adequate amount of ascorbic acid. Eight of 10 cases showing active tuberculosis did not respond with increased excretion of the vitamin, but 14 inactive cases showed a significant rise in the excretion level. The data show that between 55 and 140 mg. of ascorbic acid were required daily by patients with active tuberculosis for the maintenance of a normal urinary level of the vitamin. In one patient 200 mg. daily were not sufficient to cause a normal excretion level. The contrast of these figures with the 20 to 40 mg. daily of ascorbic acid required by a normal adult is striking. It is not yet clear whether the high rate of ascorbic acid destruction in tuberculosis represents a specific requirement, or one generally associated with chronic infectious diseases.

The data of other workers indirectly support these findings. For instance, Steinbach and Klein ('36) claim that daily injections of ascorbic acid increased the tolerance of tuberculous guinea pigs to repeated large doses of tuberculin. Also, scorbutic guinea pigs fed tuberculous sputum are more susceptible to ulcerative intestinal lesions of tuberculosis than in normal animals (McConkey and Smith, '33; Greene, Steiner and Kramer, '36).

However, there appears to be no experimental basis for the assumption that large quantities of ascorbic acid will increase the resistance of normal persons or animals to tuberculous infection. In support of this Heise and Martin ('36) found that large daily parenteral injections of ascorbic acid failed to protect guinea pigs injected with virulent tubercle bacilli. This plan of study should be extended, since it is possible that the infecting dose of organisms was too high. Also the use of large doses of ascorbic acid should be investigated with reference to survival of animals subjected to various degrees of infection.

Diphtheria: As early as 1920 it was observed that latent scurvy in infants is associated with a relatively high incidence of nasal diphtheria. Hess ('20, l.c.) attributed it to a local immunizing effect of the antiscorbutic vitamin on mucous membranes. At present there is incontrovertible evidence that ascorbic acid plays a significant role in protection against diphtheria toxin. Indirect evidence of this was afforded by Bessey, Menten and King ('33, l.c.) who found that in severe scurvy, heart muscle develops patches of fatty degeneration similar to those seen in diphtheria.

It is neither possible nor necessary to review all the work on this subject. It suffices to mention only a few of the many investigations. Prominent among these are the contributions of King and associates. For instance, King and Menten ('35) fed guinea pigs abundant, pro-

tective, and sub-protective amounts of ascorbic acid and gave them subcutaneous injections of diphtheria toxin in various doses below the minimum lethal amount. Animals partially depleted of their ascorbic acid reserves, but showing no signs of scurvy, survived about half as long as animals with normal reserves receiving the same dosage of diphtheria toxin. Menten and King ('35) noted that the ascorbic acid-depleted animals, injected with the toxin, developed diffuse hyperplastic arteriosclerosis in lungs, liver, spleen, and kidneys. Such animals also showed degeneration of the islets of Langerhans, which was associated with high blood sugar and low glucose tolerance. The findings of Pfleger and Scholl ('37) give added significance to this report. In diabetic patients who were not given insulin, the administration of ascorbic acid had no effect on the sugar content of blood or urine, but the combustion of acetone bodies was favorably influenced. When the tissues were saturated with ascorbic acid the action of insulin was noticeably intensified, so that the carbohydrate metabolism could be regulated with smaller amounts of insulin. They state that all diabetic patients, irrespective of the modification of the sugar metabolism, were improved as respects general condition, disappearance of fatigue, and increased vitality, by the administration of ascorbic acid.

When diphtheria toxin and ascorbic acid are mixed *in vitro* there is a loss of reducing capacity proportional to the time of contact, and the toxin loses its lethal effect after a certain interval (Jungeblut and Zwemer, '35; Polónyi, '35). Greenwald and Harde ('35) confirmed this observation and stated that contact of ascorbic acid with antitoxin or toxin-antitoxin mixtures causes no loss of antitoxic potency.

Otto ('36) and other workers have investigated the uses of ascorbic acid in the treatment of diphtheria, as well as its metabolism in this infectious disease. Otto found that the retention was especially great when in cases of toxic diphtheria from 500 to 700 mg. of ascorbic acid was administered daily by intravenous injection. Patients to whom large doses of the vitamin were not given excreted very small amounts in the urine, indicating that the requirements in this disease are much higher than normal. But the administration of ascorbic acid, as a therapeutic measure, does not appear to affect the time of disappearance of the diphtheric membrane and of the elevated temperature, nor does it influence the number of fatalities. It was noted, however, that a favorable influence was exerted on hemorrhages, particularly nosebleeds. According to Otto, a definite therapeutic value can be ascribed to it only in cases of diphtheric hemorrhages. But, as King and associates have emphasized, there is a wide zone of ascorbic acid deficiency, without the appearance of scurvy, where physiological processes are interfered with and the body is more sensitive to injury from toxins. The evidence suggests unequivocally that ascorbic acid should be fed

in generous amounts in diphtheria, as a means of preventing a deficiency of this nutrient in the tissues, if not as a definite therapeutic agent.

Pneumococcus Infection: As in the case of rheumatic fever, pulmonary tuberculosis, and diphtheria, pneumonia also appears to cause a large increase in the ascorbic acid requirement. Bullowa and associates ('36) found that in the high-fever stages of pneumonia, tissue depletion of ascorbic acid occurs, as estimated by the "saturation" test.

In summary, the evidence suggests that the ascorbic acid requirements are augmented by all diseases in which an elevated temperature occurs.

Ascorbic Acid in Relation to Cataract.—Although numerous papers have been published on the relation of ascorbic acid to cataract formation and in particular the metabolism of lens, it is impossible to draw final conclusions on the subject. Dietary factors other than ascorbic acid certainly affect the lens. Lactose excess and riboflavin deficiency, in the rat, as discussed elsewhere, are reported to cause cataracts.

Josephson ('35) states that the administration of 15 to 300 mg. of ascorbic acid to patients with cataract caused marked improvement, and within a week of treatment mature cataracts became sufficiently transparent to permit examination of the eye grounds and even permit some vision. Cataracts caused by poisoning with dinitrophenol were said to respond very rapidly to treatment with ascorbic acid, and other toxic effects of the drug were also ameliorated. Bellows ('36) reports confirmation of the observations of others in respect to cataract formation in rats fed large amounts of galactose. He states that ascorbic acid given with the galactose delays the onset of changes in the lens. Lens of galactose-fed animals contained less ascorbic acid than normal. Also, yeast, which is rich in glutathione but devoid of ascorbic acid, delayed the appearance of cataracts. The latter observation is significant since Bakker ('36) states that the transparency of the lens is not dependent upon its content of ascorbic acid and that a normal rate of respiration is possible when the amount of this vitamin is low. Johnson ('37) also has published evidence which indicates that ascorbic acid has no direct bearing on the etiology of cataract in guinea pigs.

It may be concluded that there is not sufficient evidence to warrant generalizations regarding the role of ascorbic acid in lens metabolism, particularly as it concerns cataract formation. The foregoing data are suggestive of the need for further investigation of this subject.

Ascorbic Acid in Relation to Gastrointestinal Ulcers.—For several years it has been suspected by a significant number of workers that gastrointestinal ulcers are in some instances caused by chronic

ascorbic acid insufficiency. There is scarcely any reason to believe, however, that ascorbic acid deficiency is generally related to such disturbances. Smith and McConkey ('33) noted that guinea pigs suffering from prolonged partial deficiency of ascorbic acid tended to develop peptic ulcers which were believed to be similar to those seen in human subjects. Hirsch ('35) reports the occurrence of peptic ulcers, previously diagnosed as neuroses, in two adolescent boys. He believes that ascorbic acid is essential in the dietetic management of such cases. According to Stepp ('36), bacteria of the coli and paratyphoid types destroy ascorbic acid so that depletion from this cause might be a contributory cause of peptic ulcers.

Although there is not adequate basis for any suggestions of a significant causal relationship between ascorbic acid insufficiency and gastrointestinal ulcers, it should be emphasized that the type of diets which have been used in treating such conditions generally contain very little ascorbic acid. This most certainly would be expected to retard the healing of lesions and interfere with the general physiological functions. As shown by Archer and Graham ('36), 6 out of 9 patients with gastric and duodenal ulcers were in the sub-scurvy state, owing apparently to the use of customary bland diets which did not supply this nutrient. Lazarus ('37) reports a high incidence of ascorbic acid deficiency in patients with hematemesis and melena who had been given typical dietary treatment. Gratifying remission of bleeding was obtained in several cases that were treated with pure ascorbic acid.

Ascorbic Acid in Relation to Anemia.—There is evidence that iron utilization is defective when the body's reserves of ascorbic acid are depleted. That anemia occurs in scurvy is well known, but it has been attributed by some to general undernutrition and hemorrhage, though increased blood destruction has been suggested as an additional factor. Mettier, Minot and Townsend ('30) state that in adults with scurvy the pronounced anemia often encountered can be promptly relieved by providing foods rich in ascorbic acid. There is marked reticulocytosis and rapid regeneration of blood. Neither large doses of iron nor the substance potent in pernicious anemia appear to accomplish these effects in the scorbutic state. The efficacy of ascorbic acid in promoting normal hematopoiesis is further indicated by the studies of Dunlop and Scarborough ('35), who reported marked blood regeneration after administration of ascorbic acid to a scorbutic subject whose diet contained only 10 mg. of iron per day. The nature of the relation to anemia is not known.

Ascorbic Acid in Relation to Addison's Disease.—The normally high content of ascorbic acid in the adrenal glands, and the conclusive evidence that its presence in any tissue is in some manner associated with an oxidation-reduction system, suggest that depletion of this

substance in the tissues may contribute to the occurrence of metabolic disturbances of a specific character. The prominence of the adrenal glands as sources of epinephrine and cortin (perhaps several different sterols or steroids of much physiological importance), as well as an ascorbic acid-rich tissue, in species which require a dietary source of ascorbic acid and those which do not, naturally has led to reflection on the significance of ascorbic acid in these glands.

By means of the urine titration method, Wilkinson and Ashford ('36) found ascorbic acid deficiency in three cases of Addison's disease. There was some increase in urinary excretion of the vitamin following reported oral dosage in large amounts. When therapy was discontinued the increased excretion fell rapidly to values characteristic of the control period. In these few cases it was claimed that the degree of ascorbic acid depletion paralleled the severity of the disease. They expressed the view that depletion of ascorbic acid may be a feature of the disease, or it may be an entirely non-specific index to the extremely low state of health of the patient. Whether there is an increased need for ascorbic acid or an increased destruction of it in Addison's disease cannot be stated on the basis of present evidence. As has been noted above, there is increased destruction of ascorbic acid in febrile conditions, but in Addison's disease fever is not a prominent feature.

Siwe ('35) found a high tolerance of ascorbic acid in patients with Addison's disease. The oral administration of 600 mg. daily did not increase the urinary excretion. He states that ascorbic acid therapy decreases pigmentation but without relieving the symptoms of the disease. It has been reported that ascorbic acid reduced pigmentation in the case of Addison's disease in a 12-year-old boy (Harnapp, '36). Wilkinson and Ashford noted a decrease in the pigmentation of two patients who had been given liberal doses of ascorbic acid intramuscularly. However, they hesitated to ascribe the effect to ascorbic acid since the patients had been given adrenal cortex extract. The present meager evidence merely suggests that pigmentation, of the kind associated with Addison's disease, and perhaps certain physiological conditions, are related to a deficiency of ascorbic acid. Further studies of this subject should be made.

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CHAPTER

XVIII

Chemical Nature of Thiamin (Vitamin B_1)

THE STUDIES of Lunin (1881), referred to in an earlier chapter (Chapter II), brought clearly to the attention of physiologists the existence of a specific problem concerning the nature of the substances which were lacking in a diet composed of purified proteins, carbohydrates, fats, and inorganic salts, without which normal nutrition could not be attained. The essential repetition of his experiments by Pekelharing (1905) and by Hopkins (1906) has already been discussed. Likewise, the studies of Takaki (1887), which resulted in the demonstration that beriberi could be eradicated from the Japanese Navy by a proper dietary reform, and the epoch-making discovery of Eijkman (1897) that the cardinal symptoms of beriberi could be induced in birds by restricting them to polished rice, his postulation that this disease was caused by a toxin which could be neutralized by a substance present in rice polishings, and the correction of this error by Eijkman's successor, Grijins (1901), have been previously discussed. Between the years 1913 and 1916 McCollum and his coworkers demonstrated that, in addition to their "fat-soluble A," young rats required another factor which they called "water-soluble B." They suggested that the new factor was identical with the antiberiberi substance.

In 1926 Smith and Hendrick showed that the water-soluble factor was of dual nature. This important discovery was utilized immediately by Goldberger (1926) in the extension of his researches on pellagra, his hypothesis being that the heat-stable factor, demonstrated by Smith and Hendrick, was the antipellagra dietary factor. Thenceforth the heat-stable component of the "B-complex" became known in England as vitamin B_2, and in America, as the P-P factor (Goldberger) and also as vitamin G. Likewise the English workers adopted the term

vitamin B_1 for the more heat-labile, antiberiberi factor, but workers in America tended to designate it as vitamin B, or the antineuritic vitamin.

Nomenclature of Vitamin B.—In the older literature the following names have been employed to designate the substance which prevented or cured polyneuritis, the analog of human beriberi, in birds or mammals: Activator (Schaumann), vitamine (Funk), oryzanin (Suzuki), antiberiberin (Tsuzuki), torulin (Edie), eutonine (Abderhalden), antineuritin (Hofmeister), water-soluble B (McCollum and Kennedy), vitamin B (Drummond), antiberiberi vitamin (Funk, Jansen), antineuritic vitamin (Mitchell), vitamin B-P (Salmon), and vitamin F (Sherman and Axtmayer). In the more recent literature vitamin B_1 (Vitamin Committee of Medical Research Council), aneurin (Jansen), and thiamin (Williams) are used. Aneurin seems to be the preferred name in Europe, whereas in America, thiamin or thiamin chloride (bromide, sulfate, etc., depending upon the acid radical combined with the base), is preferred. Commercial preparations of the crystalline vitamin have been introduced under such names as Betabion, Betaxin, Betanerva, etc. The multiplicity of names causes confusion and is deplorable. Thenceforth, in this book the name thiamin will be employed, it being understood that the hydrochloride is the substance referred to. There is adequate precedent for this usage since the Council on Pharmacy and Chemistry of the American Medical Association is in favor of the name thiamin as a common designation for vitamin B_1 (American Medical Association, '37), and it has been adopted by the American Association of Biological Chemists and the American Institute of Nutrition.

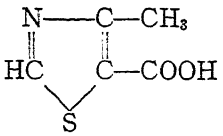
Isolation and Identification of Thiamin.—The early chemical research on the isolation and identification of thiamin was marred in several instances by over-enthusiasm and lack of discretion in the promulgation of premature and uncritical work. Advancement was slow owing in part to the lack of dependability in much of the chemical information published from 1911 to about 1920, and the inherent difficulty of the problem. By 1911 Funk ('12) had shown that the factor is soluble in water, alcohol, and acidified alcohol, is dialysable, relatively stable in the presence of strong acids, and precipitable by phosphotungstic acid. Funk's insight placed the substance in the class of pyrimidine bases which has been substantiated, as further discussion will show. Vedder and Williams' ('13) studies suggested further its pyrimidine-like nature.

Seidell's ('17) arduous investigations, begun in 1916, were based on adsorption of the vitamin from acid solutions by means of fuller's earth. Some of his concentrates had an activity 100 times that of dried brewer's yeast in the cure of polyneuritic pigeons. A comprehensive

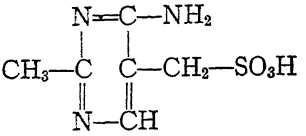
review of these and other early attempts to isolate the vitamin has been made by Kruse and McCollum ('29).

Jansen and Donath ('26), employing a modified Seidell adsorption method, isolated from rice polish small amounts of a highly active hydrochloride. It melted at 250° and the free base had the formula $C_6H_{10}ON_2$. Windaus and associates ('31) employed essentially the same procedure and obtained crystalline material with practically the same potency but differing chemically in that the formula was $C_{12}H_{17}OH_3S$. This epochal report soon was confirmed and proof was adduced that the preparation of the Dutch workers, Jansen and Donath, also contained sulfur. However, there remained some doubt that the Dutch and German crystals were the pure vitamin. In particular the bio-assays of O'Brien and Peters suggested that even concentrates of the vitamin, prepared in Peter's laboratory, were more potent than the crystalline materials given to them by Jansen or Windaus. Moreover, absorption spectra of the various crystalline preparations indicated lack of complete homogeneity. At this point the notable work of Williams and coworkers ('30) began to clarify the problem. Williams et al. ('34) eventually developed a method for securing consistent yields of the vitamin, being able to obtain approximately 5 gm. per ton of rice polishings. This appeared to be about 25 per cent of the total amount present. The relatively large yield was due, in considerable measure, to the use of quinine sulfate, instead of barium hydroxide, as an eluate of the activated fuller's earth.

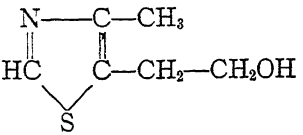
Synthesis of Thiamin.—Elucidation of the nature of thiamin and the accomplishment of its synthesis were dependent upon the painstaking study of minute amounts of material since only small amounts were available. By means of oxidation with nitric acid, Windaus, Tschesche and Grewe ('34) succeeded in a cleavage of the vitamin into two compounds. The assigned empirical formulae were: $C_7H_{11}N_3O_5$ and $C_5H_5NO_2S$ (I). Shortly thereafter Williams et al. ('35) announced the important discovery that cleavage, without complicating reactions, could be obtained by treatment of the vitamin with sodium sulfite at room temperature in slightly acid solution. The products were a sulfonic acid (II), $C_6H_9N_3O_3S$ and a basic compound, C_6H_9NOS. The latter was shown to be 4-methyl-5-hydroxyethylthiazole (Clarke and Gurin, '35). It was synthesized by Buchman ('36). Oxidation with nitric acid converted it into 4-methylthiazole-5-carboxylic acid, which is identical with the substance (I) of Windaus. Potentiometric titration revealed that the thiazole moiety of the vitamin is present in the form of the quaternary salt. In view of this, and other important facts which cannot be enumerated here, the structural formula (IV) was assigned to the vitamin. The formulae of these products, and of the vitamin itself, are as follows:



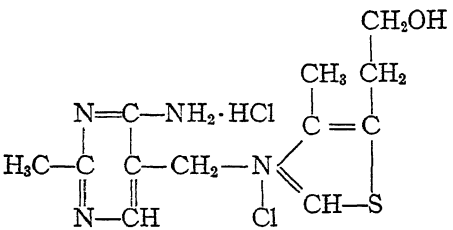
(I)



(II)



(III)

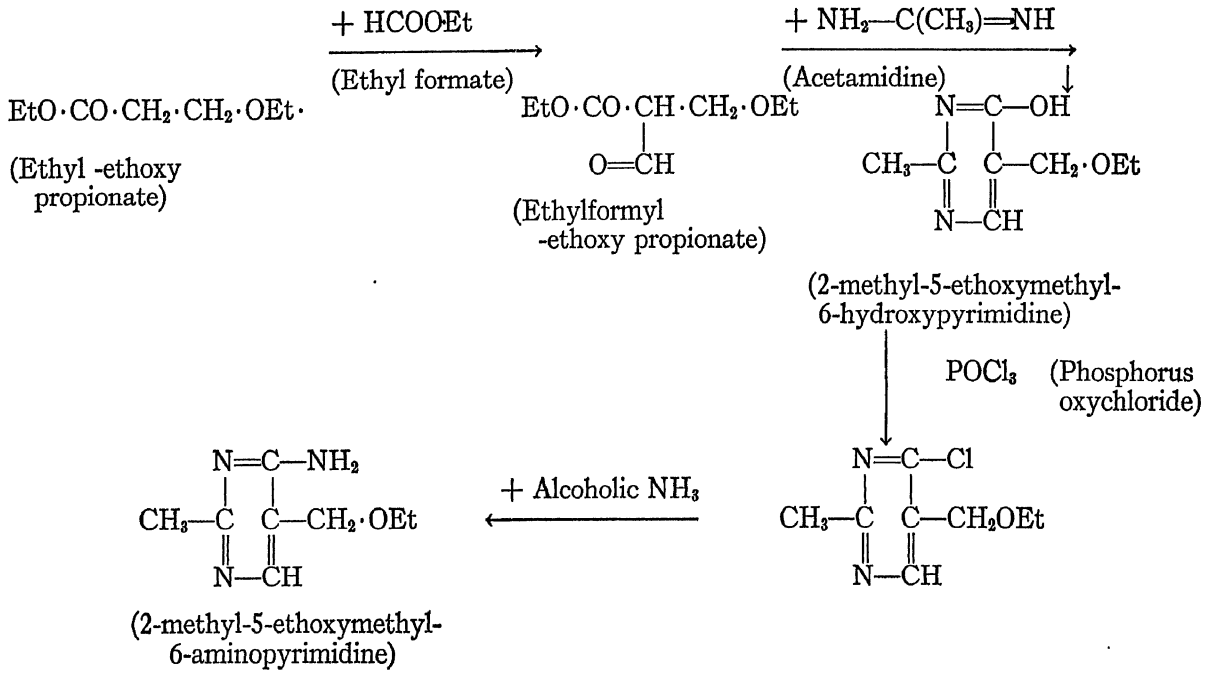


(IV)

More than one method of synthesis is known at present (Williams and Cline, '36; Grewe, '36; Todd and Bergel, '37), but it is sufficient here to outline only one procedure, that developed by Williams and Cline (pp. 445-446).

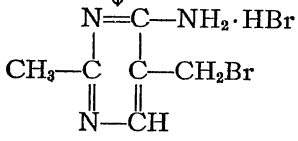
The chemical name for vitamin B_1 chloride hydrochloride (thiamin) is 2-methyl-5-(4-methyl-5-beta-hydroxyethylthiazolium chloride) methyl-6-aminopyrimidine hydrochloride.

The achievement of Cline, Williams and Finkelstein, and of Clarke and coworkers, in decomposing the vitamin obtained from rice polishings into simpler cleavage products, the molecular structures of which were known, and the subsequent building up of a molecule from relatively simple organic compounds to form a synthetic product identical with that formed by plants in nature, furnishes an excellent illustration of the spirit of organic chemistry. The history of chemistry is replete with comparable achievements by chemists who have taken the products of the plant and animal kingdoms, systematically decomposed them into known fragments, and have then put together simple com-


+ HCOOEt
(Ethyl formate)
+ NH₂—C(CH₃)=NH
(Acetamidine)
EtO·CO·CH₂·CH₂·OEt·
(Ethyl -ethoxy propionate)
EtO·CO·CH·CH₂·OEt
O=CH
(Ethylformyl -ethoxy propionate)
N=C—OH
CH₃—C C—CH₂·OEt
N—CH
(2-methyl-5-ethoxymethyl-6-hydroxypyrimidine)
POCl₃ (Phosphorus oxychloride)
N=C—Cl
CH₃—C C—CH₂OEt
N—CH
+ Alcoholic NH₃
N=C—NH₂
CH₃—C C—CH₂·OEt
N—CH
(2-methyl-5-ethoxymethyl-6-aminopyrimidine)

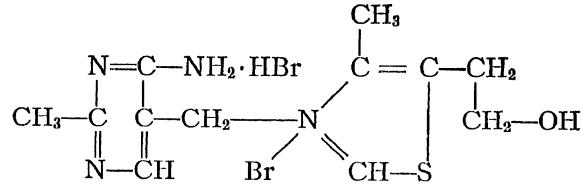
(2-methyl-5-ethoxymethyl-
6-aminopyrimidine)

HBr (Hydrobromic acid)



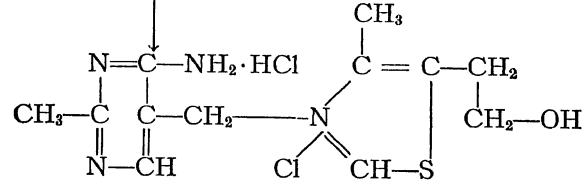
(2-methyl-5-bromomethyl-
6-aminopyrimidine hydrobromide)

4-methyl-5-β-hydroxy-
ethylthiazole



(Thiamin bromide hydrobromide)

Alcoholic
AgCl

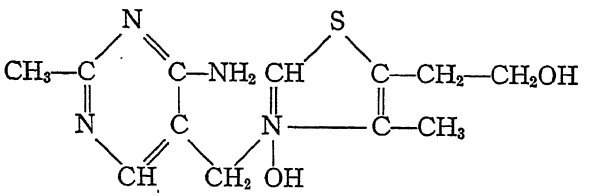


(Thiamin chloride hydrochloride)

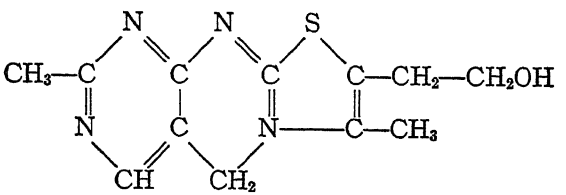
pounds to build up a complex molecule identical with the natural product. Sugars, amino acids, polypeptides, fatty acids, fats, alkaloids, odors of flowers, respiratory pigments, synthetic rubber, etc., have all been produced in this manner in the laboratory.

Chemical Properties of Thiamin.—According to Cline, Williams and Finkelstein ('37), thiamin crystallizes from alcoholic aqueous solutions as the hemihydrate, $C_{12}H_{17}ON_4SCl.HCl.\frac{1}{2}\ H_2O$. It melts at 248-250°. The corresponding bromide hydrobromide forms rosettes of needles which melt at 229-231°. Two sulfates and a nitrate of the base have been prepared by Kinnersley, O'Brien and Peters ('35). They have shown that blue fluorescent oxidation products of pure thiamin are produced at room temperature very slowly by the action of permanganate and manganese oxides at pH values more acid than pH 6.0. The reaction products form more rapidly about pH 7.0. The substances formed are yellow in acid solution. The reactions do not split off sulfur as sulfate but the state of combination of sulfur changes. It is significant that the fluorescent oxidation products show biological activity even in absence of the normal color reaction (formaldehyde-azo-reaction).

Relation to Thiochrome: The above work of Kinnersley et al. is important in relation to the studies of Kuhn, Wagner-Jauregg, van Klaveren and Vetter ('35) who isolated from yeast a yellow basic substance, $C_{12}H_{14}N_4OS$, which, in alkaline or neutral solutions, shows intense blue fluorescence. This substance was named thiochrome by Kuhn et al. Its structural similarity to thiamin is indicated in the following formulae:



Thiamin (Free base)

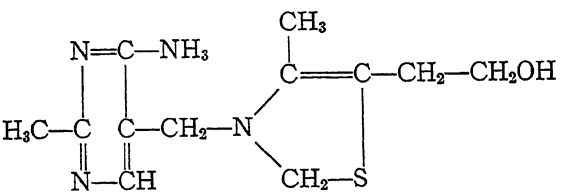


Thiochrome

That thiamin may be changed into thiochrome with relative ease is indicated by the recent literature reports. Barger and coworkers ('35) have formed thiochrome by the oxidation of an alkaline solution of thiamin with potassium ferricyanide. Kuhn and Vetter ('35) report the formation of thiochrome from thiamin by means of other oxidizing agents such as hydrogen peroxide, selenium dioxide, and potassium permanganate. Also Kinnersley et al. observed the formation of small amounts of thiochrome in alcoholic solutions of thiamin allowed to stand at room temperature for several months. The reaction is accelerated in solutions approaching pH 7.

Thiamin has a pK, 4.8, corresponding to a basic group and pK_2, about 9.0, corresponding to a pseudo-acidic or pseudo-basic group. The potentiometric behavior of thiochrome is somewhat similar, having a basic pH of 5.6 and, like thiamin, a tendency for a drifting titration value to the alkaline side of pH 7. Thiochrome has no thiamin activity as indicated by the catatorulin test (Ogston and Peters, '36).

Reduction: Lipmann ('36) has found that thiamin is readily reduced when treated with platinum black or hydrosulphite. In a phosphate solution at pH 7.5, 0.94 mol of H_2 was absorbed per mol of thiamin. It is believed that the reduction product has the structure:



The mode of reaction with hydrosulphite may be regarded as indicative of a function in the living cell, namely, that of a hydrogen acceptor.

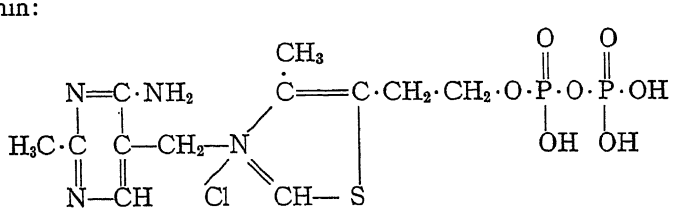
Physical Properties of Thiamin.—Wintersteiner, Williams and Ruehle ('35), among others, have studied the ultraviolet absorption of thiamin and report that two bands are present at 235 μ and 267 μ respectively whether alcohol or water is used as solvent. Narasimhamurthy ('36), by means of electrophoresis and microcataphoresis, has found that the isoelectric point lies between pH 9.0 and 10.0.

The reaction to heat has been the subject of inquiry almost since the biological discovery of thiamin. By means of heat, Smith and Hendrick, in 1926, differentiated the "vitamin B" of that period into "heat-labile" and "heat-stable" fractions. The former corresponded to the present thiamin. The vitamin, in foodstuffs, withstands ordinary cooking with practically no destruction, if the pH is not more than 7. However, several studies have shown that temperatures above 110° C.,

which may be reached in a pressure cooker, cause progressive destruction. As a general rule the temperature reached in the processing of canned foods causes very significant destruction of thiamin; hence these may be regarded as of little value as a source of this substance unless actual tests prove the contrary. It should be emphasized that foods cooked or preserved in an acid medium are much more resistant to heat than those prepared in alkaline media. Various experimental data attest to this conclusion. For example, Sherman and Burton ('26) heated samples of tomato juice during four hours at 100° C., after adjusting the reaction to pH values of 4.28, 5.2, and 7.9. The thiamin destruction amounted to 20, 30, and 70 per cent respectively. Guha and Drummond ('29) state that boiling a solution of thiamin during 24 hours at pH 1 caused no significant inactivation, but at pH 5 about 50 per cent was inactivated. In view of the present indication that thiamin functions in the body as a pyrophosphoric acid ester, to be discussed subsequently, it is not improbable that the stability in foods, at acid reactions, is attributable to its occurrence as an ester. Such a mode of combination might cause the vitamin to be more resistant to heat.

Miscellaneous Reactions of Thiamin.—For many years it has been known that alkali destroys vitamin B_1 activity. Kinnersley et al. ('35, l.c.) have shown that hot alkali splits off sulfur as hydrogen sulfide. They have also shown that thiamin reacts very slowly if at all with nitrous acid. Morgan's ('35) observation that the thiamin activity of sulfured fruits is lowered might be correlated with the high lability of the substance in the presence of sulfite.

Cocarboxylase.—With respect to the function of thiamin in tissue respiration it is of outstanding significance that a coenzyme, called cocarboxylase by Auhagen ('33) and required in the decarboxylation of pyruvic acid, should contain thiamin. It was isolated by Lohmann and Schuster ('37) who showed that it is a pyrophosphoric ester of thiamin:



Cocarboxylase

The coenzyme has been synthesized by enzymatic means (Tauber, '37; Euler and Vestin, '37) and by means of phosphorus oxychloride (Stern and Hofer, '37). As in the case of inorganic pyrophosphate, one

phosphate group is readily split off by means of acid hydrolysis, but the other is more resistant.

The International and Other Units of Thiamin.—Before any degree of success was attained in the chemical estimation of the antineuritic vitamin, many attempts were described to estimate the substance by means of biological tests upon pigeons and rats. Consequently, various criteria of potency have been employed and a number of different "units" have been defined on these bases. The need for uniform assay procedures, an international unit, and an international reference material, was met by the Second International Conference on Vitamin Standardization, held in London in 1934. The Committee adopted as the International Standard an adsorbate prepared by Jansen using the method of Seidell. A special fuller's earth is the adsorbant and 3 kg. of earth contains the thiamin which is extracted from 100 kg. of rice polish by water acidified to pH 4.5 with sulfuric acid and containing 0.2 per cent of salicylic acid as a preservative against bacterial decomposition. The International Unit recommended by the Committee is the "vitamin B_1 activity of 10 milligrams of the International Standard adsorption products." In animal assays the lack of precision in determining the potency of any preparation containing thiamin is shown by the statement of the Committee that, "A daily dose of 10 to 20 milligrams of this (International adsorbate) preparation is required to maintain normal growth in a young rat on a diet deficient in vitamin B_1 but complete in all other respects. The curative day dose for a 300 g. pigeon exhibiting head retraction on a diet of polished rice is about 10 to 30 milligrams." These statements are an expression of the great differences in the response of individual animals, some requiring two or three times as much as others to induce a comparable response. The biological assay for thiamin is, therefore, only a rough approximation of the potency of a preparation. Some advance has been made in the accuracy of reporting assays since crystalline thiamin has become available as the synthetic product. Probably poor response to a given dose in individual animals is in part to be explained by the failure of their digestive tracts to elute the vitamin from the earth. This view is supported by the observations of Sampson and Keresztesy ('37) who found that a quinine sulfate extract of the International standard earth is about twice as effective a source of the vitamin as is the earth itself. They state that rats can utilize only about 60 per cent of the vitamin administered as fuller's earth adsorbate.

Several attempts have been made to translate crystalline thiamin chloride hydrochloride into terms of International Units on the basis of biological tests. The following data show how great are the discrepancies in the results:

INTERNATIONAL UNITS ESTIMATED IN ONE MILLIGRAM OF CRYSTALLINE THIAMIN
(Chloride Hydrochloride)

Waterman and Ammerman ('35) (rat growth assay)	200
Moll ('35) (pigeon day dose, and rat curative method)	500
Smith and Seidell ('36) (rat curative method)	333
Kinnersley and Peters ('36) (pigeon curative method)	500
Jansen ('36) (Smith's modified curative method-rat)	300
Sampson and Keresztesy (l.c.) (curative method-rat)	200
Sampson and Keresztesy (curative method-rat-using quinine eluate)	400
Leong and Harris ('37) (Bradycardia method)	333–357

From data now available it is evident that no procedure has yet been devised for the accurate assay of thiamin in a foodstuff.

Approximate Equivalence of the Various Units.—Cowgill ('34) has carefully discussed the problem of conversion of various units. It is concluded upon unequivocal evidence and logic that equivalence between various units is, by whatever means of calculation, at best a mere approximation. Nevertheless it is possible to make rough estimations of the thiamin content of biological materials by means of biological assay and the conversion of results from a given assay procedure to the unitage of another. With emphasis therefore upon these limitations it is possible to state that one gram of pure thiamin equals: 150,000 Smith curative units; 600,000 Chase-Sherman units; 300,000 Roscoe units; and 300,000 International units. One International unit (fuller's earth adsorbate) equals 3.333 micrograms (3.333 millionths of one gram) of thiamin; 20 milligram-equivalents (Cowgill); 1 Roscoe unit; 2 Chase-Sherman units; and 0.5 Smith curative unit.

Biological Tests for Thiamin.—Rats and pigeons have been generally used in animal assays of biological materials. With rats the principal criteria of potency have been those of growth and the cure of polyneuritis. In practically every instance the basal diet has contained autoclaved yeast as a source of water-soluble vitamins other than thiamin. Unfortunately the growth of animals receiving autoclaved yeast plus an abundance of thiamin is not as good as in animals receiving a similar diet with yeast which has not been autoclaved. This suggests that a basal test diet, using autoclaved yeast for the purpose above indicated, is "incomplete" with respect to factors other than thiamin. This, in addition to variability in coprophagy, genetic differences in the ability to conserve thiamin in the body, variability in the stock rations employed in producing test animals, and other factors, tends to introduce considerable variability in results. Consequently, analytical data obtained by means of biological tests are, at best, only approximations of the truth.

In the case of pigeons, and other birds which have been used, the basal ration is usually polished rice. Since this food is deficient in many

respects it is apparent that results obtained thereby might be somewhat variable, thus making it impossible to ascertain the exact thiamin content of the assayed material.

Rat Procedures.—Two general methods are used, namely, growth and curative. Sherman and his coworkers (Sherman and Smith, '31) have made elaborate studies on the estimation of thiamin in foods by determining the smallest amount of the assay material which, added to the basal ration, will maintain growth in young rats at the rate of 3 grams per week during 4 to 8 weeks. This unit is the Chase-Sherman unit, or the Chase unit. Chick and Roscoe ('29) modified this method in that the experimental rats, on weaning, are restricted to a thiamin-free diet until they cease to gain weight. The material to be tested is then added to the diet in graded doses in different groups of animals, and the amount which will restore growth at the rate of 10 to 14 grams per week is found. This is known as the Roscoe unit. It is equivalent to approximately 2 Chase-Sherman units.

In the development of a curative test, Smith ('30) modified a procedure introduced by Hofmeister in 1922. In this method young rats are brought into the polyneuritic state on a suitable basal diet and the minimum dose of test material is given which will effect a cure of the acute neuromuscular symptoms. The criterion of a definite polyneuritic state is the tendency of the rat to develop a convulsion after being suspended by the tail and rotated. In this method a single dose of the test material is given and a cure must be effected within 24 hours. This measures the Smith curative unit.

Pigeon Procedures.—Three general methods have been used, namely, protective, weight maintenance, and curative. The protective method is not at all satisfactory since it involves the determination of the amount of test material which must be added to a basal ration of polished rice in order to prevent neuromuscular symptoms. The principal objections to this procedure are inherent in the weight maintenance method developed by Williams and Seidell in 1916 and elaborated by Cowgill (l.c.). Cowgill's pigeon unit, obtained by this method was estimated to be equivalent to 10 Chase-Sherman units. His unit is approximately equivalent to the Kinnersley and Peters' "pigeon curative unit." The curative test of Kinnersley, Peters and Reader ('28) is based upon the alleviation of acute head retraction, and other polyneuritic symptoms, after administration of the test material in graded doses, to pigeons made severely polyneuritic by restriction to the thiamin-free diet. The curative unit is the weight of the curative dose divided by the number of days from cure to the return of polyneuritic symptoms. Although this procedure is recognized as superior to other pigeon methods, it is obviously lacking in the analytical precision which is desired for thiamin determinations.

The Electrocardiographic "Bradycardia" Method for Thiamin Assay.—Drury, Harris and Maudsley ('30) showed that rats in thiamin deficiency have slow heart rates and that provision of the vitamin produces rapid cures, the effect on the heart rate being proportional to the amount of the vitamin administered. The normal heart rate of the rat is 500 to 550 beats per minute. When they are restricted to a diet deficient in thiamin for about three weeks the rate falls to about 350 beats per minute. A single dose of the test material is given, and the electrocardiogram is again taken after 24 hours. The increase in rate and the time during which the increase lasts are the criteria on which the dose of the vitamin is estimated. The assay of an unknown product is carried out by testing the effect of several levels of dosage, using 4 to 6 rats, and a comparison is made simultaneously with the amount of the standard reference material necessary to produce a comparative effect. In spite of pertinent objections this procedure appears to be the most satisfactory yet devised for the assay of thiamin in foods and other materials, particularly when the concentration is very low and time is limited.

The rapidity with which thiamin affects the heart rate of deficient animals is indicated by the studies of Zoll and Weiss ('36) who showed that elevation of the heart rate and disappearance of the abnormal electrocardiographic changes occur as early as within 4 hours after administration of thiamin. Birch and Mapson ('36) have found that adenylic acid, which is associated with the nucleotides of foods, has an accelerating effect on the heart. Hence, when natural foods rich in nuclear material are assayed by this material, errors of considerable magnitude may occur. Robertson and Doyle ('37) have pointed out certain difficulties in the conduct of the bradycardia test. They could not secure consistent results.

The Catatorulin Test for Thiamin.—Peters and coworkers (O'Brien and Peters, '35) have shown that the brain of an animal deficient in thiamin has a low *in vitro* uptake of oxygen in lactate solutions. It is suggested that the vitamin acts as a coferment in its effect upon oxygen utilization by the brain. The addition of a source of thiamin accelerates oxygen uptake by brain tissue under proper conditions, and distinct effects are produced by as little as 0.1 gamma of the vitamin in 3 cc. of solution. The name of the test had its origin in the use by the investigators of the name torulin for vitamin B_1, and the observed catalytic effect described above. The results obtained by this method, when the isolated vitamin is employed, appear to be reliable, but it is not applicable to foodstuffs.

Mould Growth Method of Assay for Thiamin.—Schopfer ('35) and Schopfer and Jung ('36) state that the mould, *Phycomyces blakesleeanus,* cannot grow on a medium consisting of 10% glucose, 1%

asparagine, 0.5% $MgSO_4$, and 1.5% KH_2PO_4, but grows appreciably after the addition of 0.0005 microgram of thiamin. Within certain limits the growth is directly proportional to the concentration of the vitamin. Schopfer and Jung were able to obtain results with wheat germ preparations which agree very well with those obtained by the rat growth test, but the test is not specific since autoclaved sources of thiamin are active.

Fermentation Assay for Thiamin.—Schultz, Atkin and Frey ('37) find that thiamin exerts a powerful action on the rate of alcoholic fermentation. In the presence of a suitable sugar-salt buffer mixture as little as one gamma (0.000001 gm.) of the vitamin may be detected. They suggest that this phenomenon might be employed for thiamin assay.

There are inherent difficulties in these suggested methods as a basis of quantitative assay of a foodstuff. In the assay of various kinds of foods it must be shown that absorption of essentially all of the thiamin is accomplished. Especially in the case of certain vegetable foods this question requires careful study. In respect to the methods dependent upon the preparation of thiamin concentrates it has been shown that, in general, even thorough extraction with water removes far less than the total amount of the vitamin in most foods. It appears that the problem of thiamin assay of foods and biological substances cannot be adequately solved until considerably more is learned concerning the chemical combinations of this substance in such materials, and methods of quantitatively removing the vitamin therefrom.

Qualitative Tests for Thiamin.—One of the first tests with some semblance of usefulness was that described by Kinnersley and Peters ('34). Diazotized sulfanilic acid is added to an alkaline carbonate solution and, after a short interval, the unknown solution containing a trace of formaldehyde, added immediately before mixing, is added to the diazo solution. The color, which is yellow at first, but turns pink in a short time, is stable for many days. Unfortunately highly purified test solutions must be used, thus causing loss of the thiamin in purification. Acetone and reducing agents, in particular, interfere with the test.

Jansen (l.c.), on the basis of thiochrome formation when thiamin is oxidized with potassium ferricyanide, devised a procedure for estimation of the vitamin by measurement of the fluorescent thiochrome in a comparator for fluorescent solutions. Karrer and Kubli ('37) modified the procedure with a standard of comparison prepared by the oxidation of thiamin to the fluorescent thiochrome. There are inherent sources of error in these thiochrome methods which render them less reliable than the standard biological procedures. However, Westen-

brink and Goudsmit ('37) claim that they have been able to adapt the procedure to urine analysis and that the results are satisfactory.

Another test which appears to be quite specific is that described by Prebluda and McCollum ('36), who found that when a solution of para-aminoacetanilide or methyl-para-aminoacetophenone is treated with nitrous acid and the resultant reagent is added to a solution of thiamin, a characteristic purple-red compound is produced which is stable and highly insoluble in water. The compound is soluble in acetone, amyl alcohol, and other such solvents and when dissolved therein can be compared in a colorimeter with known amounts of thiamin similarly treated.

Melnick and Field ('38) have confirmed this test and report that the purple-red compound can be quantitatively extracted with xylene and that this extract lends itself to colorimetric evaluation. Also, they have used permutit as a means of isolating thiamin from interfering substances. However, this colorimetric test, like all other chemical methods, cannot as yet be quantitatively applied to the analysis of foodstuffs and biological materials owing to the lack of methods for separation of the vitamin from all interfering substances such as protein and some other materials.

Several reagents are known which precipitate the vitamin. Among these are bismuth potassium iodide, reported by Naiman ('37), Reinecke's salt, and potassium tetranitritodiaminocobaltiate (Rosenthaler, '36). Under suitable conditions thiamin is precipitated by picrolonic acid, gold chloride, mercuric chloride, Mayer's reagent, and phosphotungstic acid.

Thiamin in Relation to Plant Growth.—The mechanism of thiamin synthesis in, or associated with, plants has yet to be described. However, several investigators have shown that thiamin is essential for plant embryo development. It has been known for some time that tomato root tips require something for growth which yeast extract supplies in addition to an otherwise suitable medium composed of certain salts and sugars. Bonner ('37), and Robbins and Bartley ('37) found that yeast extract can be replaced by thiamin either as prepared from natural sources or as the synthetic substance. Bonner states that 0.2 gamma per cent of thiamin will replace the yeast extract optimum, and 0.002 gamma has a marked influence. It is of considerable interest that Kögl and Haagen-Smit ('36) correlate the relative thiamin content of peas with the thiamin required to produce good growth of the excised embryos. Robbins and coworkers have tested on tomato root tip growth the thiazole and the pyrimidine portions of thiamin into which it is cleaved by sulfite, and from the coupling of which the vitamin results in synthesis. They find that only the thiazole

portion is needed by the root tips. It appears, therefore, that when the thiazole is provided the tips can synthesize the pyrimidine portion of the molecule, and can combine the two to produce thiamin. This assumption is warranted on the basis of the universal presence of the complete molecule of thiamin in the growing parts of plants. The fungus *Phycomyces* requires both the thiazole and pyrimidine intermediates which serve it as well as does thiamin. Another fungus, *Aspergillus,* grows without thiamin or either of the intermediates.

Thiamin in Relation to Bacterial Growth.—It has been known for many years that certain bacteria, particularly those normally occurring in the digestive tracts of ruminants, are able to synthesize thiamin. But the precursors in such syntheses are not known. Also thiamin acts as a stimulant to the growth of some bacteria. The work of Tatum, Wood and Peterson ('36) is of interest since they have secured unequivocal evidence that thiamin stimulates the growth of propionic acid bacteria. Inositol, pantothenic acid, ascorbic acid, riboflavin, nicotinic acid amide, and indoleacetic acid could not replace thiamin in this function. Owing to the availability of thiamin, various compounds related to this vitamin, and other important nutritive factors in pure form, it may be predicted that mechanisms of microorganism growth will be revealed in clearer perspective during the next few years.

Experiments on the Substitution of Thiamin Intermediates for Thiamin in Pigeon Polyneuritis.—Robbins and Kavanagh ('37) state that polyneuritic pigeons may be cured by the oral administration of sufficient amounts of both the thiazole and the brompyrimidine intermediates. These results seem to indicate that thiamin may be synthesized from its intermediates in the crop or in the tissues of the pigeon. Williams stated that, theoretically, one would suspect such a combination of the pyrimidine and the thiazole to occur, though perhaps at a very slow rate unless catalyzed.

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CHAPTER

XIX

Nutritional Significance of Thiamin (Vitamin B_1)

THE EARLIER observations on beriberi and on its analogue, polyneuritis in fowls, pigeons, dogs, and rats, have been excellently summarized by Vedder ('13) and by Funk ('22). Scheube (1881) and Baelz ('03) demonstrated the existence of polyneuritis in beriberi, and believed that the central nervous system was unaffected. Their studies led to the general belief that the disease was a peripheral neuritis. The cardinal symptoms of beriberi are those of peripheral neuritis, cardiac insufficiency, and a generalized tendency to edema. Of the two forms of the disease, the wet and the dry beriberi, it now appears that the former, characterized by edema, represents the effects of protein deficiency superimposed upon vitamin deficiency. Vedder suggested that the enlargement of the heart in beriberi is the sequel to weakening of muscle fibers and consequent hypertrophy to meet the necessity of circulation. Also, the relationship between thiamin and carbohydrate metabolism was noted early in the history of beriberi. In 1914 Funk observed that polyneuritis follows more rapidly if the carbohydrate level is increased in diets deficient in the antiberiberi factor. Subsequent researches have demonstrated a very definite relationship between thiamin and carbohydrate metabolism. This will be discussed in the present chapter in addition to various other nutritional aspects of thiamin which include the pathologic effects of thiamin deficiency, storage and excretion, requirements of various age groups, the extent of thiamin deficiency in human populations, and therapeutic uses of thiamin.

The Principal Effects of Thiamin Deficiency.—As pointed out above, the cardinal symptoms of thiamin deficiency are degeneration of the nervous system, cardiac enlargement and dysfunction, edema, gastrointestinal disturbances, muscular atrophy, and anorexia. A de-

tailed account of the pathologic and physiologic changes which characterize these general symptoms, in variable degree, would not be profitable here since we are concerned only with those general effects of thiamin deficiency which suggest, with scant equivocation, the functional nature of this nutrient. The rationality of this point of view is emphasized by Wolbach ('37), whose pathologic studies of vitamin deficiencies have contributed in an important way to our understanding of the roles of vitamins in the body.

Nervous system: Before polyneuritis was produced experimentally in animals by depriving them of thiamin, beriberi was recognized as a disease involving the nervous system. But even today it is not yet clear whether the neuritic symptoms are directly attributable to lesions of the nerves or functional interference owing, perhaps, to the accumulation of metabolic products, particularly pyruvic acid and perhaps lactic acid.

Some of the nervous symptoms of thiamin deficiency in humans are numbness and tingling sensations, particularly affecting the fingers and usually associated with a gnawing pain which runs up the arm, especially at night. The tingling sensation is of the "pins and needles" type. Usually one side of the body is more affected than the other. There are cases, however, in which cardiac dilation and sudden death occur before evidences of nerve dysfunction are manifested.

In birds and rats marked symptoms of lesions in the nervous system appear soon after restriction of such animals to a thiamin-deficient diet. Sherman and Elvehjem ('36a) produced polyneuritis in young chicks after two weeks on the experimental ration. In rats (Davison and Stone, '37) thiamin deficiency is marked by a paralysis of the extremities, equilibratory disturbances, priapism, convulsions, and tonic retractions of the head. Pathologically, the outstanding effect is that of disintegration of the myelin sheaths of the peripheral nerves and vacuolation and liquefaction necrosis of the ganglion cells of the mesencephalon, metencephalon, and anterior horns of the spinal cord. Zimmerman and Burack ('34) believe that disintegration of myelin sheaths is not primarily related to the clinical symptoms. In support of this view is the fact that rats on thiamin-deficient diets may die without developing nervous lesions.

As shown by Davison and Stone, among others, inanition alone may cause as marked change in the peripheral nerves as thiamin deficiency. Moreover, the lesions are essentially similar in both instances. Finally, as shown by Wolbach (l.c.), no differences could be found in the nerve lesions present in pigeons allowed to succumb to polyneuritis and those in which functional recovery had been induced by thiamin administration. One can conclude, therefore, with Wolbach

that, "On the whole, it seems best to regard the primary pathologic effects of vitamin B_1 (thiamin) deficiency as not demonstrable at present and to regard all the pathologic changes thus far recorded, including the myelin sheath lesions, as secondary effects."

Cardiac enlargement and dysfunction: The cardiac enlargement generally characteristic of human beriberi was ascribed by Aalsmeer and Wenckebach ('29) to edema of the cardiac musculature, but Newcomb ('30) was unable to note any significant difference in the water content of cardiac muscle from cases of human beriberi and pigeon polyneuritis. Thus the latter worker concluded that cardiac enlargement in the former case is a true hypertrophy.

More recently Wenckebach ('34) has described the beriberi heart as characterized by enlargement with rapid rate and a throbbing systolic impulse, large bounding pulsations in the peripheral arteries, venous engorgement, warm extremities and edema. As shown by Weiss and Wilkins ('36) the electrocardiograms in such cases are abnormal, being characterized by changes in the T waves, low amplitude, and prolongation of the electrical systole (Q-T). Birch and Mapson ('36) believe that observed bradycardia is due to a failure in the deaminase mechanism, resulting in increased accumulation of adenylic acid in the tissue.

The relationship between cardiac changes and the composition of the blood is indicated in the above, since there is a tendency toward low plasma protein values, hyperglycemia, increased pyruvic acid, and possibly increased lactic acid. These blood changes are suggestive of failure in the metabolism of carbohydrates, as subsequent discussion will definitely verify, and in view of this it is easy to conclude that the heart does not function normally owing to the interference with carbohydrate metabolism in that organ. The changes in heart rate and electrocardiographic behavior have already been discussed in relation to thiamin estimations (Chapter XVIII).

Edema: Human beriberi is frequently accompanied by edema. In all probability dietary deficiencies other than thiamin are related to this condition, as emphasized by Youmans ('36) and discussed by Wilder and Wilbur ('37). Probably protein inadequacy is of considerable importance.

Gastrointestinal dysfunctions: Gastrointestinal disturbances are frequent accompaniments of human beriberi but perhaps more attributable to other factors such as intestinal parasites, dysentery, malaria, etc., than to a direct deficiency of thiamin. Cowgill ('34) has adequately discussed this aspect of beriberi and he points out that conditions such as amebic dysentery, commonly found in beriberi localities, may constitute the precipitating cause of the deficiency disease. His convincing argument is that beriberians, in many instances, might

have sufficient amounts of thiamin in the diet, although at a critically low level, to prevent the disease if they were not afflicted with diarrhea and attacks of fever. The latter increases the tissue requirements for thiamin and the former causes an inefficient utilization of the vitamin owing to its loss in the watery stools.

In experimental thiamin deficiency the complicating factors of the above nature are largely ruled out and here also dysfunction and lesions of the gastrointestinal tract are said to be found. Cowgill et al. ('26) noted loss of tone in the gastric musculature of thiamin-deficient dogs. This is accompanied by gastric hypochlorhydria, according to Cowgill and Gilman ('34), who believe that the characteristic anorexia is related to this condition. Sparks and Collins ('35) have repeated in substance the studies of Hargreaves, Fletcher and Dickson ('31) and have confirmed the latter in the conclusion that thiamin deficiency in rats causes a marked increase in the volume of the colon. This demonstrates that thiamin has some role, either directly or indirectly, in the maintenance of intestinal tone. The claims of several workers, including Eddy and Dalldorf ('37), to the effect that thiamin deficiency causes erosions and ulcers of the stomach, are not substantiated by Simpson ('36). The latter's studies are convincing and at least they place doubt on the occurrence of gastric lesions under such dietary conditions.

The evidence at present is only presumptive concerning thiamin in human gastrointestinal dysfunctions. Vorhaus, Williams and Waterman ('35) noted beneficial results when 8 cases of gastrointestinal hypotonia were treated with 10 mg. daily doses of thiamin given by mouth.

Without citing further from the numerous reports on this subject, it may be stated that the gastrointestinal changes associated with thiamin deficiency have not been completely demonstrated as having direct origin in the deficiency of this vitamin. Nearly all the studies thus far reported have been complicated by inadequacy of other nutritive factors and in practically all instances the sources of thiamin were concentrates or merely yeast, wheat germ, or other such materials containing relatively large amounts of this vitamin. In conclusion, therefore, thiamin deficiency undoubtedly affects the gastrointestinal tract, causing considerable anatomical and functional changes, but further investigation is needed, using improved test diets and pure thiamin, in order to clarify the problem.

Muscular atrophy: Although muscular atrophy is a prominent, but nonspecific, symptom of thiamin deficiency, scarcely anything worth while can be recorded about it. Since anorexia and resultant inanition are marked, it may be assumed that the fall of body weight and muscular wasting are attributable to these factors.

Anorexia: Loss of appetite is one of the first observable symptoms of thiamin inadequacy. This occurs in various other deficiency diseases but in less degree. In fact, we do not know of any dietary inadequacy which occasions such a marked degree of anorexia. As pointed out by Sherman ('34), the role of this vitamin certainly is not that of making the food appetizing ". . . for when the vitamin is given separately the experimental animal will return with appetite to the same food which it had previously refused." That the mediation of thiamin in the mechanism of appetite is not through repair of structural injuries, caused by deficiency of the vitamin, is indicated by the dramatic restoration of desire for food when thiamin is administered (Cowgill, '21). This may occur in 24 hours. Some suggestion of the general relationship between thiamin and appetite is indicated by the significant correspondence between the caloric needs of the body and desire for food. In thiamin deficiency carbohydrate metabolism appears to be essentially blocked owing to failure in the breakdown of pyruvic and lactic acids. It appears that appetite for food is influenced in some manner by the blood level of intermediates of carbohydrate metabolism. Therefore, it may be postulated, an interference with this essential process, as occurs in thiamin deficiency, causes a loss of appetite. This, of course, is mere speculation but it should be suggestive of the scope of an important problem in nutrition, namely, the nature of appetite.

Other effects of thiamin deficiency: There is lack of clear-cut evidence concerning thiamin in relation to the sexual status of animals and man. Evans' ('28) work suggests that sex interest is decreased in the absence of this vitamin, but there is no indication in his results that the anatomical and functional integrity of at least the male reproductive system is directly affected. Evans and Burr ('28) report decrease in lactating ability and suppression of ovulation has been noted in rats by Evans and Bishop ('22).

Wolbach (l.c) has discussed the enlargement of the islands of Langerhans, noted by several workers, and relates it to the disturbance in carbohydrate metabolism. McCarrison ('19) observed that the adrenals hypertrophy. This may be of significance in relation to carbohydrate metabolism and sexual physiology. However, in most of these studies it is possible, if not probable, that complicating factors interfered owing to the lack of pure thiamin.

Blood changes are especially significant, since as subsequent discussion will reveal, they suggest unmistakably the general nature of thiamin action in the body. The most important of these appear to be an increase in glucose and pyruvic acid.

Effects of Thiamin in Massive Amounts.—It was not possible to make conclusive determinations of the effects of thiamin in massive amounts until this vitamin became available in pure form. Since that

time several valuable investigations have been made with the result that practically all the evidence demonstrates complete non-toxicity in all practical uses of the vitamin. Vorhaus, Williams and Waterman (l.c.) reported that the oral ingestion by man of single doses of thiamin up to 90 mg. (27,000 I. U.) were without the slightest unfavorable effects. Weiss and Wilkins ('37) were not able to note harmful effects although they administered average doses of 50 mg. daily and, at times as much as 130 mg. daily. Hecht and Weese ('37), on the basis of studies on the cumulative effect of repeated massive doses of thiamin, concluded that there is no danger from prolonged ingestion of thiamin unless the dosage is exceedingly high, perhaps about 7200 times a normal dose of 0.08 mg. of thiamin per kg. of body weight.

Physiological Role of Thiamin.—An analysis of all the evidence at present suggests that thiamin is definitely concerned with tissue respiration, particularly as it relates to the metabolism of pyruvic acid. It demonstrates that there was justification in the early suggestions that disorders observed in polyneuritis may be due to an accumulation of intermediate substances arising from carbohydrate degradation, which cannot be metabolized in the absence of thiamin. Especially significant now is the early observation of Funk ('14) that an increase in the carbohydrate ingestion causes a more rapid production of polyneuritis in pigeons on a thiamin-deficient ration. Another significant advance was the finding of Collazo ('23) that the introduction of considerable amounts of carbohydrate into the crops of normal and thiamin-deficient pigeons invariably caused death in the latter but not in the former, and it was shown by Rubino and Collazo ('23) that the carbohydrate thus introduced was actually absorbed.

The more recent studies have shown that thiamin deficiency is accompanied by a progressive diminution in carbohydrate tolerance (Kauffmann-Cosla, et al., '32). Lewinson ('37) noted that thiamin deficiency causes a derangement of carbohydrate metabolism which precedes the nervous disturbances and is manifested as hyperglycemia. Also, it was shown by Roche ('31) that in polyneuritis there is an accumulation in the blood of intermediate products of carbohydrate metabolism and an increase in the C:N ratio in the urine. On the one hand this derangement of carbohydrate metabolism has been attributed to reduction in the amount of oxidizing enzymes, or to interference with their activity; on the other, it has been attributed to inanition. The latter view was championed by Drummond and Marrian ('26), who insisted that the rapid diminution in body weight, the fall of body temperature, and the hyperglycemia are the result of inanition rather than of direct deficiency of the vitamin. In accordance with the more recent findings, particularly those of Lohmann and Schuster ('37), this view is not easily reconciled with the evidence. Lohmann and

Schuster made the important observation that a pyrophosphate of thiamin isolated from yeast is cocarboxylase. Since this coferment increases the *in vitro* oxygen uptake of brain tissue from thiamin-deficient animals it is indicated that the vitamin does play an active role in tissue oxidations, and that hyperglycemia, loss of weight, etc., are directly attributable to a deficiency of the vitamin in the tissues.

A full discussion of this significant subject would exceed the limitations of space in this chapter. It suffices to briefly mention the important contributions of Peters ('36) and coworkers at Oxford University, whose persistence in investigations of thiamin action has added greatly to an understanding of this problem. They have unequivocally demonstrated that thiamin is a catalyst in the oxidative removal of the lower degradation products of glucose metabolism. At first the Oxford workers (Kinnersley and Peters, '29) favored the lactate oxidase theory of thiamin action, basing their conviction, in part, upon an apparent increase in the amount of lactic acid in the brain of pigeons deprived of thiamin. Later a test for pyruvic acid was found (Peters and Thompson, '34) and it was demonstrated that pyruvic acid accumulates in the blood of thiamin-deficient pigeons (Johnson, '36). More recently Peters (l.c.) has discarded the lactate oxidase theory and now concludes that thiamin ". . . is related specifically to pyruvate oxidase in its aerobic reaction." This specific action manifests itself in two ways, (a) by increasing the oxygen uptake under precisely defined conditions, the so-called catatorulin effect, and (b) by decreasing markedly the pyruvate formed by thiamin-deficient brain tissue.

Sherman and Elvehjem ('36b), by means of a thiamin-deficient diet which produces polyneuritis within two weeks in chicks, further elucidated the subject when they showed that heart and kidney tissue acts similarly to brain tissue, with respect to pyruvic acid. Moreover, they have shown that the removal of pyruvate injected intravenously in polyneuritic chicks is much slower than in normal chicks. These data further suggest that in thiamin deficiency there is a failure in the tissue metabolism of pyruvic acid. The findings correlate beautifully with the demonstration of Lohmann and Schuster that a pyrophosphate of thiamin is cocarboxylase. Since this coferment has the catatorulin effect it strengthens the view that thiamin is indispensable in the metabolism of carbohydrate.

Finally, it is of importance to note that methyl glyoxal, the aldehyde of pyruvic acid, has been found in the blood and urine of beriberians and animals on a thiamin-deficient diet (Arakawa, '30; Chiba, '32; Uga, '35). Geiger and Rosenberg ('33) noted this carbohydrate degradation product in infants with toxic symptoms. It has been suggested by Platt and Lu ('36) that a determination of bisulfite-

binding substances (B.B.S.) such as methyl glyoxal and pyruvic acid in blood and urine may be used as a criterion in the diagnosis of thiamin deficiency. This is plausible but, as shown by Wilkins, Taylor and Weiss ('37), an increase in B.B.S. of the blood occurs in conditions probably unrelated to thiamin deficiency. It would seem that a promising procedure in the determination of mild degrees of deficiency is the estimation of B.B.S. before and after the adminstration of a standard dose of thiamin. Any decrease in the level, after thiamin dosage, might plausibly be attributed to an inadequacy of thiamin. The extent of this diminution would measure the degree of deficiency.

State of Cocarboxylase in Tissues.—Since the very recent discovery that cocarboxylase, essential in the decarboxylation of pyruvic acid, is the pyrophosphate of thiamin, a precise consideration of thiamin in carbohydrate metabolism must be in terms of its relation to cocarboxylase. The function of cocarboxylase in yeast fermentation is clear but its role in animal metabolism has not been satisfactorily elucidated. A question of prime importance is the form in which thiamin occurs in plant and animal tissues since this is significant as respects its availability in metabolic processes as well as its mode of action. Tauber ('37) has claimed that much of the thiamin in plants is unphosphorylated. According to Lipmann ('37) all of the thiamin in mammalian tissues is in the form of cocarboxylase. But the evidence in both instances is only indirect. Tauber ('38) has adduced evidence that phosphorylation of thiamin, i. e., formation of cocarboxylase, occurs in the presence of a duodenal enzyme preparation. The enzyme system cannot hydrolyze the coenzyme. Kidney tissue, according to Tauber, rapidly hydrolyzes cocarboxylase. The studies show that coenzyme is dephosphorylated by the kidneys before being excreted in the urine. Lipschitz, Potter and Elvehjem ('38) believe that thiamin and cocarboxylase exist in dynamic equilibrium *in vivo*. If thiamin must be phosphorylated before it can participate in metabolic processes it is conceivable that under some conditions phosphorylation is inhibited and, as a consequence, thiamin is not utilized. As shown by Lipschitz et al. (l.c.), phosphorylation is inhibited by iodoacetic acid. The action of cocarboxylase is not affected by iodoacetic acid.

Storage and Excretion.—The investigations previous to 1923 were inconclusive regarding storage and excretion of thiamin. In 1923 Osborne and Mendel found that the content of thiamin in the livers of rats is greatly reduced when the animals are deprived of that vitamin. Thus it was early learned that tissues must be constantly supplied with thiamin if they are to contain it even in moderate amounts. An indication of the rapid loss of the vitamin, when it is not replenished by the diet, is given in the work of Westenbrink ('32) who found that in rats the heart, liver, kidneys, muscle, lung, spleen,

stomach, and intestine become thiamin-depleted after only 5 weeks on the deficient diet. And, as claimed by Graham and Griffith ('32), the rapid loss of thiamin, under such dietary conditions, has no analogue with respect to components of the vitamin B-complex, assayed as a whole.

Leong ('37), using the bradycardia method of assay, has reported balance studies in which the test animals, adult rats, were given doses of thiamin ranging from 0 to about 700 I. U. per rat daily. The quantity in the feces was 0.5 to 1.5 I. U. per day, depending upon the bulk of the feces. But the urinary excretion became larger in proportion as the intake was increased. When scarcely any thiamin was given it amounted to 0.1-0.4 I. U. daily, a fraction of this being derived from the tissue stores. When the intake ranged from 7.5 to 31 I. U. daily, the urinary output increased from 0.6 to 12.8 I. U. When 250 I. U. were injected subcutaneously the amount excreted was about 75 per cent of the intake. A large percentage of this was in the urine. But with oral ingestions of 100 to 700 I. U. daily, about 30 I. U. were unaccounted for in the excreta, thus indicating the approximate amount of destruction in the body. Leong estimates, however, that in cases of thiamin deficiency of the diet a greater percentage of the vitamin withdrawn from the tissues is destroyed.

The significance of these data is indicated in relation to the utilization and retention of thiamin in man. For example, Knott ('36), on the basis of 23 balance studies conducted with 8 children from 4 to 7 years of age, concluded that the body is not capable of building up a significant reserve of thiamin, owing to the rapid depletion which occurs on low ingestion levels. The results indicate a definite trend toward higher retentions of thiamin when the level of intake is relatively high. The levels which resulted in highest retentions were found to be about 27 I. U. per kilogram of body weight. These findings are supported by those of Roscoe ('36) and Harris and Leong ('36). The former worker, using the rat curative test, could not find more than traces of the vitamin in urine from normal adults receiving a hospital diet. The addition of 720 I. U. of thiamin daily to this diet was followed by the daily excretion of 167 to 333 I. U. in the urine. It seems probable that a considerable amount of the remainder was retained, owing to inadequacy of the hospital diet in meeting the tissue needs for the vitamin. Harris and Leong, in their search for a simple method of estimating the state of thiamin nutrition in human subjects, also found a marked correspondence between the amount of thiamin in the diet and the level of excretion in the urine.

Undoubtedly the next few years will witness notable advances in knowledge of the storage and excretion of thiamin, since data of such nature are essential to an understanding of human and animal re-

quirements for the vitamin. We may safely conclude at present that appreciable storage of thiamin within the body has not been demonstrated and it probably cannot occur. This suggests the need for adequate amounts of the vitamin in the diet at all times.

Factors Affecting the Requirements for Thiamin.—At least three important factors may operate in determining the amount of thiamin that should be in the diet in order to satisfy the body's need for this vitamin. They are (a) the absorptive status of the gastrointestinal tract, (b) the degree of thyroid activity and (c) the amount and quality of the fat in the diet.

Scarcely anything can be recorded at present concerning absorption of thiamin since there is a paucity of clear-cut experimental work on this subject. Instances are on record, however, where parenteral dosage was more effective than oral ingestion of the vitamin. Examples of poor absorption are conditions in which food is not well digested, or in which there is diarrhea or inflammatory diseases of the stomach or intestines. An adequate discussion of the problem is given by Cowgill ('34, l.c.).

A considerable amount of data is accumulating regarding thyroid activity and thiamin. This is briefly reviewed by Carpenter and Sharpless ('37), whose studies suggest that some factor present in yeast, which is lost when the yeast is autoclaved and which is not supplied by a vitamin B-containing extract, causes an increase in the iodine content and concentration of the thyroid.

Sure and Buchanan ('37) have published results which show that in experimental hyperthyroidism protection can be afforded by ingestion of large amounts of pure thiamin. On the basis of these relationships Sure and Smith ('34) suggested that thiamin might be used in the treatment of toxic goiter, particularly in non-operative cases. There is considerable experimental basis for this view, particularly the thorough work of Cowgill and associates (Cowgill, '34, l.c.) which unequivocally demonstrates a high correlation between the total energy metabolism and the thiamin intake necessary to maintain good health.

Probably of relatively less practical importance is the so-called sparing action of fats on thiamin. Several years ago Evans and Lepkovsky ('29, '31, '32, '35) found that liberal inclusion of fat in the diet will enable an animal to withstand for many weeks the withdrawal or omission of thiamin from the diet and that in the presence of fat better growth will occur at any given level of thiamin ingestion than in the case with a fat-free diet. It was found that myristin and caprylin are more effective than others, and that natural fats are superior to any of the single glycerides. Tristearin exerted no sparing action; this they suggested was due to its poor absorption, its bulk

in the food causing partial starvation. Substantiation of their general findings has been published by several workers including Salmon and Guerrant ('30) and Whipple and Church ('36). But Kemmerer and Steenbock ('33), Sure and Buchanan ('35), Westenbrink ('35) and others have reported conflicting or contradictory results. Recently Salmon and Goodman ('37) have claimed that the effectiveness of esters of single fatty acids in alleviating the symptoms of thiamin deficiency in rats depends upon the length of the carbon chain of the fatty acid. The effectiveness was maximum at the 8-carbon acid and decreased in each direction from this point.

The significance of these data as it concerns the mechanism of thiamin action is, as yet, obscure. But the evidence that the vitamin acts as an essential factor in carbohydrate metabolism, at the pyruvic acid stage, raises the question as to the role which it may play in the synthesis of fat. Whipple and Church have shown that the main factor in the weight increase, following the administration of thiamin to deficient animals, is the accumulation of fats which, in their experiments, apparently could only be formed from carbohydrate. Moreover, the synthesis of fats by rats during response to thiamin therapy was indicated by the high respiratory quotient of such animals. McHenry ('37) has confirmed this observation. It is indicated, therefore, that in the absence of thiamin pyruvic acid accumulates, whereas in the presence of the vitamin fat is formed, presumably with pyruvic acid as an intermediary substance. The latter is supported by the discovery of Krebs and Johnson ('37) that hydroxybutyric acid can be formed from pyruvic acid by the tissues. On the basis of these observations it is at least very interesting that the thiamin content of pork is relatively much higher than in beef. The hog is exceptionally able in the conversion of carbohydrate into fat.

Other factors affecting the requirements for thiamin might be discussed but it suffices to mention the work on riboflavin as a sparer of the vitamin. Evans, Lepkovsky and Murphy ('34), as well as others, had noted that autoclaved yeast, presumably without thiamin activity, appeared to spare thiamin in the rat. Ellis and Zmachinsky ('37) studied the question and found that growth and length of survival in rats deprived of thiamin could be increased by supplementation with riboflavin.

Thiamin Requirements of the Body.—Owing to the apparent lack of ability for storage of thiamin in the body, and in view of its rapid loss from the tissues during short periods of deficiency, it would seem that optimum nutrition, with respect to this factor, cannot be maintained unless the diet regularly contains it in adequate amounts. At present, however, although there are many published estimates of requirements for various bodily functions, based in only

a few instances upon carefully controlled and dependable procedures, there is no final basis for opinion as to the thiamin requirement for any bodily function. Without the assemblage of lengthy arguments in support of this view, one has only to cite the fact that no method is yet known for the reasonably accurate estimation of border-line degrees of thiamin deficiency. Without even a good criterion for such a status it is presumptious to discuss with certainty thiamin requirements in terms of absolute units. But it is important to utilize such information as is available for it is useful and dependable for very rough estimations.

The present knowledge is based in considerable measure upon Cowgill's ('34, l.c.) comprehensive investigations of many types of diets. In general he has found that the minimum thiamin requirements tend to vary greatly between individuals, and in the same individual under different physiological conditions. Cowgill's attempts to express mathematically the thiamin requirement has resulted in the proposal of a formula which embodies the body weight and energy requirement as variables. Probably the formula anticipates important information which is not yet established but as an approximation of the general truth it does appear to be of some value. It is:

Thiamin requirements = Body weight + Caloric requirement × a constant.

Possibly important practical factors which are not represented in the formula are: (a) ratio of carbohydrate in the diet to fat and protein, (b) water excretion, (c) state of thiamin in the food, and (d) absorptive capacity of the gastrointestinal tract.

It is of some interest and value to cite certain opinions regarding thiamin needs. The Council on Pharmacy and Chemistry of the American Medical Association ('36) concluded, on the basis of knowledge available in 1936, that the minimum daily requirement is not less than 50 I. U. for infants and 200 I. U. for the average dult. Daniel and Munsell ('37), in evaluating the adequacy of diets with respect to thiamin, have used 200 to 300 I. U. per adult per day as a tentative standard. For average adults 400 to 500 I. U. per day is regarded by Rose ('38) as a liberal intake. But Baker and Wright ('36) claim that they have observed actual cases of beriberi in persons whose diets presumably contained over 300 I. U. per day. In all probability gastrointestinal absorption in these persons was very inefficient. Thus we see that present numerical standards are essentially expressions of opinion inadequately substantiated by facts.

The question of numerical standards for children, pregnant and lactating women, etc., is even more confusing than in the case of

normal adults. On the basis of present indications it would seem that infants, children, and adolescents require larger daily intake than adults in proportion to the caloric needs.

Sure ('28), Macy et al. ('30), and others have found that the "vitamin B" requirements of the rat for lactation are three to five times the amount necessary for the support of normal growth in young rats. Although milk is relatively low in thiamin, as compared with whole grain cereals, Daniels and associates ('29) did not observe symptoms of thiamin deficiency in young rats suckled by mothers restricted to a milk diet. The young were debilitated but this was attributed to the inability of the maternal rat to ingest sufficient milk to meet the caloric requirement of lactation. It is possible, however, that the maternal rats failed to ingest sufficient milk owing to poor appetite caused by thiamin deficiency. The evidence undoubtedly suggests that women during pregnancy, and especially during lactation, should receive considerably greater amounts than normal adults.

Since there is no reason to believe that this vitamin is injurious unless ingested in amounts far beyond the range of probability, it would be a safe practice, where conditions permit, for all classes of people to take some rich source of thiamin in the diet.

The Extent of Thiamin Deficiency in Western Countries.—The general experience of mankind shows that many different types of diets, forced upon people by reason of geographic and soil conditions, have nourished them adequately in so far as the prevention of beriberi is concerned. It is only in those regions where polished rice has been consumed in excessive amounts, with consequent restrictions of the intake of other and better constituted foods, that this disease has been endemic. However, with the advent of the machine age, inventive genius has perfected the technic of refining wheat, corn, and other cereals to a degree hitherto unknown. And in addition, in Western countries, the consumption of sugar has increased from a few pounds per person per year a little over a century ago, to upwards of 100 pounds per capita today. This increase is not uniform as respects individuals. Many adults eat sweets little or not at all, and have no craving for sweet foods, but individuals who go to great extremes in the consumption of sweet foods are numerous. It has been frequently pointed out during the past 25 years that, as a nation, we are eating too large a proportion of our nutrients in the form of refined and manufactured products. This practice constitutes a new experience for the race, and there are numerous records which demonstrate that Western dietaries are frequently of poor quality in respect to several nutrients, among them, thiamin. As a basis for consideration of the problem, a few diets which have produced beriberi may be considered.

Chamberlain ('15) reported that among 5,000 Philippine Scouts there were always 100 to 600 incapacitated from beriberi. Their diet consisted essentially of:

	Ounces Daily
Beef	12
White flour	8
Potatoes or onions	8
Polished rice	20

On changing the diet by substituting 16 ounces of unpolished rice and 1.6 ounces of beans for the 20 ounces of polished rice, and including 20 ounces of sweet potatoes, the number of cases of the disease soon decreased to 50; during the following year to 3 cases, and during the third year 2 cases, and thereafter none. Hehir ('17) reported that during the siege of Kut-el-Amara, from December, 1915, to April, 1916, the British troops ate white flour biscuits, canned meats and fresh cooked horse flesh as their sole food. Many cases of beriberi appeared. No scurvy was diagnosed. The Indian troops used dhal or dry pulses of various kinds and either atta or barley flour, and refused meat. They were free from beriberi but suffered severely from scurvy. In the light of our present knowledge the freshly cooked horse meat protected the British against scurvy, and the unrefined cereals protected the Indians against beriberi. Little ('12) called attention to the frequent occurrence of beriberi in Newfoundland and attributed it to the excessive use of refined wheat flour. Aykroyd ('33) states that beriberi in Newfoundland is closely associated with poverty. The cheapest food there is white flour. The severity of winter allows little opportunity for hunting and fishing, and most poor families lay in a store of foods to last from November till June. If money is short, owing to a poor summer of fishing, they include more white flour and less meat, milk, peas, and potatoes. In May, after they have subsisted for six months on white bread and tea, and small other additions, they develop beriberi. To cite a further example, Scott and Herrmann ('28) described the occurrence of beriberi among farmers in Louisiana. They produce animals for winter meat, chickens, eggs, milk, and vegetables of various kinds, but they sell these and buy polished rice as their staple cereal. These farmers are rice growers, and sell all their rice crop instead of keeping some of the unpolished grain for food. In autumn, it is said, they eat little else than polished rice and bacon grease, their "riz et sauce." When winter comes they add meat from home slaughtered animals, potatoes, and bread, after which no new cases of "maladie des jambes" (beriberi) occur. Those who have confidence in appetite as a guide to the selection of food should note that these farmers eat their "riz et sauce" three times a

day, virtually excluding all other foodstuffs except during the winter months.

Cowgill ('34, l.c.) made a critical study of the "vitamin B" (thiamin) content of typical human dietaries in different parts of the world, and concluded that deficiency of thiamin is relatively common. He recommended to physicians the consideration of thiamin therapy in gastrointestinal disorders, cardiac dysfunctions, various neurological conditions, anemias, infant nutrition, anorexia, restriction of growth, and in conditions of heightened metabolism. As shown recently by Vorhaus ('37) and others, thiamin has been used successfully for each of these purposes.

Borderline States of Thiamin Deficiency.—McCollum has repeatedly called attention to the tendency, in industrialized communities especially, for wage-earning people to subsist largely upon "ready to serve" or "grocery store" foods. Many foods of such selection are semi-perishable products composed largely of refined cereals, sugar, and slightly rancid fats. Moreover, they have been subjected to high heat and are aged for a considerable time before they are sold. Such foods tend to be deficient in all vitamins, and their liberal consumption crowds out of the diet foods such as milk, fruits, vegetables, and fresh meats. The regular consumption of such staple foods is likely to bring about partial deficiency of any or all of the vitamins. Ill health may result from a slight deficiency of thiamin, or any other essential nutrient, much less severe than is necessary to bring about the typical syndrome associated with its complete absence from the diet. Thus there are, for example, many degrees of iron or iodine starvation, recognizable as anemia or disturbance of thyroid function, respectively. It is with such borderline states of thiamin deficiency and their consequences that clinicians are now becoming increasingly concerned.

In human experience it seems certain that in most cases where thiamin deficiency occurs, but of a degree which falls short of inducing well-marked signs of beriberi, the diet is generally of a sort which causes multiple deficiency involving other vitamins, iron, calcium, iodine, protein, or protein of poor quality, etc. In such cases the remedy is not a source of thiamin alone but a better food supply.

Minot ('36) has given us illuminating examples of patients whose disorders arose from improper diet, but whose illnesses were extremely difficult to diagnose correctly, because the description of their diets was such as to suggest that they were satisfactorily nourished. But on careful inquiry it was found that although a nutritious diet had been placed before them, they had been fussy and particular about eating it. Although they ate a little of many kinds of food, they had, over long periods, selected principally dextrinized food, biscuits pre-

pared for invalids, and had eaten meat but scantily, while fruits and green vegetables had never been taken in more than minimal amounts, the latter particularly in puréed form. The "tea and toast" diet is notorious in the case of the chronically ill. Persons who suffer from digestive disturbances generally assert that a number of the better class foods do not agree with them, and accordingly decline to eat them. Thus a vicious cycle tends to become established. It has also become evident that nutritional deficiencies may arise even though the individual has an adequate diet. This is possible in conditions of abnormal digestion, absorption, utilization, and metabolism. Such conditions are seen in chronic diarrhea, achylia gastrica, dysentery, vomiting of pregnancy, or other causes.

Thiamin Craving in Rats.—Richter, Holt and Barelare ('37) noted that rats deficient in thiamin ate dried baker's yeast with keen relish, and inferred that the incentive was due to its richness in thiamin, the ingestion of which relieved the deficiency. Following this they have demonstrated that such rats show an overmastering appetite for thiamin in the pure form. The odor as well as the taste of thiamin aroused great interest, and the rats found at once the bottle containing it in solution when as many as 12 containers were available, furnishing different foods or solutions. Once they had tasted it they were avid in drinking the solution. One rat drank 11 cc. or 5,500 I. U. in less than half an hour; another drank 29 cc. or 14,500 I. U. in 24 hours. Efforts to remove the bottle were met by fierce resistance, the bottle being held tightly with paws and teeth. Richter and associates suggest that the craving for thiamin does not depend upon the experience of a beneficial effect from taking it since the ravenous appetite for it is immediately manifested when the nutrient is placed before the deficient animals. Although this work is interesting and important, it should not be concluded, on the basis of it that humans will select foods in accordance with their nutritive requirements, even though free choice is provided. The higher intelligence of humans is accompanied by all sorts of acquired ideas, bizarre indeed in some instances, and powerful, which repress "instinctive" demands if they potentially exist.

"Vitamin B" Supplements in the Feeding of Infants and Children.—There are several direct experiments on record which support the opinion that typical dietaries of infants and children would be markedly improved by the use of some rich source of thiamin. It appears likely that anorexia which is prevalent in early years (Bartlett, '28), might be attributable in many instances to an inadequacy of thiamin, although faulty training and environment are undoubtedly considerably responsible for "feeding problems." As stated several years ago by Schultz ('25): "A complaint for which

the child is brought to the physician more often than any other is persistent anorexia with cessation of gain of weight. . . . A problem is to provide abundant vitamin supply, particularly the water soluble vitamin B." It will be recalled that Cowgill has shown that anorexia is the first symptom of thiamin deficiency in animals.

Morgan and Barry ('30) reported a study of two groups including from 31 to 47 underweight children from 11 to 13 years of age who were compared as to growth in weight, height, and certain other physical indices of health over three periods, totaling 30 weeks. Each child in one of these groups in each period was required to include in the noon meal 3 ounces (85 gm.) of rolls made with 50 per cent wheat germ and 50 per cent of white flour. The control group took the usual white flour rolls; thus the former group ingested a total of 5 ounces of wheat germ each week. The weight increases in each of the wheat germ periods were about three times as great as in the control periods. The difference in all cases was five or more times greater than the probable error of the difference. The height increases were significantly greater in two of the wheat germ periods than in the corresponding control periods. These investigators point out that there may be a relation of the decreasing "vitamin B" content of the modern diet to decreased appetite in children, nervousness, constipation, and possibly other gastrointestinal disturbances. This view appears to be substantiated by the observations of Elias and Turner ('36) that infants without extra "vitamin B" in the diet had a slightly greater incidence of anorexia than other infants fed supplements of wheat germ and yeast.

Summerfeldt ('32) reported that when a group of normal children were given a special cereal mixture rich in "vitamin B" and the essential mineral elements, in place of the commonly used cereals, during a ten-week period, a marked increase in weight resulted. Ross and Summerfeldt ('35) substituted 3 ounces of a special cereal mixture rich in these nutrients for an equivalent amount of ordinary cereals in the diet of normal children, and observed an increase in weight 2.3 times the expected gain over a period of six months. However, they did not attribute the beneficial effects exclusively to the high "vitamin B" intake. In another experiment normal children fed a "good" diet showed an increase in hemoglobin content during six months from 10.4 to 10.7 gm. per 100 cc. of blood; whereas when a vitamin concentrate free from copper and iron was added to the diet the hemoglobin increased from 10.1 to 11 gm. Replacement of the ordinarily used cereals by the special cereal mixture resulted in an increase of hemoglobin from 10.2 to 12 mg. Their ordinary cereal consisted of oatmeal or cracked wheat. The special cereal consisted of the same, but supplemented with 6 gm. of a "vitamin B" con-

centrate, made from wheat germ and brewer's yeast, for each 3 ounces.

It is obviously impossible to translate the present results of feeding studies on infants and children into terms of thiamin since all of the "vitamin B" sources that have been used contained other factors which might have affected the results. However, the available facts seem to suggest that thiamin is a significant limiting factor in the diets of many infants and children.

The Therapeutic Uses of Thiamin.—Conscientious and thoughtful nutrition investigators regard the application of the knowledge in nutrition to clinical problems as a responsibility to be shared by both the clinician and nutrition specialist. Without this attitude of mutual dependence, mistakes of over-enthusiasm and misinterpretation of facts are likely to occur. It is hoped, therefore, that we can strictly maintain this essential point of view in the following brief discussion, which represents our interpretation as nutrition specialists of present information and trends in certain clinical possibilities of thiamin. Moreover, it is our intention that this view shall obtain in all other discussions in this book concerning clinical applications of modern nutrition.

The previous discussions have indicated the observable effects of thiamin deficiency, and have reviewed the physiological action of this nutrient, in addition to the normal bodily requirements and some of the factors which affect the requirements for it. Thus the factual bases are laid for the present consideration.

Vorhaus (l.c.) has recently reviewed the subject of thiamin therapy and has differentiated the various clinical conditions in which the vitamin appears to be of particular use from those in which its use in large amounts is apparently without value. Some of the conditions in which it has been employed with definite success are: neuritis associated with pregnancy; heavy metal neuritis, such as lead, and arsenical neuritis occurring during intensive treatment for syphilis; trigeminal neuritis; certain so-called diabetic conditions; certain alleged gastrointestinal disorders; and various types of anorexia, particularly those associated with long standing illnesses. To this list may be added the important observations of Weiss and Wilkins ('37, l.c.) and others, that thiamin gives striking improvement in certain types of cardiovascular dysfunctions. Also, the value of this vitamin in the treatment of alcoholic polyneuritis (Jolliffe et al., '36) and the indications of its value in hyperthyroidism (Means, et al., '37) must be mentioned. Space is too limited to discuss at length the use of thiamin in these various conditions, but it is profitable to give some attention to certain ones of them. The subject has been thoroughly reviewed by Williams and Spies ('38).

Pregnancy.—Strauss and McDonald ('33) point out that it is customary to refer any and all pathologic conditions developing during pregnancy as manifestations of some occult toxemia, relief from which may be secured by termination of the gravid state. These involve the anemias, the polyneuritis, the vomiting, and eclampsia of pregnancy. Whitfield (1889) appears to have first attributed polyneuritis of pregnancy to persistent vomiting. Berkwitz and Lufkin ('32) reviewed the literature relating to 52 cases of polyneuritis of pregnancy, and concluded: "The clinical and pathological picture of the nerve changes in pregnancy is the same as that resulting from alcoholism, infectious conditions, and diet deficiency disturbances such as beriberi and pellagra. *The exact nature of the toxins producing neuritis is still unknown.*" (Italics ours.) Strauss and McDonald conclude from their studies that the polyneuritis of pregnancy is probably a dietary deficiency disorder similar to beriberi, and that the rational therapy should aim at provision of the deficient nutrients, especially the vitamin B complex. This view is supported by Plass and Mengert ('33), Theobald ('36), Vorhaus ('37, l.c.) and Schwochow ('37), all of whom have noted in many cases marked improvement or disappearance of the polyneuritic manifestations following thiamin treatment.

The polyneuritis of pregnancy would seem to have its origin in several causes or associated factors, among which are anorexia and vomiting of pregnancy. Plass and Mengert have pointed out that in the forcing of high carbohydrate diets on patients with vomiting of pregnancy there would seem to be increased risk of producing vitamin deficiencies. Depletion of thiamin, of course, might be expected since carbohydrate foods tend to increase the requirement for this nutrient.

Cardiovascular Dysfunctions.—Although Weiss and Wilkins ('36, l.c., '37, l.c.) have reported striking effects from thiamin therapy in several cases of cardiovascular disturbance, it is not advisable to discuss the subject here more than to emphasize the general correspondence between the heart symptoms and electrocardiographic data in these clinical cases and those of experimental animals whose diet was deficient in thiamin. The question is naturally raised as to whether chronic deficiency of thiamin over a period of many years might not be related, in part, to the high incidence of cardiovascular dysfunctions occurring long before the end of normal life expectancy.

Alcoholic Neuritis.—Jolliffe and Colbert ('36) have reviewed the literature relating to the etiological factor in the development of polyneuritis in the alcohol addict. Thiamin deficiency was first suggested in this connection by Shattuck ('28). Later Strauss ('34) and Blankenhorn and Spies ('35) showed that patients with alcoholic neuritis improved if treated with a diet rich in several vitamins, even

while they were given one to two pints of whiskey daily. Hence, Strauss concluded that alcohol, taken as whiskey, has no demonstrable toxic effect on the peripheral nerves. More convincing evidence that thiamin deficiency is the etiological factor was furnished by Jolliffe and Colbert, who obtained dramatic alleviation of polyneuritis in one patient, and at least good response in another, treated with pure thiamin. Many other cases studied by Jolliffe et al., as reported in their numerous papers on the subject, have responded favorably to treatment with thiamin concentrates.

Cowgill's important work, which definitely establishes a direct relationship between caloric intake and thiamin requirement, constitutes a basis for the explanation of the efficacy of thiamin in prevention and cure of alcoholic neuritis. Since alcohol has a relatively high caloric value and alcoholics tend to subsist haphazardly on foods low in thiamin, the combination of these two antagonizing factors readily causes thiamin deficiency—consequently alcoholic neuritis.

Gastrointestinal Disorders.—Mackie and Pound ('35), among others, have discussed the changes in the gastrointestinal tract in nutritional deficiency states. They emphasize that disease may result from a defective dietary or from abnormal physiology of the gastrointestinal tract, and that defective absorption constitutes one of the outstanding features of sprue, as was earlier pointed out by Castle and Rhoads ('32). Atrophy, resulting in impaired absorption, occurs without extensive changes in the structure of the small intestine apart from the atrophy that is not disproportionate to the general wasting of the body and the viscera. In a series of 75 cases of chronic ulcerative colitis they found evidence of nutritional deficiency in 63 per cent. Their appraisal of the diets indicated that the deficiency was multiple, including not only vitamins but also protein, and essential inorganic elements. The changes which they were able to find in the intestinal mucous membrane were those associated with edema, disorganization of the normal motor activity, and reduction in tone of the intestinal musculature.

Borsook and associates ('38) have reported their clinical experience with a series of 227 cases of chronic gastrointestinal malfunction which were treated for periods of 3 months to 4 years by means of a special dietary regimen. They eliminated starchy and sweet foods and instructed the patients to take daily at least a pint of milk, 1 glass of orange juice or tomato juice, 1 egg, 1 liberal helping of such vegetables as spinach, carrots, string beans, broccoli, lettuce or asparagus. The water in which the vegetables were cooked was added to the food. If more food was desired, it was allowed in the form of more milk, meat, and vegetables. In addition, each patient was given a daily supplement of 100 gm. of a cereal containing 10 to 15 per cent of

wheat germ and the finely ground bran layer, and assaying 5 to 10 I.U. per gm. of thiamin. Also, it was rich in riboflavin and other "B complex" factors. Since the improvement in gastrointestinal function, including elimination, was so marked, they concluded that the results indicate the existence of a widespread partial "B complex" deficiency, and that most people require for normal gastrointestinal function several times the minimum amounts of the water-soluble vitamins required to prevent severe deficiency diseases such as beriberi and pellagra.

But, as pointed out by Vorhaus (l.c.) symptoms of gastrointestinal dysfunction are often vague and it is difficult to elicit a reliable history from patients with complaints of this sort. Hence, it is a problem to secure objective and trustworthy evidence of the role of thiamin in this class of disturbances. At best it would seem, from present indications, that constipation, flatulence, etc., are not specifically attributable to thiamin deficiency, although it may accompany such conditions.

Likewise, the role of thiamin in treatment of anorexia is not clear. Undoubtedly many instances of poor appetite are attributable to an inadequacy of thiamin, but owing to the inability to clearly define or measure appetite, it is not possible to determine the role of this nutrient in the problem.

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CHAPTER

XX

Riboflavin

Historical Development.—Until 1932 no one suspected that the natural water-soluble, yellow-greenish fluorescent pigments occurring in plants and animals were of unique significance in nutrition.

The first report of the occurrence of one of these was that of the yellow pigment of milk whey detected by Shoepf in 1784 (cited by Blyth, 1896) in a study of the composition and analysis of foodstuffs. In 1879 Blyth obtained this pigment from whey in the form of an impure resinous orange-red mass and named it "lactochrome." The first chemists to study the problem of isolation and identification of the yellow-green fluorescent pigment of milk whey were Bleyer and Kallman ('25). They obtained a crude preparation of this naturally occurring substance.

Isolation of the substance in pure form progressed slowly until the significance of yellow pigmented compounds obtained from whey (Bleyer and Kallman, l.c.) and heart muscle (Banga and Szent-Györgyi, '32) was indicated by the fundamental studies reported by Warburg and Christian ('32a), concerning a "yellow respiratory ferment" which they had prepared from bottom yeasts. These workers ('32b) succeeded in splitting the ferment into a protein component and a pigment component and noted that neither fraction alone was catalytically active.

In 1933 a number of naturally occurring, water-soluble, nitrogenous dyes had been isolated independently from various sources by different groups of investigators and their growth-promoting properties recognized. Ellinger and Koschara ('33) had separated from milk, liver, muscle, kidney, urine, yeast, and various plants three crystalline dyes, reddish-yellow in color, which were rich in nitrogen and oxygen and showed strong yellow-green fluorescence in aqueous solution.

They suggested that these compounds were related to Warburg-Christian's oxidation ferment.

While attempting to isolate vitamin B_2 from whey and egg white, Kuhn and associates ('33) had separated in pure form nitrogenous, water-soluble, yellow pigments displaying strong green fluorescence. These dyes resembled spectroscopically Warburg-Christian's pigment obtained from yeast. They noted the similarity in distribution and properties of vitamin B_2 and these yellow pigments and called attention to their probable relation to the yellow enzyme.

At the same time, Booher ('33, '34) also confirmed the fact that the "vitamin G growth promoting activity of whey is associated, at least partially, with its water-soluble, yellow fluorescent pigment."

Nomenclature.—There is much confusion in the literature relating to the vitamins of the B_2-complex. This state of affairs will probably continue until the nature of all the components of this complex is made clear.

Vitamin G and vitamin B_2 are terms which were employed to designate the heat-stable fraction of the B-complex after Smith and Hendrick ('26) had shown that the B-complex consisted of a heat-labile and a heat-stable portion. Goldberger and coworkers ('26) named the latter the P-P (pellagra-preventive) factor. In America it has generally been called vitamin G, whereas in Europe the same substance was known as vitamin B_2. During the intervening years these terms have been employed to designate the antipellagra factor.

When Kuhn and his associates and other investigators proved by biological tests that the isolated yellow pigment is an indispensable nutrient, they applied the generic term "flavin" to these water-soluble yellow pigments isolated from various sources and grouped together under the term "lyochrome." Specific designation has been assigned to the flavin isolated from different compounds, e. g. lactoflavin from milk, ovoflavin from egg, hepatoflavin from liver, etc. Subsequent experiments definitely established that all these flavins are identical, and it appears that there is but one biologically important flavin. These investigators were wrong, however, in their assumption that flavin was B_2 or G, and in assigning the term B_2 to designate flavin they made the literature most confusing. There were two main reasons for this confusion—one was that B_2 and G in the minds of all research workers at that time meant the same as Goldberger's P-P or antipellagra factor, which the flavin is not; the second was that the rat which was the experimental animal employed in these studies presented a symptomatology of rat pellagra generally believed to be the analog of human pellagra. But this assumption was incorrect, for rat pellagra turned out to be caused by a deficiency of what is now known as vitamin B_6. Space does not permit the recording of

the details of this confusion in experimental data. The history of these erroneously interpreted experiments has been discussed by Sherman and Sherman ('37) and Hogan ('38).

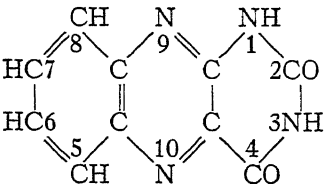
With the further investigation of the flavins and the elucidation of their chemical nature, The Council on Pharmacy and Chemistry of the American Medical Association ('37) proposed the use of the term d-riboflavin or simply riboflavin as the name for the nutritionally essential flavin and as a substitute for the terms vitamin G or vitamin B_2. This designation, based on the chemical nature of the substance, was later approved by the American Society of Biological Chemists and the American Institute of Nutrition. In Europe this substance is still generally called lactoflavin, irrespective of its source.

Isolation and Synthesis.—The isolation of riboflavin from many naturally occurring substances was first reported by Kuhn and his associates ('33, l.c.) and has been confirmed independently by the work of several laboratories. The methods of isolation used by different investigators are basically similar in principle. The general procedure consists of:—extraction with aqueous acid solutions, with alcohol, or with water-alcohol mixtures; precipitation or adsorption of the flavin (the adsorption methods are regarded as more reliable and greater yields are obtained); elution of the pigment with aqueous or slightly alcoholic mixtures containing alkaline compounds such as ammonia, pyridine, sodium hydroxide or diethylamine; removal of impurities from the eluate and concentration, precipitation, and purification by means of thallos or silver salts of the riboflavin.

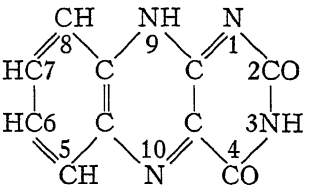
Warburg and Christian ('33) while studying their yellow enzyme or its pigment component isolated a photoderivative from the pigment of the yellow enzyme and had made clear many of its more outstanding properties. They observed that when the pigment component was exposed in alkaline solution to ultraviolet or ordinary light it underwent a decomposition in which the water-soluble substance was changed into a yellow photoderivative (lumiflavin) which is with difficulty soluble in water, but readily soluble in chloroform. They separated it by acidifying the solution at intervals and shaking with chloroform. This new pigment had the empirical formula $C_{13}H_{12}N_4O_2$. Its absorption spectrum resembled that of the original yellow one. The photoderivative readily lost its yellow color on reduction with hydrogen in the presence of palladium, and when warmed in a solution of barium hydroxide, it liberated urea.

Kuhn and Rudy ('34) followed up this observation and found that irradiation in alkaline solutions caused the splitting off of a hydroxyl-rich side chain, and the formation of Warburg and Christian's insoluble yellow lumiflavin. Since the original lactoflavin had the composition $C_{17}H_{20}N_4O_5$, and the photoderivative the composition

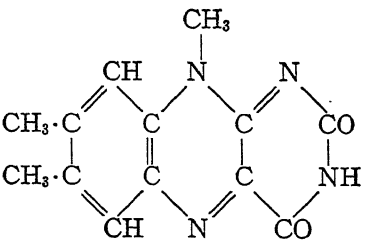
$C_{13}H_{12}N_4O_2$, it was evident that the hydroxyl-rich portion split off by light must have the composition $C_4H_8O_4$. That there were in the side chain thus split off four hydroxyl groups was shown by the fact that lactoflavin formed a tetra-acetyl derivative. Booher ('38) has reviewed the steps by which Kuhn and associates arrived at the conclusion that lactoflavin and lumiflavin belonged to the class of compounds known as alloxazines, which had been discovered and studied by Kühling (1891). Another excellent review of the synthetic work on the flavins is furnished by Turner ('34).



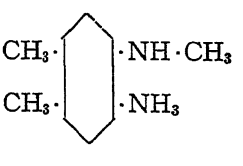
Alloxazine



Isoalloxazine

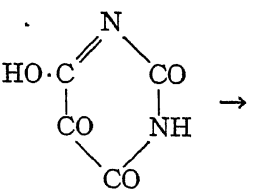


6, 7-dimethyl-9-methyl-isoalloxazine
Lumiflavin.

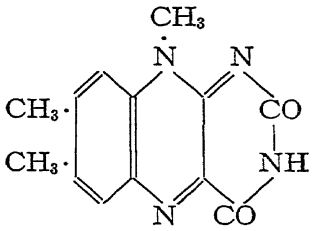


N-methyl-4, 5-diamino-
o-xylene

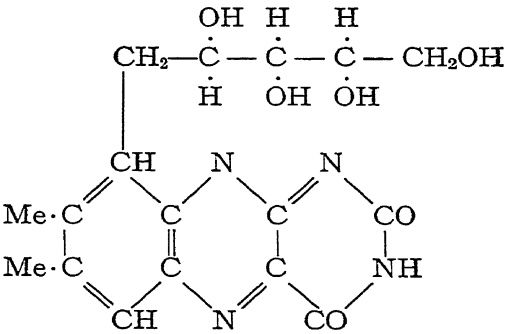
+



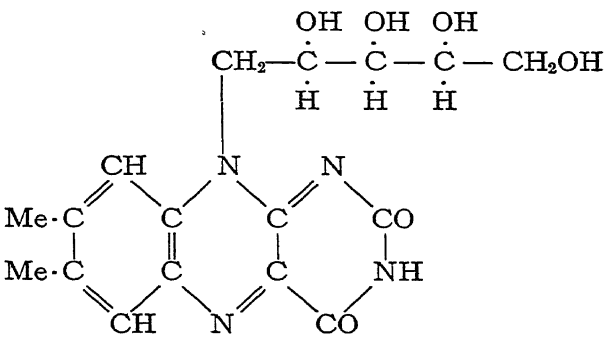
Alloxan



Lumiflavin



6, 7-Dimethyl-9-*l*-araboflavin
Araboflavin



6, 7-dimethyl-9-(*dl*'-ribityl)-isoalloxazine
Riboflavin

The synthesis of l-araboflavin and d-riboflavin was effected by Kuhn and coworkers ('34, '35) by a method similar to that employed in preparing lumiflavin, by starting with m-diamino-xylene, in which one hydrogen of an amino group was replaced by the sugar group instead of a methyl group. It is of interest that the two apparently inactive methyl groups in the nucleus of the flavin molecule are essential for the biological activity of the compound. Both araboflavin and riboflavin have been shown by animal assays to be biologically active, but the naturally occurring flavin from various foodstuffs is d-riboflavin. Araboflavin is only about one-third as potent as riboflavin.

A second synthesis of flavin compounds has been successfully accomplished by Karrer et al. ('35a, '35b, '36) with results substantially in agreement with those of Kuhn. The methods employed by Karrer for the preparation of the necessary intermediates are somewhat different and give better yields. The final stage of the synthesis is practically the same as that of Kuhn.

Chemical and Physical Properties of Riboflavin.—Riboflavin crystallizes as yellow-brown needles which form clusters of slender prisms and is bitter to the taste. It has the empirical formula of $C_{17}H_{20}N_4O_6$ and is a 6, 7-dimethyl-isoalloxazine with a sugar-like (d-ribose) side chain in position 9, as represented above. The isoalloxazine nucleus is responsible for the color of flavin. The constitution of riboflavin demonstrates a relation to different groups of organic substances; namely, to the sugars, to the purines, to the azine pigments, and to benzene derivatives. It does not melt sharply but darkens at 240° C. and decomposes at 267-294° C.

Riboflavin is soluble in water (25 p.p.m. at 25° C.) and slightly soluble in alcohol. It is insoluble in the usual fat solvents. When the d-ribityl side chain has been split off by illumination in alkaline solution, the resulting methyl derivative, lumiflavin, is soluble in fat solvents. Riboflavin is relatively highly heat-stable. It is sensitive to change in alkaline solution at elevated temperatures but is stable in even strong acids. It is not altered by atmospheric oxygen or other oxidizing agents including the halogens. Riboflavin is "reversibly reduced," that is, it takes up hydrogen from reducing agents and gives it up easily under the influence of atmospheric oxygen. It is destroyed by visible and ultraviolet light. In neutral or acid solutions, irradiation produces lumichrome, which is like lumiflavin except that the 9-methyl group is replaced by hydrogen. Lumichrome exhibits an intense blue fluorescence. Aqueous solutions of riboflavin are yellow in color and show yellow-green fluorescence in natural light. This fluorescence is at a maximum between pH 6 and pH 7 and is markedly intensified when examined in the ultraviolet rays.

Riboflavin, lumiflavin, and Warburg's yellow enzyme show well-

defined absorption maxima which are closely similar. The maxima for riboflavin are at 220, 267, 366, and 446 millimicrons. The green fluorescence lies in the region from approximately 500 to 630 millimicrons.

Riboflavin yields with sodium bisulfite, sodium hydrosulfide, zinc dust and acid, or hydrogen and platinum, a leuco-derivative which reverts to the original dye on oxidation.

The Nature and Function of Riboflavin.—The relationship between certain vitamins of the B-complex and some enzymes is of prime importance in metabolism. Several of the enzymes which are concerned in the oxidation-reduction reactions of the cells are conjugated proteins in which the conjugating or prosthetic groups contain one of the vitamins of the B-complex. In each of these cases the vitamin, or a molecule of which the vitamin is a part, has been coupled with a protein carrier. The combination exhibits either enzymatic activity or, in certain instances, may be a member of a system which is necessary for the activation of an important enzyme. Riboflavin is a member of the flavin system, an important oxidative chain in the organism.

The Warburg-Christian yellow oxidation pigment was shown by the researches of Kuhn et al. ('36), Theorell ('34a, '35a), and others to consist of two parts: (a) a flavin phosphoric acid capable of undergoing reversible oxidation and reduction, and (b) an enzyme-like protein component with which the flavin phosphoric acid is in intimate association. Warburg now gives the name "flavin enzyme" to the combination of these two components.

Warburg ('34) has demonstrated that riboflavin is the active group of the yellow oxidation enzyme which appears to be present in varying amounts in practically all living cells and tissues. Its vitamin activity is believed to be due to the formation of riboflavin-phosphoric ester (Rudy, '35; Theorell, 34b, 35b; Kuhn and Rudy, '35; Theorell et al. '35). This ester, also known as the yellow coenzyme, forms with a specific protein (Theorell, '35a, l.c.) the yellow oxidation enzyme which has a significance in cell respiration. Flavin is present in certain tissues such as liver, kidney, heart, etc., in the form of the phosphoric ester bound to protein, while in substances such as milk it is believed to exist in the free form.

Recently, Corran and Green ('38) isolated a flavin-protein compound from cow's milk. The colored prosthetic group of the compound can be split off from the colorless protein. A solution of the prosthetic group exhibits the characteristic yellow-green fluorescence of flavin. The absorption spectrum is practically identical with that of riboflavin. The prosthetic group does not seem to be riboflavin, but evidence indicates that it may be the corresponding phosphorylated compound. From their observations Corran and Green believe that

milk flavoprotein acts catalytically not by serving as an intermediary between reduced coenzyme and the carrier, but by forming a complex with the reductant and the oxidant and thus facilitating the transfer of hydrogen.

Riboflavin is an important factor actuating cell combustion processes as the oxidation of sugars. It also complements the iron-containing respiration enzyme. Its growth-promoting property is partially or totally destroyed by the photochemical transformation of flavin by visible light into the blue fluorescent photochrome.

Evidence indicates that riboflavin is not synthesized by the animal cell; consequently it must be supplied by the diet. Hence the collapse of animals depleted of riboflavin is, no doubt, due to the cellular asphyxiation resulting from the lack of a cellular oxidation catalyst.

Development and Symptomatology of Riboflavin Deficiency in the Rat.—The production of an uncomplicated riboflavin deficiency was very difficult owing to the confusion existing as to the preparation of a diet free from this factor but adequate in all other respects. The symptomatology produced by the lack of riboflavin in the diet is still not entirely clear.

A survey of the early literature shows that Goldberger and Lillie ('26), who were attempting to produce pellagra in the rat, were probably the first to describe the symptoms of riboflavin deficiency in the rat. The main symptoms encountered in their experiments were arrest of growth; following this, a tendency of the lids of one or both eyes to adhere together with, in some cases, an accumulation of dried secretion on the margin of the lids. Subsequent to or at the time of the appearance of the ophthalmia, alopecia developed in most of the rats, leading in some of the animals to almost complete denudation of the head, neck, and trunk. The denuded areas were sharply outlined and bilaterally symmetrical. In addition to these as described in Chapter XXI the rats on this experimental diet also showed a characteristic dermatitis, stomatitis, and other pellagra-like symptoms. It is now apparent that most of the symptoms of the rat disease which the early investigators attributed to a lack of the P-P factor were due to a deficiency of riboflavin. Further work (Hogan and Hunter, '28; Hogan and Richardson, '33, '34; György, '34; Chick et al., '35; Harris, '35) definitely established that the vitamin G (B_2) complex was composed of several factors, of which riboflavin was one.

The Bourquin-Sherman diet ('31) has been used for studies on riboflavin deficiency. Some investigators believe that this method of bio-assay measures the riboflavin activity because the basal ration contains ample B_1 and therefore the growth response that follows the addition of the material under assay is an indication of the riboflavin content. However, this is not a justifiable deduction since other

factors in the B-complex affect growth in rats and this method is a measure of the growth-promoting activity in rats of the vitamin G (B_2) complex content of substances. The diet used by Richardson and Hogan ('36) appears to be well adapted for this purpose.

György first ('35) used basal diets devoid of the water-soluble vitamins except the chemically pure vitamin B_1 (thiamin). When this diet was supplemented by riboflavin the rats still showed the characteristic dermatitis which was promptly cured by other supplements. The work, therefore, demonstrated that riboflavin has no dermatitis-preventing activity and hence plays no role in rat pellagra. Bender et al. ('36) further noted that flavin does not prevent or cure dermatitis in the rat.

P. L. Day and associates ('31) described the development of cataract in rats, mice, chickens, and monkeys deprived of vitamin G (B_2). The condition begins with the appearance of a conjunctivitis and keratitis after 7-8 weeks on the diet, followed by a dullness of the eyeball and finally a definite opacity. The incidence of cataract in their experiments was almost 100 per cent. This group of investigators ('37) have presented evidence pointing to riboflavin as the substance that prevents this type of cataract.

Bourne and Pyke ('35) repeated Day's work and produced cataract in but 31 per cent of his animals. On the other hand, neither György ('35, l.c.) nor Richardson and Hogan ('36, l.c.) observed cataract in their rats deprived of riboflavin. The reason for the divergent results is not apparent. It seems probable that Day's observations are correct and that the diets of the experimenters who failed to produce cataract may not have been completely depleted of riboflavin.

György's ('38) most recent observation is that riboflavin deficiency in the rat is often associated with pediculosis and that administration of riboflavin to the lice-infested animals is followed by complete disappearance of the parasites. This phenomenon does not seem to be a result of a generally diminished vitality of the rats preventing their keeping themselves clean, for most of these lice-infected rats were neither weakened nor inactive. This may be an indication that flavin is essential in the normal sensory acuity of the skin, and if in flavin deficiency skin sensitivity is reduced, the rats are not able to keep themselves free from pediculosis in the manner of normal animals.

Riboflavin Deficiency in the Dog.—Sebrell ('29, '33) appears to have been the first to recognize as a distinct pathological entity a condition in dogs which has since been shown to be due to deficiency of riboflavin. Lillie and Sebrell ('37) described the pathology of the disease under the name "yellow liver." It occurred in certain but not all of their dogs fed diets designed for the study of the etiology of

blacktongue. Sebrell and Onstott ('38) have shown conclusively that "yellow liver" is due to riboflavin deficiency.

The symptoms characteristic of the acute attack of this deficiency state developed rapidly, and death occurred within a few hours. There was a sudden onset of weakness and ataxia, and very soon the animal was unable to stand or move its legs. There were at this time varying degrees of spasticity, which was sometimes marked. The dogs appeared to be fully conscious, since they followed activity in the room with their eyes and attempted to wag their tails and move their heads. There was no evidence of pain or discomfort. Bradycardia and an exaggerated sinus type of cardiac arrhythmia (the heart speeding up on inspiration and slowing on expiration) probably due to vagotonia occurred. The respiration was slow and regular. Within an hour the animal had usually passed into a deep coma, in which there was no response to stimulation. It remained in this condition for several hours until death supervened. These workers did not note survival beyond 12 hours unless the animal was given riboflavin. Preceding death, the heart slowed and the respirations became shallow and labored. Death appeared to be caused by respiratory failure.

Pathologically the most striking changes in riboflavin deficiency in this animal occurred in the liver. This organ was regularly yellow in color, with fine red lobular markings. It was firm, usually quite friable and often distinctly greasy to the feel. There was no evidence of enlargement. The yellow color and fine red markings were evident on the cut section. The liver cells were filled with fat droplets. In the kidneys, fatty infiltration of the epithelium of the limbs of Henle's loops, which they state are often seen in normal dogs, was practically constant in "yellow liver" animals. Other pathological findings in their animals were less constant and may have been due to complications arising from incipient blacktongue (dog pellagra) or from former attacks of this disease. In the brain, injection of the meninges was seen in about half the animals. Severe, diffuse, quite general fatty degeneration of the white substance and the cord, disintegration of the chromophil corpuscles (tigrolysis) in the brain-stem ganglia, nerve-cell atrophy and perinuclear edema in the basal ganglia and cortex were observed in a sufficient number of dogs to indicate that these changes are probably a feature of the deficiency state due to lack of riboflavin.

Riboflavin in the Nutrition of the Chick.—Hauge and Carrick ('26) were the first to definitely demonstrate that in addition to the antineuritic substance (thiamin), the chick required a growth promoting factor for normal growth and development. Norris and associates ('29-30, '30-31, '33, '36), and Bethke, Record and Kennard ('30-31, '33) confirmed and extended the work of Hauge and Carrick. These

studies showed that the requirements of the growing chick for the growth promoting factor now known to be riboflavin are very great.

From their experiments Norris and associates ('36, l.c.) concluded that chicks need approximately 290 micrograms of riboflavin per 100 gm. of feed in order to attain normal weight at the age of 8 weeks. Hens require about 230 micrograms per 100 gm. of feed in order to produce eggs that will hatch well. As small an amount as 130 micrograms is sufficient for normal egg production. They found that the rate of growth in chicks is closely correlated with the amount of riboflavin ingested, which indicates that this pigment is intimately associated with growth processes. This is easily visualized on the assumption that each increment of new tissue must have in it a certain quota of Warburg's yellow respiratory pigment. These investigations also show that, for the chick, ordinary components of rations for growth employed in America and Europe do not provide enough riboflavin for development, hence riboflavin-rich supplements must be provided. Lack of such information has, in the past, caused enormous economic loss to poultry producers.

Riboflavin and Pellagra.—There are considerable data indicating that riboflavin is not concerned in the etiology of pellagra. Treatment of pellagrins with riboflavin was unsuccessful (Dann, '36; Fouts et al., '36). However, riboflavin deficiency may occur in man simultaneously with or independently of pellagra.

Storage and Excretion.—Kuhn, Kaltschmidt and Wagner-Jauregg ('35) found that the riboflavin content of animal organs cannot be increased to any degree even by feeding ten times the normal amount. These investigators made the interesting observation that in rats deficient in B_6, but receiving an abundance of riboflavin, the liver contained, on a weight basis, about 30 times as much riboflavin as muscle. Another noteworthy observation is that the riboflavin content of the liver is affected by the length of the period of flavin deprivation. The longer the rats were kept on the B_6 deficient diet, supplemented with this nutrient, the lower the liver riboflavin values became. There was no change in the flavin content of the control animals.

The organism apparently holds tenaciously to its store of riboflavin, for even in rats that die as a result of flavin deprivation, the content of this substance in the liver, kidney, and heart still remains about one-third of the normal level (Kuhn et al., '35, l.c.; Vivanco, '35). When flavin is added to the diet of depleted animals, growth starts before the reserves in the various organs are restored to normal.

Under adequate dietary conditions riboflavin is a normal excretory product. The total excretion of flavin in the urine and feces of the rat ranges from 3 to 5 micrograms daily, the feces containing about

twice as much flavin as the urine (Vivanco, l.c.). After a 14-day period of total deprivation of "vitamin G (B_2)," no flavin was excreted in the urine and growth was suspended.

Emmerie ('36, '37a) has conducted an extensive investigation of riboflavin excreted by man. He found the daily output of this substance in the urines of normal men to vary between 819 and 1250 micrograms. However if the intake of the nutrient was greatly increased, there was a corresponding increase in the amount excreted. When the consumption of flavin was considerably decreased, the excretion exceeded the intake; with an increased intake, there was both an increased retention and excretion. Roscoe ('36) reported similar findings. The rate of destruction of riboflavin by the mammalian organism is not known; experimental data indicate, however, that some destruction occurs.

Biological Activity of Riboflavin.—The data from several laboratories showing the growth increment in the rat with various intakes of riboflavin are so little in accord that they do not afford a basis for establishing a quantitative relationship.

Kuhn and associates ('33, l.c.) using the Bourquin-Sherman "vitamin G (B_2)" deficient diet ('31, l.c.), supplemented by vitamin B_4, found that 7 micrograms of riboflavin supported growth at the rate of 3 gm. per rat per week. Ansbacher and associates ('36) found that when rats were fed a basal diet free from all water-soluble vitamins, supplemented with thiamin and a B_6 concentrate and varying amounts of riboflavin, the rate of growth differed with the amount of riboflavin fed. With daily doses of 5, 10, and 20 micrograms, weight increases during a period of 49 days were 0.26, 0.57, and 0.77 gm. respectively. With an intake of 3 micrograms crystalline riboflavin per rat daily, Booher ('38, l.c.) secured weight increases of 3.1 gm. per week. Von Euler and coworkers ('34) using a different diet found that daily administration of 5 and 10 micrograms of riboflavin induced an average daily gain of 1.1 gm. and 1.3 gm. respectively. Itter, Orent and McCollum ('35) fed 100 micrograms daily and obtained weight increases of 1.1 to 1.4 gm. per rat daily. Lepkovsky and associates ('35) using 100 and 300 microgram supplements of flavin daily observed increases of 2.9 and 3.0 gm. per day respectively. Stare ('35) found a daily dose of 50 micrograms of the nutrient resulted in a daily gain of about 0.6 gm. Karrer et al. ('33) feeding 3 micrograms of the substance reported a daily increase of 0.75 gm. per animal. Kuhn and associates ('35, l.c.) state that 10 micrograms daily of natural riboflavin induced a daily gain of 1.45 gm. and a similar dose of the synthetic product resulted in a gain of 1.43 gm.

Riboflavin Assay.—Chemical and physical methods for the determination of riboflavin have been developed (Morton, '35; Supplee

et al., '36; Weisberg and Levin, '37, Emmerie, '37b). The fact that riboflavin exhibits a blue-green fluorescence in ultraviolet light serves as a basis for its determination by fluorometric methods.

The various methods of estimating riboflavin consist of the following processes:—extraction of the flavin with water, dilute alcohol, and dilute acetone; adsorption on fuller's earth and elution with dilute pyridine; estimation of the riboflavin content of the eluate by comparing the fluorescence with that of solutions of known riboflavin content or by converting the riboflavin to lumiflavin by exposing it in alkaline solution to the action of light and then determining the lumiflavin colorimetrically. The existing methods need still further refinement, for the chance of loss of material at the various steps of these procedures is considerable. Therefore the results thus obtained are undoubtedly low and must be considered only approximate.

Measurement of riboflavin by biological means has been conducted by a number of investigators, but in only one case (Whitnah et al., '37) was the flavin content of the foods compared with that of pure riboflavin.

There is no officially recognized procedure for the assay of riboflavin. The important factor in the biological assay of this nutrient is the selection of a basal diet absolutely devoid of flavin and the inclusion in this ration of all the other essential factors of the vitamin B-complex free from riboflavin. Some of these factors are now available for this purpose in highly purified forms. The rat at the weaning age is the animal commonly used. The feeding of such a basal diet will cause cessation of growth, one of the earliest symptoms of the deficiency, and if the deprivation is continued the characteristic denudation will appear. When the flavin-containing material is added the animals begin to grow and the denuded areas show new fur.

A biological method of assay using young chicks has been described by Norris and associates ('36, l.c.). Norris as a result of these experiments defines a chick unit of riboflavin as one microgram. However there is as yet no recognized unit of riboflavin.

Dietary Requirement for Riboflavin.—The riboflavin requirements of the animal organism appear to be dependent on the size and activity of the body tissues. Reliable data for the absolute amounts of riboflavin required for normal nutrition are lacking at this time.

Stiebeling ('36-'37) and Rose ('37) using the Bourquin-Sherman unit of vitamin G which is regarded by them as the riboflavin unit, estimated the normal human requirement of riboflavin. Stiebeling proposed 450 units for boys under 6 and girls under 7 years of age, 540 units for boys from 7 to 10 and girls from 8 to 13 years of age, and 600 units for older children and adults. Rose has suggested for children

up to 10 years of age at least 400 units a day or 20 units per 100 calories if more than 2000 calories a day are consumed; for adults she recommended 20 units per hundred calories. However, the Bourquin-Sherman unit as a measure of riboflavin is, at least, equivocal as we have discussed previously in this chapter.

Distribution.—Riboflavin is most abundant in milk, egg white, liver, and leafy vegetables; other good sources are heart, pancreas, beef muscle, veal, chicken, apricot and tomato. Lesser amounts are present in grains and legumes. Yeast, although probably the richest natural source of the vitamin B-complex, varies in its flavin content according to the particular sample. Fish liver contains but little of this substance and fish muscle is almost devoid of it.

The retina of fish eyes contain flavin and von Euler and Adler ('34) suggested that this flavin may be of special significance in vision.

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CHAPTER

XXI

Pellagra and Animal Deficiency Diseases Related to Pellagra

IT IS NO EASY MATTER to trace far back the history of indubitable pellagra, because in the past it was confused with many other diseases such as scurvy, leprosy, syphillis, erysipelas, etc.

Don Gaspar Casal, the learned and able physician of King Philip the Fifth of Spain, as early as 1735 was the first to grasp the individuality and significance of pellagra. In Italy it was first recognized by Pujati in 1740, but nothing was published until 1771 when Frapolli described it under the popular name of "pellagra," by which it has long been known to the peasantry. In France the disease was first observed by Jean Hameau in 1818 and in Rumania by Theodori in 1833. The history of the discovery of pellagra in other countries shows everywhere again and again the same late and fortuitous recognition, notwithstanding its antiquity and wide distribution of the disease. Until about 1907 pellagra was looked upon as non-existent in North America and was not even mentioned in medical text books. Yet it existed, and two physicians in different sections of the country, Grey in Utica, New York, and Tyler in Summerville, Massachusetts, had both recorded in 1864 one case each. But it was not until 1907 that pellagra which began to develop with alarming speed in the Southern States became a subject of inquiry in the United States. Numerous and severe cases were observed in the Austrian Tyrol between 1875 and 1905. The first description of pellagra in England appeared in 1912. Sporadic cases occur in many countries and epidemics have been observed in France, Spain, Italy, the Balkan countries, and in Egypt. At present the largest number of cases of pellagra occur in the southern parts of the United States, where 200,000 are said to be afflicted. The mortality is also higher in the United States than abroad.

Pellagra occurs among all races. Although not inherited the disease may affect children at an early age; the larger number of patients, however, develop the symptoms between the close of the second and fifth decade of life. It occurs in both sexes, but in the United States the subjects of the disease are in larger proportion married women. This suggests a relationship between pregnancy and lactation as predisposing influences.

Pellagra is a disease of poor people in the rural districts who subsist on a limited choice of food. It is rarely seen among the well-to-do who enjoy a more varied diet. It is seasonal in its outbreaks, 90 per cent of the cases having their onset in the period from April to July, with remissions in the late fall and winter. The duration of the attack is anywhere from 6 weeks in acute cases to 20-30 years or even longer in the chronic forms. It is generally confined to warm countries. Both the southern portions of Europe and the United States have suffered the most.

The most typical symptom is erythema, which at first resembles sunburn but later becomes brown and makes the skin rough and scaly. It attacks only certain parts of the body surface, particularly the backs of the hands in adults and of the feet in children. Other parts not infrequently attacked are the sides or front of the neck, the face, elbows, or knees. Another marked peculiarity is its tendency to appear at about the same time on both sides of the body. Accompanying the skin eruption are soreness of the mouth, redness of the tongue, indigestion and diarrhea, as well as disturbances of the nervous system leading in the severest cases to insanity. On the whole the pathology of this disease is not distinctive and does not offer much aid in determining the possible cause of the disease.

The etiology of pellagra is still not entirely clear. A number of views have been proposed to explain its cause. Marzari more than a century ago was the first to express the belief that it resulted from a dietary insufficiency. He observed that the pellagrins in Italy used excessive amounts of maize and he believed that maize lacked sufficient albuminous material to be a good food.

The two theories which have had in the past the greatest number of adherents were the maize toxin theory and the infection theory. According to some proponents of the toxin theory, the toxin was produced by molds and other microorganisms with which maize may be infected. According to others, a toxic substance was produced in the animal body as a result of digestion and absorption of maize products. However, the experimental work supporting these theories is confusing and difficult to interpret. In more recent years Jobling and Arnold ('23) in the United States observed an increase in the fungus, hyphomycetes, in pellagrins and supposed that these molds

were in themselves toxic or produced a toxin when grown on corn. They isolated from laboratory cultures of fecal fungi from pellagrins a fluorescent dye which sensitized albino rats to light so that exposure caused erythema, thickening and scaling of ears and other exposed skin areas. They were never able to identify these molds as a causative factor in pellagra. No further work appears to have been done since, hence it is doubtful as to how much significance may be attached to these findings. Some of the objections to the toxin theory are as follows: a toxin is not apt to cause recurring seasonal attacks long after the patient has ceased to take it in his food; the attacks of pellagra continue to recur in pellagrins after they have ceased to eat corn. The disease also occurs in persons who do not eat corn or who have eaten it but rarely.

The infection theory has little to substantiate it although extensive epidemiological studies have been made. Exhaustive studies of pellagrous material have yielded no evidence that an infectious agent was associated with the disease, and thorough efforts to transmit the disease from man to man have also failed.

In 1910 Sambon, an Italian, after a number of investigations both in Italy and France, advanced the parasitic theory stating that the disease is due to an infection with some living organism probably transmitted and propagated by an infected sand fly. During the period of 1913-1914, the Thompson-McFadden Commission made an epidemiological study of pellagra in Spartansburg County, S. C. Their investigations included an examination of the dietaries of pellagrins and non-pellagrins of this region. Their conclusions from this survey were that there is no relation between the character of the diet and incidence of the disease. As a result of these observations they suggested that the stable fly displayed certain characteristics which seemed to qualify it as a vector of pellagra.

The objections to the infection theory are the failure to find in the blood or in the tissues and body fluids of pellagrins any parasites or specific bacteria and the failure to reproduce the disease when the blood of pellagrins is injected into the body of monkeys or healthy men.

The modern era of pellagra research began in 1913 when Goldberger of the U. S. Public Health Service undertook an investigation of the disease in the South. The epidemic was extremely widespread and the morbidity high (in 1917 170,000 cases were recorded). Goldberger and associates ('15) first planned three sets of investigations which he conducted at the Carolina State Asylum, the Georgia State Asylum, and the State Sanatorium at Jackson, Mississippi. During these surveys he noted that the staffs of these institutions although living in the same buildings and supposedly getting the same dietaries were immune from pellagra. It seemed to be only the inmates who

were afflicted with the disease and died of it. His first conclusion was that since the nurses, doctors, orderlies, and other members of the staff in spite of the fact that they handled the patients and even lived and slept in the same wards with the pellagrins, appeared to be immune, the disease could not be contagious and was not infectious. In addition, he had observed that the difference between victims of the disease and those who were not afflicted with it was that pellagrins consumed diets mainly composed of cereals and almost devoid of animal proteins. He therefore looked into the matter of the dietaries of the asylum staffs and inmates and found that theoretically both groups were supposed to get the same food, but actually, the staff were getting the choice foods of the dietaries and furthermore, the staff members also obtained additional foodstuffs from outside sources, which privilege was not enjoyed by the patients. Thus it was apparent that the diets of the two groups were different.

Next Goldberger and his coworkers ('15, l.c.) performed a conclusive experiment in an orphanage in Mississippi where pellagra was rife,—recurring with regularity every spring. In this institution, the children from 6-12 years of age were receiving the poorest type of foodstuffs. Those below that age were given in addition a glass of milk daily; whereas, those older than 12 had a more varied diet since they earned a little money to supplement their diet with more choice foods. Goldberger added liberal quantities of eggs and milk to the orphanage diet and the next spring there was no reappearance of pellagra in the institution, whereas in other similar ones where no dietary change was made, the recurrence rate of the disease was as high as in previous times. Goldberger and his staff followed this experiment with a series of similar ones to discover what foods actually prevented pellagra. They established the fact that lean meat, milk, eggs, yeast, tomato juice, tinned salmon, and a number of other foods if given in sufficient quantity will prevent the disease. Then ('15a) he attempted to induce pellagra by dietary means in healthy men. On promise of a pardon, 11 convicts on the Rankin Prison Farm volunteered to serve as subjects. These men were restricted to a diet composed of maize meal, white wheat flour, potatoes, salt pork, and syrup. At the end of 6 months, 7 of them were found suffering from the disease. The addition to this diet of 30 gm. of yeast, 200 gm. of meat, or 2 pints of milk daily cured the pellagra and prevented its recurrence.

Subsequently, Goldberger set out to determine the manner in which these foods exercise their protective function. What factor did they supply which is lacking in maize? Goldberger ('22), and also Wilson ('21) and Bigland ('20), who worked in Egypt, believing the protein of corn to be of poor biological value, first thought that pel-

lagra was caused by an amino acid deficiency. Zein, one of the proteins of maize, is deficient in tryptophane and lysine and the pellagra-preventive foods are rich in these amino acids. However, the discovery that a yeast extract practically devoid of amino nitrogen was as effective in curing pellagra as was yeast, meat, and milk, put an end to that theory.

To finally settle the question of infection, Goldberger ('16) and his associates then tried a number of ways to transmit pellagra to themselves by inoculations with blood, nasopharyngeal secretions, feces, urine, and desquamating epithelium. But the results were entirely negative. This, therefore, served as further proof that pellagra is not an infectious disease.

Spencer ('16) was the first to suggest that spontaneous canine blacktongue was analogous to human pellagra. The first report of experimental pellagra appeared in 1917, when Chittenden and Underhill produced by dietary means a condition in dogs strikingly suggestive of pellagra in man. Chittenden ('07) had observed and described this syndrome as early as 1905 in connection with his nutritional studies on protein, but he did not, at that time, recognize its relation to pellagra. This pellagra-like syndrome was characterized by a sudden loss of appetite and marked apathy. After refusal to eat for a day or two the dogs developed a severe stomatitis, characterized by pustules on the inner surfaces of the cheeks, lips, and edges of the tongue, which simulated a mass of rotten flesh. The odor was foul, and the mucous lining of the mouth could be wiped away by stroking it with absorbent cotton. There was intense salivation and bloody diarrhea. Pustules on the thorax and upper abdomen were characteristic. At autopsy of these animals, which died early from convulsions, the chief feature was an intense hemorrhagic condition of the large intestine and ulcers of the duodenum. This pathologic condition was produced by feeding a diet of cooked peas, cracker meal, and cottonseed oil. A similar condition was produced, but with greater difficulty, by a diet of meat, cracker meal, and lard. In continuing the study, Underhill and Mendel ('25, '28) found that some substance present in butter fat, egg yolk, or carrots cured this condition. Cod liver oil was not effective, neither was yeast nor meat, but crystalline carotene cured the disease. Cary ('20) was the first to suggest that the Chittenden-Underhill syndrome was identical with the canine disease known as blacktongue.

Wheeler, Goldberger and Blackstock ('22) described the pathology of a disease in dogs which had been variously designated as blacktongue, typhoid in dogs, and canine diphtheria, which closely resembled the syndrome of Chittenden and Underhill. They had discovered this disease at various places in the South and they expressed

the belief that it represented the analog of human pellagra. They quoted several observers who had met with the condition in dogs in widely separated areas.

In 1926 Goldberger and Lillie found that the typical diet of pellagrins would cause blacktongue in dogs. They also produced experimental blacktongue in dogs by means of a diet adequate in all respects but deficient in pellagra-preventive foods. This important finding opened the way to the effective study of the etiology of pellagra in man. They cured this experimental condition by feeding yeast and red meat. Experimental blacktongue is similar to the blacktongue that occurs spontaneously in dogs, which in turn is similar to human pellagra as to its geographical distribution, symptomatology, and histology. The stomatitis produced by Underhill and Mendel ('25, l.c.) corresponded to these but a number of the symptoms in the Underhill-Mendel dogs were different; scrotal lesions and the dermatitis of the chest characteristic of Goldberger's dogs and in spontaneous blacktongue were absent from the Underhill-Mendel animals. Since carotene completely controlled and prevented the Underhill-Mendel syndrome and since proof of the identity of the two conditions such as histologic studies is lacking, it seems probable that the two types of diseases are essentially different though grossly similar in certain respects. The Underhill-Mendel syndrome seems to be very similar to the combined deficiency of vitamin A and B-complex which has been described in man by Wright ('32).

Smith ('32), in a review of the literature referring to oral spirochetes and related organisms in fusospirochetal disease, points out that this group of organisms, which grow so profusely in the mouths of sick dogs, is capable of producing severe infection in normal animals when inoculation is made with minimal trauma. He showed that there is true symbiosis of these organisms as the causative factor of Vincent's angina, pyorrhea, and fusospirochetal disease of the lungs. Miller and Rhoads ('35) observed the characteristic flora described in blacktongue in dogs. They were unable to infect normal dogs by inoculating masses of these organisms under the labial mucous membrane. Smith and associates ('37) produced both the Underhill-Mendel syndrome and Goldberger's blacktongue in dogs and concluded that the clinical and bacteriological findings in dogs fed these diets are identical. They demonstrated that fusospirochetal organisms exist in enormous numbers in the oral lesions of dogs on both diets. They attach significance to this observation because similar organisms are found in the lesions of human pellagra, and because upon treatment of both blacktongue and pellagra these organisms disappear. In addition, this seems to be further proof of the analogy between experimental blacktongue and human pellagra.

It appears that the primary deficiency of the Chittenden-Underhill-Mendel dogs was vitamin A and that of Goldberger's dogs was the antipellagra factor. The mouth condition which was regarded by both groups of investigators of dog pellagra as clinically significant, was in fact due to overgrowth of the mucous membranes by the fusospirochetal flora as the result of debility of the animals. This observation is of additional outstanding importance since it makes clear the fact that microbial agencies, which in the well individual are not able to establish themselves upon mucous surfaces and become a menace to health, may easily do so when the vitality is lowered through malnutrition. In this case the overgrowth of fusospirochetal organisms occurred equally menacingly in two distinct deficiency states.

In his dietary studies, Goldberger observed that most foods rich in vitamin B would cure pellagra. Consequently when in 1926, Smith and Hendrick showed that vitamin B was made up of at least two substances, one thermolabile, the other thermostable, Goldberger et al. ('26) repeated these experiments on rats and confirmed Smith's observations. Then they proceeded to test the effect of the thermostable portion of the vitamin B-complex in the form of autoclaved yeast and water extracts of autoclaved yeast on both blacktongue dogs and cases of human pellagra and obtained beneficial results. Goldberger therefore designated this fraction of the vitamin B-complex as the P-P (pellagra-preventive) factor.

The Role of Sunlight in Pellagrous Dermatitis.—The relationship of pellagra to light has frequently been commented on in the clinical literature. In the early days, sunlight was believed by some to cause the disease. However, the sunshine was neither hotter nor different in the pellagrous regions than in other sections of the world where the disease did not exist; and furthermore even in the early days, it has been observed that the dermatitis may occur on parts of the body covered by clothing. In those exposed to direct sunlight the dermatitis in pellagra seems to appear earlier in relation to the other symptoms. Various experiments have been performed with fenestrated gloves which seemed to show protection from the dermatitis when the parts were protected from light. The clinical experience of Smith and Ruffin ('37) has shown that the dermatitis of the exposed surfaces of human pellagrins is closely dependent upon the action of sunlight on the patient's skin. Spies ('35) recently has also studied this phase of the disease and has reported that pellagrous lesions occur in the absence of sunlight or ultraviolet radiation. Spies emphasized that pellagra should be considered a systemic disease and not a cutaneous condition and that under certain circumstances, still matters of conjecture, sunlight might act as an irritant and precipitate the cutaneous lesions of the disease. The role of light in

pellagra is extremely confusing but it is certain that the influence of the solar light is only of minor importance,—a patient must first have the disease internally before the sun can cause or influence the dermatitis. The deficiency state and not the sun is the cause of the pellagrous dermatitis.

Rat "Pellagra" and Vitamin B_6.—Goldberger and associates ('26, l.c.) found that the typical pellagra-producing diet of man likewise caused in rats the development of a syndrome characterized by arrest of growth, ophthalmia, some denudation of hair, and dermatitis at one or more of the following sites: ears, front of neck and upper part of chest, forearms, backs of forepaws, shins, and the backs of the hind paws. The matted fur on the backs of the hind paws desquamated, leaving a denuded, pale pink, glistening skin. In some instances there was fissuring or ulceration at the angles of the mouth, together with a lesion at the tip of the tongue, beginning as a small roughly circular, grayish opacity or bleb; or as an ulceration, which in some animals went on to the formation of a localized yellowish slough. Inflammation of the anterior part of the floor of the mouth and diarrhea were also seen in some animals.

Goldberger et al. ('26, l.c.) found that lean meat as well as yeast or yeast extract would cure the condition in rats. They were also able to clear up this syndrome, like blacktongue in dogs, by the provision of an earth adsorbate of yeast extract which had been freed from the antineuritic substance (thiamin) by heating. Goldberger concluded that in both the dog and the rat the lesions observed represented pellagra and that the deficiency was prevented by the same substance which had a preventive or curative effect in human pellagra. It appeared then that these deficiency states in the three species must have a common origin.

In 1927 Boas described in rats a syndrome caused by feeding Chinese egg white as a dietary source of protein, the chief characteristics of which were eczematous dermatitis, accompanied by alopecia, blepharitis, spasticity, and in some cases edema of the feet and hemorrhages in the skin. The similarity of this condition to pellagra was noted. The efforts to discover the nature of this egg white injury has been discussed in Chapter VI.

Even as recently as 1934 the great difficulty in making further progress in the study of the etiology of pellagra lay in the fact that there were several water-soluble vitamins which had not been clearly distinguished as to their effects, and had not been prepared in sufficient purity to permit the producing of separate and distinct deficiency states due to each of these factors. The first advance in this direction came through the discovery by Kuhn and associates (see Chapter XX) that riboflavin is an essential nutrient, and a com-

ponent of the B-complex. As has been mentioned in the discussion of riboflavin, Kuhn found that when rats which were restricted to the Bourquin-Sherman type of diet had ceased to grow, and were approaching the rat-pellagra stage, the provision of crystalline riboflavin induced a prompt growth response. This they wrongly interpreted to mean that riboflavin possessed intense vitamin B_2 (G) effect. György ('34) found that rats, under these dietary restrictions, develop pellagra-like symptoms even when liberally provided with riboflavin. György ('37) describes the symptoms of this uncomplicated deficiency as involving symmetrical dermatitis of the peripheral parts of the body, the paws, nose, mouth, and ears. There is no pruritus. Scaliness and edema are evident. Alopecia usually does not occur; at most it is slight. Abscesses form, particularly around the mouth and cheeks. Sublingual necrotic ulcers are not uncommon. In advanced stages scaliness over the trunk is seen, and slight or no loss of hair accompanies the scale formation. The symptoms appear in rats in 6 to 15 weeks. It now became possible to prepare diets containing two known factors in the water-soluble, or B-complex group, and so to make further progress in characterizing the remaining ones.

Birch, György and Harris ('35) made a critical study of the response of rats and dogs to experimental diets designed to show whether these species react alike or differently when different foods or preparations were the source of vitamin B_2, this term now being used to signify those essential nutrients of the old B-complex other than thiamin and riboflavin, both of which had become available in pure form for experimental purposes and were supplied in all diets. They knew, on the basis of clinical literature, that pellagra is essentially confined to maize-eating communities; that molasses is one of the principal constituents of the human pellagra-producing diet; that fish has preventive value in pellagra and in blacktongue; and that administration of riboflavin does not benefit human pellagrins. On the basis of these facts they prepared experimental diets which clearly led to the following conclusions:

Maize, which forms a large part of the diet of pellagrins, is highly effective in preventing or curing the condition hitherto called rat pellagra, rat dermatitis, or rat acrodynia. Pellagra and blacktongue-producing diets contained considerable of the nutrient required to prevent rat dermatitis, hence the rat syndrome is etiologically distinct from human pellagra. In conformity with the earlier suggestion of György ('34, l.c.), the antidermatitic substance needed by the rat was called vitamin B_6.

György ('35) showed that thiamin together with riboflavin failed to prevent dermatitis in rats. Peters' eluate (Kinnersley et al., '33) on the other hand contained the factor that prevents rat pellagra.

Thus by 1935 it was established that the vitamin G (B_2) complex was composed of riboflavin, a growth-promoting substance, and B_6, a dermatitis-preventive factor essential for the rat but different from the P-P factor required by man. Vitamin B_6 has no effect on black-tongue in dogs or on the so-called chick-pellagra. Dann and Subarrow ('38) reported further confirmation of the characteristic components of the vitamin G (B_2) complex. György et al. ('34, l.c.) and Edgar and Macrae ('37) believe that vitamin B_6 may be identical with factor Y of Chick and Copping ('30). (See Chapter XXII.)

Dann ('36) confirmed the B_6-deficiency theory of rat dermatitis and further showed that rats on a B_6-deficient ration develop dermatitis as readily in the dark as in the light, which is further evidence that this syndrome is not analogous to the dermatitis of human pellagra.

György ('38a) has reported that environmental factors, such as temperature, may seriously affect the appearance of symptoms of vitamin B_6 deficiency. During the summer when the temperature ranged from 80°-100° F. the incidence of rat acrodynia was decidedly on the decline. With lower temperature conditions the production of the acrodynia was increased. Acceleration in the production of the dermatitis was achieved by exposing rats to low temperature (40° F.). In these experiments a high food intake was characteristic of the rats exposed to cold without a corresponding increase in weight. In spite of its earlier appearance, the acrodynia in the rats exposed to cold progressed slower and exhibited certain peculiarities such as prevalence of erosions of the lips and angles of the mouth, and the extremities exhibited the signs of deficiency at a comparatively later stage of the disease. Peters' eluate cured and prevented the disease.

György calls attention to the possible clinical significance of these results. Striking similarity between this disease in the rat and chilblain in man suggests that vitamin B_6 reserves in the body may function in the predisposition to chilblain, and that vitamin B_6 treatment might be used in chilblain. He also calls attention to the similarity of the angular stomatitis to that of perlèche common in British India. Aykroyd and Kushman ('36) concluded that the etiology of the human disease lies in a nutritional deficiency, probably a factor of the B_2-complex. György suggests that, in the light of his observations, the etiology of perlèche should be correlated to B_6 deficiency.

Birch ('38) presented evidence pointing out that two factors are concerned in rat pellagra. One is the water-soluble vitamin B_6 and the other is fat-soluble and is found in the fatty acid fraction of maize oil. This factor appears to be similar to Burr and Burr's "fatty acid factor" and to the fat-soluble antidermatitis factor of Hogan and

Richardson. He suggests that the physiological function of vitamin B_6 is connected with the utilization of the unsaturated fatty acids.

Vitamin B_6 and Microcytic Hypochromic Anemia in Puppies.—Fouts, Helmer, Lepkovsky and Jukes ('38) produced severe microcytic hypochromic anemia in puppies by feeding them a synthetic diet containing thiamin, riboflavin, the chick filtrate factor, and nicotinic acid, and which was apparently deficient only in vitamin B_6. Addition of B_6 to the experimental diet cured the anemia.

The Isolation of Vitamin B_6.—A number of experimenters have attempted the isolation of vitamin B_6. The chemical studies of Birch and György ('36) indicate vitamin B_6 to be of a basic nature (although it contains no primary amino group) and probably attached as a prosthetic group to protein, since it is not easily extracted from tissue until after digestion.

Keresztesy and Stevens ('38a) report the isolation of B_6 from rice bran. It is a nitrogenous base, the hydrochloride of which is freely soluble in water and sparingly in alcohol and acetone. They describe the compound as white platelets melting at 204-206° C. with decomposition. Daily doses of 0.050 mg. caused disappearance of symptoms of B_6 deficiency. Daily doses of 0.100 mg. were highly effective both in relief from symptoms and in stimulating growth.

György ('38b) showed that vitamin B_6 can be adsorbed upon fuller's earth and eluted by barium hydroxide. It is precipitated by phosphotungstic acid. He reports the isolation of crystalline material which, in doses of 15 micrograms per day cured rat acrodynia in 2 weeks. These crystals are described as colorless rods of varying size, with rounded ends, which have a tendency to form rosettes or fan-shaped groups.

Lepkovsky ('38) announced the isolation in crystalline form of factor 1, which is the portion of the B-complex other than thiamin and riboflavin that is absorbed by fuller's earth under specified conditions. The crystals are described as colorless rods which aggregate mostly as rosettes and sometimes fan shapes. The dry crystalline material has a slight yellowish tinge which is believed to be due to some impurity. No analysis of the crystals is given. Dermatitis of the peripheral parts of the body of rats, involving the feet, paws, ears, and areas around the mouth, were promptly cured by daily doses of 10 micrograms of crystalline factor 1. When growth had ceased on the experimental diet causing these symptoms, there was an immediate growth response when the crystalline material was administered. From the symptomatology described, it appears that Lepkovsky was dealing with what others call B_6 deficiency, and that it is this factor which he has isolated.

In a later paper Keresztesy and Stevens ('38b) further describe the

chemistry of this vitamin. The empirical formula is $C_6H_{12}N_3O$ Cl. It does not contain O-CH_3 or N-CH_3 groups. The free base melts at 160° C. and has the empirical composition of $C_6H_{11}N_3O$. Both the base and its hydrochloride readily sublime. With ferric chloride the vitamin gives a reddish-brown coloration. The pure substance is stable to concentrated hydrochloric acid at elevated temperatures. It is not affected by heating with alkalies, by nitrous acid, ethyl nitrate, or Fehling's solution.

Absorption spectrum studies of the substance in acid, alkaline, and neutral solutions show that it has tautomeric properties. Well defined absorption occurs in the spectral region from 2300 to 3300 Å. In hydrochloric acid at pH 2, there is a single band with maximum absorption at 2925 Å. At pH 4.5 this band diminishes in intensity along with a new band with maximum absorption at 3275 Å. At pH 6.75 the latter band has increased markedly, while that at 2925 Å. has disappeared, and simultaneously a new band at 2560 Å. has appeared. At pH 10.2 the two bands which were present at pH 6.75 have increased in intensity and shifted toward the shorter wave lengths.

Thus we may confidently expect that full knowledge of the structure of B_6 will be soon forthcoming, and that its synthesis will be not long delayed.

Chick Pellagra and the Filtrate Factor.—In 1931 Ringrose, Norris and Heuser described a pellagra-like syndrome in chicks which were restricted to diets containing commercial casein, purified casein, or dried raw egg whites together with cereals, cod liver oil, and the necessary mineral supplements. Granulation of the eyelids, a viscous exudate which caused the lids to stick together, crusty scabs at the mouth angles, which later extended around the nostrils and under the mandible, were characteristic of this disease. The skin on the soles and between the toes thickened and cornified. Cracks and fissures also developed.

In 1932 Kline and coworkers reported that chicks fed a diet composed of yellow corn, casein, wheat middlings, cod liver oil, NaCl and $CaCO_3$ which had been heated dry at 95-100° C. for 144 hours, produced chick pellagra in about 3 weeks.

Ringrose and Norris ('36) noted that dried pork liver contained a factor which prevents the development of the pellagra-like condition in chicks in addition to another factor required for growth. The former they found to be insoluble in an alcohol-water solution and to be destroyed by dry heating in air at 100° C. for 144 hours or at 120° for 50 hours, and by autoclaving at pH 11 but not at pH 9. Their growth promoting factor was soluble in alcohol-water solution and was stable to heat in a dry atmosphere and relatively stable to autoclaving at pH 11. Yeast, dried or autoclaved, and liver extract were

ineffective in preventing the syndrome caused by the egg white or casein diet. Dried pork liver and the residue of extracted liver, on the other hand, were completely effective in this respect.

In 1936 Lepkovsky and Jukes succeeded in separating the chick dermatitis factor from the rat dermatitis factor. The term filtrate factor they provisionally used to indicate the water-soluble vitamin which prevents the dermatitis in chicks produced by feeding a dry heated ration of natural foodstuffs as described by Kline et al ('32, l.c.).

The richest known sources of the chick filtrate factor are yeast and rice bran. It is also found in egg yolk, beef, and in small amounts in cereal. This substance may be extracted with water-saturated butyl alcohol and 95 per cent ethanol. Jukes ('37) found that it is also easily extracted by hot or cold acidified water. This factor is stable to autoclaving and labile to dry heat at 120° C. for 24 hours. It is resistant to oxidizing and reducing agents. It is stable to benzoylation and is hardly precipitable by phosphotungstic acid. The rat antidermatitis factor (B_6) is readily adsorbed on fuller's earth, whereas the chick filtrate factor remains behind. This filtrate factor prevents or cures chick dermatitis.

At this time the evidence is insufficient as to the relationship between the chick filtrate factor and the pellagra-preventive factor. Fouts et al. ('36) reported successful treatment of pellagra in man with a rice bran concentrate containing the chick filtrate factor. Koehn and Elvehjem ('36) cured blacktongue with a liver extract which was also effective for chick pellagra. Sebrell and associates ('37) observed that a preparation containing the filtrate factor also contained the blacktongue preventive factor. However the distribution of the chick filtrate factor and the P-P factor shows marked differences. Jukes' (l.c.) assays indicate that this nutrient is present in the same quantity in beef, whole corn meal, and wheat germ. Corn meal on the other hand is an extremely poor source of the P-P factor, whereas beef and wheat germ are good pellagra-preventive foods. This suggests that the chick-pellagra factor is distinct from the pellagra-preventive factor of man. Further experimental evidence is necessary before final conclusions can be made.

The Human Pellagra-Preventive or Blacktongue Factor.—The evidence that vitamin G (B_2) was made up of several factors was of aid in determining the relation of the various pellagra-like animal deficiency diseases to human pellagra. It has been demonstrated, as cited in the discussions above, that the rat pellagra-preventive factor and the chick pellagra-preventive factor are not analogues of the disease in man.

Recent experimental data lead to the assumption that the pellagra-preventive factor is the blacktongue preventive also. Ruffin, Persons,

Harvey and Smith ('37) have observed that extracts of liver used for parenteral administration contain a factor which alleviates blacktongue symptoms, but which is only partially effective in curing the disease. Sydenstricker and Thomas ('37) observed that the addition of the liver residue, which is usually discarded in the preparation of the extracts, produced a curative effect in blacktongue. Pellagrins reacted similarly to the same fractions (Ruffin et al., '37, l.c.).

These observations offered additional evidence supporting the identity of the blacktongue-preventive factor with the pellagra-preventive factor.

During their study of chick pellagra Elvehjem and associates ('37) isolated nicotinic acid from potent liver concentrates. They correlated all the known facts concerning the properties and distribution of nicotinic acid and tested the effect of nicotinic acid and nicotinamide on dogs suffering from blacktongue.

Immediately upon the announcement by Elvehjem and associates ('37, l.c.) that nicotinic acid could cure blacktongue in dogs, several investigators proceeded to test its effectiveness in human pellagra. Smith and associates ('37) described a dramatic cure of a case of pellagra by the daily administration of 60 mg. of nicotinic acid. There was striking improvement of the appetite within 24 hours. The mental confusion began to improve after 48 hours, and the patient was entirely rational after 6 days' treatment. Within 3 days there was noticeable change in the skin of the face, and after 12 days the skin was normal. On the seventh day there was a striking change in the electrocardiogram, the T waves having returned to normal. On the twelfth day the abdominal and cremasteric reflexes were present.

Fouts and collaborators ('37), Harris ('37), and others have also confirmed the value of nicotinic acid in the treatment of pellagra. Spies et al. ('38) reported the cure of 11 cases of pellagra by the administration of nicotinic acid. Three of these were alcoholic pellagra, 2 were endemic pellagra, and 6, pellagra secondary to organic disease. The symptoms which they noted as clearing up promptly were glossitis, stomatitis, ptyalism, vaginitis, urethritis, proctitis, the characteristic dermatitis, and the peripheral neuritis. The recent studies by Spies, Bean and Stone ('38) and Bogart ('38) are further support of these observations. Chick, Macrae, Martin and Martin ('38) have cured a pellagra-like condition in swine by nicotinic acid.

The conclusion seems secure that nicotinic acid or nicotinic acid amide is either a part or the whole of Goldberger's P-P factor.

Woolley and associates ('38) studied effectiveness of various pyridine derivatives in the treatment of dogs suffering from blacktongue. Their experiments demonstrate that a specific structure is required for antiblacktongue potency. Nicotinic acid N-methyl amide, nico-

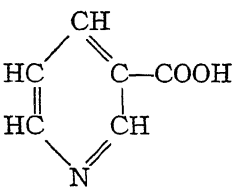
tinic acid N-diethyl amide, nicotinamide glucosidoiodide, and nicotinuric acid were effective. Wooley et al. suggest that in addition to nicotinic acid and its amide the compounds possessing antiblack-tongue activity are those which are capable of oxidative or hydrolytic conversion to these substances in the animal organism.

Although nicotinic acid, or its amide, is ineffective in rat pellagra and chick pellagra, it is essential for the health and growth of rats Elvehjem and Frost, 37; György, '38c; Euler and Malmberg, '36) and pigeons (Funk and Funk, '37).

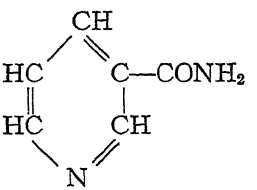
Further data pointing to nicotinic acid in the form of its amide as an important constituent of the living cell are the experiments demonstrating that this factor is also indispensable for the growth of various microorganisms (Knight, '37).

Nicotinic Acid and Nicotinamide as the Antipellagra Vitamin. —Nicotinic acid has been familiar to chemists for about 75 years. Suzuki and coworkers first isolated it from plant materials in 1912. Funk ('11) encountered it while attempting to isolate the antineuritic factor of the vitamin B-complex but since at that time the only biological test for the vitamin B-complex was the cure of polyneuritis he did not recognize its importance in nutrition. Szymauska and Funk ('26) narrowly missed making a discovery of its significance when they secured evidence that nicotinic acid exerted a food-sparing and weight-sustaining action. From time to time thereafter nicotinic acid was a subject of investigation by biochemists.

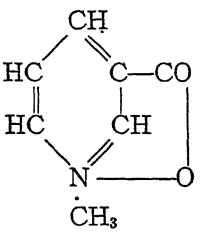
Nicotinic acid has the structural formula



Nicotinic acid



Nicotinamide



Trigonellin
(Methyl betaine of nicotinic acid.)

It melts at 228°-229° C.; it readily sublimes and is soluble in hot water and alcohol.

A number of investigators (Harris, '37 l.c.; Schmidt and Sydenstricker, '38) because of the variable results obtained upon the treatment of pellagrins, especially chronic cases, with nicotinic acid suggest that nicotinic acid may not be the only or principal deficiency in pellagra. In explaining the effects of nicotinic acid or its derivatives, Elvehjem suggests the theory that the acid is probably essential in one of the enzymes that transfers oxygen from the blood to the cells. Apparently the animal body cannot build the vitamin from the foods but must get it ready made.

Warburg's researches of the role of pyridine compounds in certain biological oxidation-reduction reactions point to the relationship between some of the vitamins of the B-complex and some enzymes of prime importance in metabolism. Warburg et al. ('35) and Schlenk and Euler ('36) demonstrated that nicotinamide is the active group in certain coenzymes. The hydrogen transporting coenzyme of the Warburg-Christian enzyme system yields on hydrolysis one molecule of nicotinic acid. The amide is evidently an essential constituent of the cozymase molecule. It is regarded as the functional group in cozymase which has a fundamentally important part in fermentation, glycolysis, and respiration. The discovery of the amide of nicotinic acid as an important constituent of living cells is at first sight very surprising and unexpected. It is of interest to note at this point that for several years the diethylamide of nicotinic acid has been widely employed under the name of "coramine" as a heart stimulant in cases of collapse. Kuhn and Vetter ('35) isolated nicotinic acid amide from heart muscle and it now appears that Warburg believed the coenzyme to be present in heart muscle.

As a portion of the cozymase molecule, nicotinic acid amide becomes an essential link in the chain of catalysts involved in certain biologic oxidations and reductions.

It appears then that nicotinic acid is either the pellagra-preventive vitamin, or a provitamin, or that it is only one, two, or more substances essential for the prevention of this deficiency disease. On the other hand, it may be conjugated with other substances in the body into a more complex compound that is essential to counteract pellagra. This possibility is suggested by the fact that Warburg's enzymes, diphosphonucleotide and triphosphonucleotide, each contains a molecule of nicotinic acid amide.

Dietary Treatment of Human Pellagra.—Sebrell ('38) points out that the essential points in the dietetic treatment of pellagra are: Inclusion in the diet of at least 1 quart of milk daily; a caloric intake of 3000 to 4000 calories per day, to be supplied in addition to milk and

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CHAPTER

XXII

Other Vitamin Factors

IN PREVIOUS chapters the nature and functions of the better known and well-recognized vitamins have been discussed (except vitamin E, to be considered subsequently). In this chapter the principal evidence which has been presented for a number of dietary factors that appear to be essential for the normal development of different species will be considered.

Vitamin B_3 is a term employed by Carter and O'Brien ('35) to designate a highly thermostable factor from yeast believed necessary for the pigeon. A number of investigators (Williams and Waterman, '27, '28; Carter et al., '30; Eddy, Gurin and Keresztesy, '30) demonstrated that when pigeons were fed polished rice supplemented with vitamin B_1 (thiamin) in the form of fuller's earth adsorbate or other sources of B_1 (thiamin), the birds gained weight at first, then suffered a decline, suggesting deprivation of a stored factor or factors. The food intake was inadequate, especially in respect to protein. This inadequacy could be remedied by addition of casein or wheat gluten. It was postulated that the extent of weight recovery varied with the degree of depletion of the store of B_3. The remarkable advances since this work was published, which have revealed the nutritive significance of riboflavin, nicotinic acid, the filtrate factor, etc., make it unprofitable to attempt an analysis of the nature of the hypothetical B_3. Reader ('29) used the term B_3 to designate a second heat-labile factor in yeast.

Vitamin B_4 and the Antiparalysis Factor.—Vitamin B_4 is the term first applied by Reader ('30) to a heat- and alkali-labile factor in yeast essential for weight maintenance in rats in addition to B_1 (thiamin). The symptoms caused by its deficiency, closely resembling pink disease in man, were described as muscular weakness, spastic

gait, swollen red paws, and a tendency to sit in a hunched position. From what has been said of the effects of deficiency of riboflavin and vitamin B_6, it appears that the condition encountered by Reader represented a multiple deficiency state involving these nutrients. Other investigators (O'Brien, '34; Harris, '35; Kinnersley et al., '35) believe that vitamin B_4 deficiency is a chronic phase of thiamin deficiency. However, Kline et al. ('36) and Elvehjem and Arnold ('36) confirmed and extended Reader's original observation with rats. Subsequently Keenan et al. ('33) employed the term vitamin B_4 to designate a factor found in liver and in acid charcoal extracts of yeast prepared by the method of Kinnersley et al. ('33). Using a basal diet composed of casein, dextrin, salts, yeast, and cod liver oil, they observed a leg paralysis, a condition which has been described by some investigators as "leg weakness," and also brain degeneration in chicks. They found that this antiparalysis factor cannot be extracted from hog liver by ether and that it remains in the extracted residue. It is soluble in water and is destroyed by autoclaving at the natural pH of liver. They regard it as probably identical with Reader's B_4. Reader's B_4 was absorbed on acid charcoal, whereas the B_4 factor of the Wisconsin group occurred in the acid charcoal extracts. Therefore these two B_4 factors are not necessarily identical. However the nature of the factors designated as vitamin B_4 is still obscure.

Keenan and associates regard the paralysis in chicks to be identical with the nutritional encephalomalacia in birds described by Pappenheimer and Goettsch ('31). In nutritional encephalomalacia there are extensive lesions of the cerebellum and occasionally of the cerebrum. Keenan states that in their chicks, gross and microscopic changes occurred in most instances in the cerebrum and in a few cases in the cerebellum. Hogan and Boucher ('32) have made observations similar to those of Keenan and associates.

Vitamin B_5 is another member of the swarm of factors which emerged from the earlier attempts to interpret the cause of weight loss in pigeons fed a polished rice diet. It is a slightly water-soluble, heat- and alkali-stable factor and is found in whole wheat and yeast (Carter et al., '30 l.c.). More searching recent investigations have left vitamin B_5 isolated in the literature as an entity of ghost-like tenuity.

Factor Y.—It is impossible to accurately appraise certain meritorious investigations which were clearly among the first to indicate that the "B-complex" is of multiple nature. Among such studies are those of Chick and Copping ('30) who showed that dermatitis developed in rats receiving a diet of purified substances supplemented with thiamin and with riboflavin, the former as Peter's purified concentrate of yeast and the latter as a preparation from egg white

representing the "B_2" group of nutrients. They found that the dermatitis was cured by autoclaved yeast and yeast extracts. These investigators employed the term "Y factor" to designate the dermatitis-preventing substance. This nutrient is relatively stable to heat, light, and alkali. It is present in autoclaved yeast, aqueous extracts of yeast, green vegetables, egg yolk, and beef liver. It is almost entirely absent from egg white, wheat germ, and lean beef. These researches were carried out before the emergence of nicotinic acid, factor W, the filtrate factor, etc., as nutrients; therefore further investigations will be necessary for an interpretation of these studies.

Vitamin H.—The term vitamin H has been employed to denote three entirely different factors. György ('35) uses the term to designate the hypothetical nutrient which prevents the so-called "egg white injury." Since György found vitamin H to be water-soluble only upon enzymatic hydrolysis of the foodstuffs containing it, it is not really a part of the vitamin B complex. This investigator ('37) has recently reported that vitamin H appears to be an ampholyte and in particular an acidic amino acid.

Booher ('37) applied the term vitamin H to designate a heat-stable and alkali-labile factor essential, in addition to thiamin and flavin, for growth and for the prevention of erythrodemic dermatosis in rats. It appears to be similar to what is now generally known as vitamin B_6. McCay, Bing and Dilley ('28) studied the problem of rearing young trout on diets of purified foodstuffs, and found that they could not be reared unless some fresh meat was provided. The unknown factor provided by the fresh meat they called factor H. The substance is heat-labile. It appears to be present in all kinds of fresh animal tissues. McCay ('37) has reviewed the literature on this subject.

Factor W, or the alcohol-ether precipitate factor, is a nutrient of the G (B_2) complex which apparently is required by the rat for riboflavin to exert its full action. During their investigations of the vitamin B complex, Elvehjem and coworkers ('36) discovered that in addition to thiamin, B_4, B_6, riboflavin, and the filtrate factor, the rat requires still another nutrient which they term factor W. This factor, precipitable from liver extracts by mixtures of alcohol and ether, is extremely active in promoting growth in rats, but has no effect upon the dermatitis of chicks. Although not adsorbed by fuller's earth, there is some doubt whether it is distinct from vitamin B_6 (Halliday and Evans, '37). Factor W is strongly stable to acid and alkali in the cold and moderately so at higher temperatures. Being heat-labile, it belongs to the group of thermolabile B factors.

In a recent publication Elvehjem and Frost ('37) present evidence suggesting a relation of this factor to the pyridine nucleotides. They obtained definite growth responses with their diet on the addition of

adenine nucleotides, nicotinic acid amide, and riboflavin. Adenine nucleotides alone produced an immediate though not continuous response. Nicotinic acid amide alone gave at first a very poor growth response, but after about two weeks there was a definite and sustained increase in rate of growth. The combination of these two supplements caused a more immediate and continuous response. These results point to the significance of adenine nucleotides as fundamental nutrients.

That some further elusive nutrient for the rat remains to be identified is suggested by the finding of Emerson and Evans ('38) who supplemented their diet No. 805, composed of extracted casein, sucrose, lard, cod liver oil, and a salt mixture, with thiamin, riboflavin, B_6 as a molasses eluate, and yeast nucleic acid. The latter contains adenine nucleotide. These animals failed to show a normal growth response, but such response was secured by provision of a supplement of a multiply adsorbed liver filtrate. Elvehjem and Frost (l.c.) secured good growth response with a daily supplement of 1.0 mg. of adenine nucleotide. Emerson and Evans ('38, l.c.) administered the equivalent of 1.0 mg. of adenine nucleotide in the form of yeast nucleic acid, yet they did not get an equivalent response. One possible explanation for the difference in their results is that adenine nucleotide in yeast nucleic acid does not become available in the animal through digestion and absorption; and that they were providing this nucleotide in the form of their multiply adsorbed liver filtrate. It is of interest, in the light of the possible nutritive significance of adenine (or its nucleotide), to note that Buell and Perkins ('28) showed that adenine nucleotide is a normal constituent of the blood of various species, and occurs there in surprisingly large amounts.

The chemical behavior of factor W towards barium and mercury salts and to the complementary action to flavin points to a similarity to the coenzymes. At present, however, it is difficult to evaluate the results on factor W owing to the non-synthetic nature of the diet used, for vitamins B_4 and B_6 and small amounts of the filtrate factor were provided in the form of white corn in this experiment.

Factor U.—Stokstad and Manning ('38) observed that yeast and wheat middlings had a growth-stimulating effect on the chick other than that produced by the filtrate factor or riboflavin. This appears to be distinct from any of the growth substances heretofore described as indispensable for the chick. The basal ration used in this investigation, composed of polished rice, water-washed fish meal, soy bean oil, sardine oil, thiamin, rice, bran filtrate, whey adsorbate, salt mixture, and ground limestone, furnished adequate amounts of thiamin, riboflavin, filtrate factor, vitamins A, D, K, and the gizzard factor. Addition of nicotinic acid had no effect on growth.

This factor is abundant in yeast, middlings, wheat bran, and alfalfa.

It is present to a lesser extent in corn and in very small amounts in polished rice. There is none in molasses.

The substance is insoluble in ether, acetone, or isopropanol. It is soluble in water and in mixtures of water and methanol. It is adsorbed on fuller's earth and on activated charcoal and can be eluted by a mixture of pyridine, methanol, and water. Autoclaving yeast does not destroy its activity, whereas autoclaving alfalfa does destroy it.

This growth factor is not identical to vitamin B_4; no paralysis is produced by a deficiency of it. Neither is it vitamin B_6 as is shown by its distribution. B_6 is abundant in yellow-corn, whereas even large amounts (55%) of yellow corn do not furnish protection to chicks deprived of the substance. Molasses, a rich source of B_6, is entirely lacking in the new growth stimulant.

Until a chemical name can be assigned, the authors have called it, tentatively, factor U.

Vitamin K, The Antihemorrhagic Factor.—The observations of Dam ('29, '30), Horvath ('30), McFarlane et al. ('31), Holst and Halbrook ('33), Dam and Schønheyder ('34), and Almquist and Stokstad ('35) have demonstrated the necessity in the nutrition of chicks, ducks, and geese, of a fat-soluble factor distinct from vitamins A, D, and E. Deficiency of this substance in the chick was brought about by feeding cereals and even by a ration containing 15 per cent yeast. This deficiency disease which resembles scurvy is characterized by subcutaneous, intramuscular, and abdominal hemorrhages, and by anemia. Because of the prolonged coagulation time of the blood in the animals deficient in this substance, the Danish investigators applied the term vitamin K (Koagulations-Vitamin) for this factor. Dam and associates ('36) and Schønheyder ('36) have shown that the failure of the blood to clot properly appears to be due to a decrease in the prothrombin in avitaminosis K.

Almquist and Stokstad ('36a) have demonstrated the transfer of the antihemorrhagic factor from hen to chick through the egg yolk. They noted indications of a certain degree of synthesis of this factor in the intestinal tract of chicks receiving a diet free from vitamin K. The vitamin appears to be synthesized by bacterial action, since its amount tends to increase in feedstuffs subjected to such action. Dam et al. ('37) also used dogs, rats, guinea pigs, rabbits, and pigs to determine their need for the antihemorrhagic substance. They studied the changes in the clotting time of blood from these animals following administration of vitamin K under experimental conditions. However, the results in mammals are not conclusive. It may be that certain species have no physiologic need for this factor or that these animals are either able to synthesize the antihemorrhagic substance or procure it from synthesis of the vitamin by intestinal bacteria. No evidence as

to its relation to hemorrhagic diseases in humans has yet been presented.

Dam and his associates further studied the possible function of this factor in man. No beneficial effects were obtained from use of vitamin K in a case of hemophilia. More extensive investigation may furnish data of greater significance as to the role of this factor in the clotting of mammalian blood.

Hog liver fat, hemp seed, soy bean oil, and rice bran are good sources of this nutrient. It is present in vegetables, particularly the leafy varieties and to a lesser degree in cereals. The fats from rye, corn, rice, sunflower, cod liver, and wheat germ oil, orange oil, fresh carrots, lemon juice, liver extract, egg yolk, egg white, and alfalfa ash were inactive as respects vitamin K activity (Almquist and Stokstad, '36b).

The antihemorrhagic factor occurs in the easily soluble non-sterol fraction of the unsaponifiable matter; its activity, however, seems to be markedly increased by the presence of the fatty acid fraction (Dam et al., '36, l.c.). It is a colorless, unsaturated compound, unstable to alcoholic alkalis even in the absence of air. It is fairly stable to light, heat, and standing, and it easily distills *in vacuo*.

Recent experiments of Klose, Almquist and Mecchi ('38) indicated that the activity of the vitamin was destroyed by oxidizing agents, strong acids, aluminum chloride and a number of reagents which have the ability to add across an ethylene linkage. It was not appreciably affected by reducing agents nor by reagents which react with alcoholic, carbonyl or phenolic groups. These findings show that there is no alcoholic or carbonyl group in the vitamin molecule and that its properties are consistent with those of a complex unsaturated hydrocarbon. Acetone is a good solvent for the vitamin and acetone or alcohol extracts are more active than the food without extraction, indicating that extraction is incomplete in the alimentary tract.

Almquist ('36) has described a method for obtaining concentrates of vitamin K by extraction of foodstuffs with hexane. Distillation of this concentrate in high vacuum gives a yellow viscous oil which is a very potent source of vitamin K. He ('37) has also described the preparation of vitamin K in crystalline form. The crystalline material is free from nitrogen, sulphur, and phosphorus but there is slight evidence that the molecule contains one or more benzene nuclei.

The Anti-Gizzard-Erosion Factor.—In avitaminosis K erosions of the lining of the gizzard were frequently observed. Early in these studies it was accepted by the investigators in this field as a part of the syndrome of vitamin K deficiency.

Almquist and Stokstad ('36, l.c.) found that erosion of the gizzard was cured by a saponifiable fraction of the hexane extract of alfalfa

or dried kale. Their anti-gizzard-erosion factor is fat-soluble and appears to be thermolabile and readily destroyed by alkalies. They succeeded in concentrating it to some extent by freezing out the inactive material from an hexane extract of wheat bran oil. This substance does not seem to be necessary for growth.

In recent experiments Almquist and Mecchi ('38) found that cholic acid is an effective dietary supplement in the cure or prevention of erosions of the gizzard lining. Dehydrocholic acid is almost equally effective, while deoxycholic acid is comparatively ineffective.

Cholic acid is an integral part of the gizzard lining and a deficiency of bile or of cholic acid in the chick leads to erosions of the lining.

In their studies of B_4 deficiency in chicks, Bird et al. ('36) and Kline and associates ('36) constantly noted gizzard erosion. They found dried lung tissue to be the most potent source of the gizzard factor. Liver and kidneys also contained it. Among the cereals, oats is the best source; wheat middlings and bran compare favorably with oats, wheat and corn are fair sources, and alfalfa is ineffective. In their experience this factor could not be extracted with alcohol or ether. Their anti-gizzard-erosion factor is labile to both dry heat and autoclaving at 120° C.

Thus a discrepancy exists between the work of Bird and associates ('36, l.c.) and those of Almquist et al. ('36, l.c.) in their findings as to the solubility of the anti-gizzard-erosion factor and as to its distribution. The former group of investigators believe that the factor is specific and that it is a water-soluble substance, while Almquist and coworkers present equally convincing data that it is a fat-soluble factor.

The Wisconsin group of investigators (Bird and Oleson, '38) were led by the observation on the richness of the anti-gizzard-erosion factor in pork lung to investigate the connective tissue of lung, and other types of connective tissue. They found that feeding 10 per cent of cartilage afforded excellent protection, but doubtful results were secured by feeding beef tendon and yellow elastic tissue. Still more surprising is their finding that protection against gizzard erosion follows feeding of 3 to 5 per cent of chondroitin, an amino polysaccharide conjugated with sulfuric acid. Glucosamine, glucuronic acid, and galactose, all components or derivatives of chondroitin, were ineffective.

Further investigation will determine whether the anti-gizzard-erosion factor is a specific one and whether more than one substance is involved in the maintenance of the healthy condition of the lining of the gizzard in chicks.

The "Grass Juice" Factor.—Elvehjem, Hart, Jackson and Weckel ('34), Stirn, Elvehjem and Hart ('35), and Kohler, Elvehjem and Hart ('37) have shown by feeding studies that green grass and grass

juice contain one or more substances which greatly enhance a diet supplemented with all known vitamins. The factor is abundant in summer milk and is present in winter milk in regions where feeding with silage is practical. The amount is so small in winter milk as to be the limiting factor in the growth of rats fed such milk, even when the known inorganic deficiencies (iron, copper, and manganese) of such milk are supplied. The following table from the paper by Kohler et al. ('37, l.c.) illustrates the distribution of this factor:

THE EFFECT OF VARIOUS SUPPLEMENTS AS ADDITIONS TO MINERALIZED WINTER MILK

(Male rats only)

SUPPLEMENT	LEVEL FED	GAIN OVER CONTROLS	GAIN IN WEIGHT
	per day	*gm. per day*	*gm. per day*
Cod liver oil	0.1 gm.	0.08	...
Orange juice	1.0 cc.	−0.52	...
Defatted wheat germ	1.0 gm.	0.27	...
Brewer's yeast	0.1 gm.	0.14	...
Brewer's yeast	0.5 gm.	0.49	...
Dried hog's brain	0.25 gm.	0.55	...
B_2 concentrate	0.5 gm. liver extract	0.55	...
Fresh lawn grass	3.0 gm.	1.68	3.91
Grass juice	0.5 cc.	0.91	...
Grass juice	3.0 cc.	1.69	4.48
Dried oat grass No. 180	0.6 gm.	1.36	3.98
Dried oat grass No. 223	0.6 gm.	0.55	3.17
Liver extract sample 1	0.2 gm.	1.38	...
Liver extract sample 1	0.5 gm.	1.23	3.40
Liver extract sample 2	0.5 gm.	0.67	...
Rice bran	0.2 gm.	1.29	3.77

These investigators point out that the grass factor is not vitamins A, D, C, thiamin, B_2, B_4, B_6, all of which were eliminated by their tests, in which inferior growth responses were secured when mineralized winter milk was supplemented with cod liver oil, orange juice, brewer's yeast, dried brain, and defatted wheat germ. Summer milks are relatively rich in the grass factor.

Hogan and Johnson ('36) found that when guinea pigs or rabbits were fed a diet consisting of ground oats, skim milk powder, wheat germ oil, cod liver oil, NaCl, $CaCO_3$, the animals could grow but not reproduce. Although it is not stated whether ascorbic acid was provided, undoubtedly it was included; otherwise growth would not have occurred. When, however, the ration was supplemented with grass

juice, a dilute alcohol extract of the juice of young cereal grasses, oats or barley, or an ether extract of dehydrated alfalfa, reproduction became possible. However the two kinds of animals responded somewhat differently to the alcohol and ether extracts of these plant tissues. In case of the alcohol extract alone, advanced pregnancy in the guinea pig was rarely observed and there was evidence of abortion. When normal litters were cast, the mortality was low but growth was not normal. With the ether extract alone, the animals became pregnant but the ration was apparently still inadequate because some of the mothers died after delivering the young. In the ones that survived, the young seemed normal at birth, but some were dead and the others grew slowly for a time and then died. These workers believe that the alcohol extract is a lactation factor and the ether extract is essential during pregnancy. A combination of the two extracts was much more effective.

L_1 and L_2 Lactation Factors.—Nakahara and Inukai ('34-35, '35-'36, '36-37, '38) report that a diet consisting of polished rice, fish protein, butter, salts, and brewer's yeast or the acid earth adsorbate of yeast extract, was adequate for growth, pregnancy, and parturition of rats but failed to support lactation. In order to secure normal lactation, two substances, which these workers designate factor L_1 and factor L_2 must be added to this diet. The two factors are different substances since one cannot be substituted for the other. They are equally essential, for both together are needed to produce the desired effect.

Factor L_1 is present in beef liver and is obtained from an extract of this foodstuff freed from its vitamin B-complex.

Factor L_2 is found in baker's yeast and is obtained from it after the removal of the B-complex.

Beef liver does not contain L_2 and baker's yeast appears to be deficient in L_1.

Nakahara and associates believe that these factors, which are effective in minute doses, are vitamins since animals are entirely dependent on the diet for their supply. In addition, they propose that vitamin L_1 and vitamin L_2 together constitute the vitamin L-complex.

Factor L_1 was prepared as an extract of beef liver in 40 per cent alcohol. The extract was adsorbed on acid earth at pH 3 to 4 and the active filtrate was precipitated with barium hydroxide, followed by phosphotungstic acid. Each of these precipitates was an active source of factor L_1. They later reported ('36-37, l.c.) that factor L_1 alone did not support adequate lactation until supplemented with factor L_2. This was prepared as described, except that the filtrate from the earth was precipitated successively with phosphotungstic acid and $AgNO_3$ + $Ba(OH)_2$. Both precipitates contained the active factor. The nature of these hypothetical factors is unknown.

The Chick Anti-Encephalomalacia Factor.—Pappenheimer and Goettsch ('31, l.c.) described a syndrome in chicks caused by the deficiency of some nutritional factor. They called this disease, characterized by extensive lesions of the cerebellum and occasionally of the cerebrum, nutritional encephalomalacia. Pappenheimer and Goettsch found that the disease can be prevented by the feeding of various oils such as Wesson (cottonseed) oil, hydrogenated cottonseed oil (Crisco), corn oil or peanut oil, soy bean oil, and olive oil. Lard, on the other hand, appears to intensify nutritional encephalomalacia (Goettsch and Pappenheimer, '36). These investigators have ruled out vitamin E as a factor in this condition.

Jungherr and Pappenheimer ('37) present some remarkable illustrations of the different reactions of various species of birds to their encephalomalacia diet. Whereas chicks suffer extensive lesions of the brain when fed this diet, ducklings do not develop brain lesions, but instead there is a widespread degeneration of the skeletal muscles. The same diet produces in turkey poults selective necrosis of the smooth muscle of the gizzard wall, unaccompanied by significant lesions in other organs or tissues. Pigeons appear to be refractory under these conditions of nutrition.

Ni ('37) using a diet of the same composition as Pappenheimer and Goettsch also produced nutritional encephalomalacia in chicks. However, he succeeded in preventing this disease by the addition of Chinese gelatin to the diet. Gelatin freed from fat was quite as effective as the original which contained fatty substances.

Thus the experimental syndrome described under the terms chick paralysis and nutritional encephalomalacia is as extraordinary as is that respecting gizzard erosion or hemorrhagic disease in chicks. As stated elsewhere, Keenan et al. (l.c.) and Hogan and Boucher (l.c.) believe that the chick paralysis produced by them is identical with the chick encephalomalacia of Pappenheimer and Goettsch ('31, l.c.). Yet Keenan and associates claim that the curative substance is present in defatted tissues and is a water-soluble factor, whereas Pappenheimer and Goettsch correct the deficiency they produce by the feeding of various oils. Goettsch and Pappenheimer ('36, l.c.) have discussed the differences of their experimental results and those of Keenan and associates and conclude that the two groups may not be dealing with the same syndrome.

Muscular Degeneration in Herbivora in its Relation to Nutrition.—Madsen and associates ('32-33) made many attempts to formulate a satisfactory diet of purified foodstuffs for herbivorous animals but without complete success. A diet composed of cellulose, cornstarch, casein, sucrose, yeast, lard, salts, cod liver oil, and orange or tomato juice daily, produced in rabbits and guinea pigs a state of malnutri-

tion from which the animals eventually died. There was evidence of failing circulation, consisting of intermuscular edema of the legs, increased fluid in body cavities, and congestion and edema of the lungs. There was frequently a severe hemorrhagic enteritis, often associated with large amounts of blood stained fluid in the lumen of the small intestine. Fatty degeneration of the liver and kidneys was a frequent finding. The skeletal muscular lesions were similar to hyaline, waxy or Zenker's degenerative changes. There was also a proliferation of fibroblastic tissue which tended to replace the degenerating muscle. The heart muscle was involved in all animals. Gross alterations consisted of dilations especially of the right ventricle. When the heart was yet normal in shape, the myocardium was usually paler than normal, suggesting degeneration and fibrosis. Histologically, hyaline and granular degeneration of the heart muscle fibers was seen, and in some areas the fibers were necrotic and in process of absorption. These changes were accompanied by an increase in connective tissue which often completely replaced portions of the degenerated muscle. Comparable changes occurred in goats fed a diet of similar composition. These heart changes being similar to the ones described by Agduhr ('26), they studied the question of cod liver oil injury, but with inconclusive results.

Pappenheimer and Goettsch ('31, l.c., '36) have described nutritional muscular dystrophy in rabbits by restricting them to a diet of rolled oats, wheat bran, casein, lard, cod liver oil, NaCl, and $CaCO_3$. One adult rabbit which successfully completed a pregnancy on this diet, when about one year old, upon the performance of a biopsy from the thigh muscles, showed necrotic fibers. Her young were scrawny and were dead within 24 hours. Histological sections from their muscles showed that the muscular dystrophy had been transmitted to the young in prenatal life.

These workers conclude that it is not justifiable at the present time to speak of this disease as a deficiency state but point out that there is some evidence that soy bean oil and cottonseed oil exercise a protective effect. Madsen ('36) found that the basal diet supplemented with the non-saponifiable fraction of cod liver oil, and without added fat, produced dystrophy to nearly the same extent as the same diet containing 6 per cent of lard. Substitution of cottonseed oil for lard caused a high degree of protection. He confirms the finding that cod liver oil causes dystrophy and concludes that the fat relationships studied are not the only factors involved in the production of muscle dystrophy.

Morgulis and Spencer ('36) using the diet of Pappenheimer and Goettsch ('31, l.c.) prevented as well as cured the muscle atrophy

by supplementing the basal diet with fresh green alfalfa, lettuce, wheat germ oil, dry alfalfa, and wheat germ oil or whole wheat germ. These experimenters believe that there are at least two substances involved in the prevention or in the cure of muscle dystrophy, both factors being present in fresh green alfalfa or in whole wheat germ. On the other hand, cold pressed wheat germ oil supplies one of these factors and lettuce or dry alfalfa furnishes the other.

Drying, extraction with water or alcohol, or ethereal-ferric chloride, readily destroys at least one of the substances.

Olcott ('38) has made the plausible suggestion that muscular dystrophy of Herbivora, produced by the experimental procedures outlined above, is due to a lack of vitamin E caused by the destructive effect of cod liver oil upon this nutrient. The skeletal muscle lesions in his young rats from females on vitamin E-deficient diets were very similar to those in the nutritional muscular dystrophy of Herbivora.

It will be seen from the foregoing that much experimentation will be necessary in order to finally establish the number and nature of the nutrient factors which different species of mammals and birds require. Specific differences in nutritive needs exist to an extent not contemplated a few years ago. Very little is known about the specific substances which are necessary for the nutrition of the lower forms of living things. McCay ('38) has reviewed some of the literature on this subject, and Knight ('36) has summarized the present knowledge of bacterial nutrition.

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CHAPTER

XXIII

Vitamin E

AN ACCOUNT of the discovery of vitamin E and the role it plays in nutrition begins in the year 1920 when Mattill and Conklin ('20), in studies of the nutritive qualities of milk, found that rats reared on cow's milk were incapable of raising young although the milk was supplemented with iron, probably containing copper, and a small amount of yeast. Two years later Evans and Bishop had made sufficient progress in their studies on the relation between nutrition and fertility to announce the existence of a dietary factor essential for reproduction (Evans and Bishop, '22). This discovery was followed by a comprehensive investigation of the "reproductive vitamin" in which Evans and associates and Mattill and coworkers contributed a large amount of our present information. The evidence for a dietary factor, apparently nonessential for growth but required for reproduction, tended to broaden the concept of the role of diet in all aspects of life. At present the chemical investigations of this group of nutrients appear to have reached the climax of painstaking and remarkable achievement in their isolation, identification, and synthesis. The physiological and therapeutic studies have made considerable progress. Now that pure materials will soon be available we may expect to witness rapid advances in such investigations. Excellent reviews of the subject have been written by Evans ('32) and Mattill ('38).

Vitamin E and Antioxidants.—For several years there was much confusion among different investigators of vitamin E owing to its peculiar relation to oxidative influences. Under certain conditions, for example, it was stable to high temperatures (up to 250° C.), but in the presence of rancid fat it was rapidly destroyed, even at room temperature. A clue to the nature of this extraordinary behavior was furnished by McCollum and associates (Simmonds, Becker and McCollum,

'27) and Jones ('27) who found that the inactivating effect of ferrous sulfate on vitamin A could be prevented by means of wheat germ oil, a rich source of E. Simultaneously Mattill ('27) showed that diets which caused sterility were subject to rapid oxidation and that wheat germ oil was efficacious in preventing such oxidative changes. Some time later Mattill and Crawford ('30) adduced evidence that the protective property of wheat germ oil was due to the presence of hydroxy groups which inhibit oxidation of fatty acids. The term antioxidant was employed to designate the protective substances in wheat germ oil, and later found in lettuce oil and tomato oil (Olcott and Mattill, '31a; Bradway and Mattill, '34).

Olcott and Mattill devoted much effort to the problem of determining whether the antioxidant effect of vitamin E concentrates and the vitamin E effect resided in the same or in different compounds. Studies of a concentrate which exhibited the properties of both E and the antioxidant furnished important information (Olcott and Mattill, '31b). When the concentrate was treated with petroleum ether and 92 per cent methyl alcohol the vitamin E activity was found in the petroleum ether fraction and the antioxidant went into the methyl alcohol solution. Thus a difference in identity was indicated. Later studies supported this belief since acetylation destroyed the antioxygenic properties but left the vitamin E activity unimpaired (Olcott and Mattill, '34). Moreover they found antioxidant effects associated with the vitamin in wheat germ oil and oils from lettuce, tomato, carrot, alfalfa, cottonseed, corn, sesame, palm, soy bean, and peanut. But there were no demonstrable antioxidant effects in yeast, lard, cod liver oil, palm kernel oil, and castor oil. It was concluded, therefore, that the antioxidant substances, which they now designate as inhibitols, are not vitamin E.

Antioxidants in the food are necessary for the preservation of vitamin E activity. Cummings and Mattill ('31) correlated the susceptibility to oxidation of several fats and oils with the reproductive behavior of rats. They showed that oxidation in the fatty constituents of the diet destroys E, that such oxidation is extensive in some common fats and oils, and that the amount of vitamin E in any diet is largely determined by the balance between anti- and pro-oxygenic substances. These findings were extended by Bradway and Mattill (l.c.) who showed that the fat-soluble vitamins in tomato, carrot, and wheat are accompanied by inhibitors which prolong the induction period of autoxidizable fats. Carotene and lycopene are pro-oxygenic, and shorten the induction period of autoxidizable fats. Such information was of great assistance to investigators in protecting vitamin E from oxidative destruction during its concentration and isolation.

Destruction of Vitamin E by Means of Ferric Chloride and Rancid Fats.—The ready destructability of vitamin E by ferric chloride or rancid fats, when the content of antioxidants is relatively low, has been of practical use in preparing diets without vitamin E activity. Waddell and Steenbock ('28) found that E is more readily destroyed by ferric chloride, under suitable conditions, than vitamin A. Several different rations produced the typical sterility in rats after a dilute ether solution of ferric chloride had been evaporated upon them. The iron treatment of rations caused the formation of some substance which actively opposed vitamin E. This was called an antivitamin. It could be extracted from the treated ration by ether.

As pointed out by Evans ('32, l.c.) the discovery of vitamin E was due in part to the accidental use of slightly rancid lard in rations which apparently contained all the necessary nutrients. Since lard is usually deficient in antioxidants, the vitamin E of the rations was destroyed. Butter fat, cod liver oil, and other fats and oils, particularly if they are in incipient rancidity, destroy E; consequently they are sometimes included in experimental rations to destroy this factor.

Isolation of Vitamin E.—As early as 1927 Evans and Burr ('27) had made potent concentrates of vitamin E. In their work the nonsaponifiable matter from wheat germ oil was treated with pentane and methyl alcohol, thus removing most of the sterols and some oily material. After removing the carotenoid pigments and some other inactive material, by means of these solvents, they obtained a red oil of high activity. Olcott and Mattill ('34, l.c.) obtained similar oils from various sources. High vacuum distillation provided them with a product considerably more potent than that of Evans and Burr. Drummond and associates ('35) also prepared highly potent concentrates. After the demonstration by Olcott ('35) and by Drummond and coworkers (l.c.) that the vitamin contains a hydroxyl group which can be readily esterified, it became possible to consider the isolation of crystalline allophanates. The reaction of cyanic acid with the hydroxyl group of alcohols forms allophanic esters which are generally favorable for identification purposes. By this means Evans, Emerson and Emerson ('36) were able to isolate three different allophanates from wheat germ oil concentrates:

1. Allophanate of β-amyrin with a melting point of 250°. The regenerated alcohol had no vitamin E activity.

2. Allophanate with a melting point of 138°. The regenerated alcohol had some activity.

3. Allophanate with a melting point of 158-160°. The activity of the alcohol regenerated from this substance was much higher, 3 mg. being sufficient to permit an E-deficient rat to bear young. The empirical formula $C_{29}H_{50}O_2$ was assigned to this alcohol. According to

Evans and coworkers it shows a characteristic absorption band at 298 A., $E\ {}^{1\ \text{per cent}}_{1\ \text{cm.}} = 90$ ca. The name *α-tocopherol* was given to it (Tokos: childbirth; phero: to bear; -ol, indicating an alcohol).

Todd, Bergel, Waldmann and Work ('37a) confirmed the findings of Evans and coworkers, and in addition they isolated a crystalline allophanate, m.p. 70°, which has not been examined biologically. Todd and associates ('37b) have developed an improved procedure for separating the allophanates of vitamin E concentrates of wheat germ oil and rice germ oil. By this means an allophanate was obtained which they designated as β-tocopheryl allophanate, m.p. 143.5-144.5°. It is optically active and the parent alcohol shows an absorption maximum at 295 mμ. They succeeded in securing only traces of the α-tocopheryl allophanate described by Evans et al. Since the vitamin E activity of their concentrates, yielding principally β-tocopheryl allophanate, showed full activity in single doses of 5 mg. per rat they concluded that β-tocopherol is the principal vitamin E in wheat germ oil. But Emerson's (38a) extensive studies show that oils vary greatly in their content of the different tocopherols, depending on the locality and on the year of the crop from which they are obtained.

Emerson, Emerson and Evans ('36) have isolated α-tocopherol from cottonseed oil, which apparently is a good source of the substance, as compared with wheat germ oil, since it contains less non-saponifiable material. They have also obtained γ-tocopherol from the same source (Emerson, Emerson, Mohammad and Evans, '37). It appears probable that there is considerable variability in the proportions of α,-β,- and γ-tocopherols in various natural substances having biological activity. It can be definitely stated that they have uneven E potency. The α-substance is more active than the β-or γ-forms. It is probable that all three compounds are isomeric, and therefore have the same empirical structure $C_{29}H_{50}O_2$. On the basis of structural considerations, it appears probable that there are more than the three known tocopherols which possess the properties of vitamin E. Definitely, vitamin E is of multiple nature.

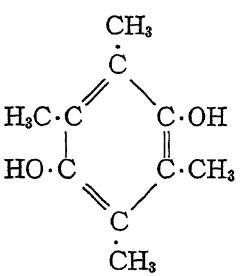
Chemical Structure and Synthesis.—At present (early summer of 1938) there is much activity in several different laboratories in studies on the structure and synthesis of vitamin E. Rapid progress is being made and it appears that the fundamental chemical investigations will be completed in a short time.

After the empirical formulae of both α- and β-tocopherol had been suggested by Evans and associates ('36, l.c.), Fernholz ('37) made the next advance when he found that α-tocopherol, isolated from cottonseed oil, decomposes when heated at 350° and a crystalline sublimate is obtained as well as an oily distillate. The crystalline material, when

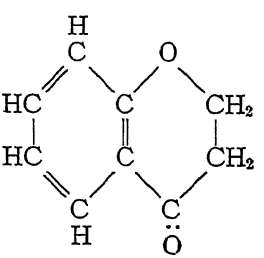
purified, led to a formula $C_{10}H_{14}O_2$. A search of the literature showed that it closely resembled durohydroquinone (i.e. tetramethyl hydroquinone), and upon comparison with a pure specimen of this substance was found to be identical with it. Somewhat similar observations were made independently by McArthur and Watson ('37) and by Todd, Bergel, Waldmann and Work ('37, l.c.). Since the reduction of a silver nitrate solution with pure α-tocopherol did not result in the splitting off of durohydroquinone it appeared that the alkyl portion of the α-tocopherol molecule is connected with the aromatic ring by means of a carbon bridge (Fernholz, '38). It was necessary, therefore, to abandon the view that the linkage is by means of an ether bond.

Various observations, particularly those based on oxidative reactions, indicated a heterocyclic structure. Bergel and coworkers ('38a) were led to believe that β-tocopherol is a coumaran derivative since the absorption spectrum and reducing properties of both heptadecylcoumaran and methylcoumaran are very similar to those of pure β-tocopherol. Also, Karrer and associates ('38) believed that coumaran is the derivative of α-tocopherol. However, the observation of Fernholz ('38, l.c.), Smith, Ungnade and Prichard ('38), Emerson ('38b), and of John and associates ('38) provide rather convincing evidence that the derivative of α-tocopherol is chroman, instead of coumaran. According to Fernholz, a lactone is obtained when α-tocopherol is oxidized with chromic anhydride in acid solution. This is explainable on the basis that α-tocopherol contains a chroman ring, but if it were a coumaran ring a beta hydroxy acid should be expected as an oxidation product. There are other evidences which suggest that chroman is the basis of vitamin E structure.

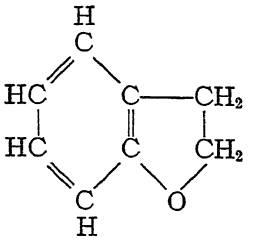
The following formulae indicate the principal compounds under discussion:



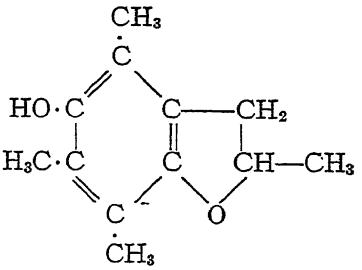
Tetramethyl hydroquinone
(Durohydroquinone)



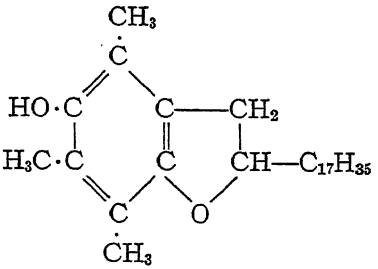
Dihydrobenzopyran
(Chroman)



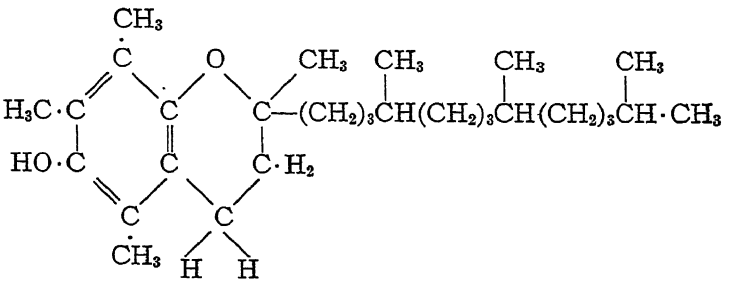
1, 2-dihydrobenzofuran
(Coumaran)



5-hydroxy-2 : 4 : 6 : 7-
tetramethylcoumaran
(Methylcoumaran)



5-hydroxy-4 : 6 : 7-trimethyl-2-n-
heptadecylcoumaran
(Heptadecylcoumaran)



α-Tocopherol (Fernholz)

α-tocopherol was first synthesized by Karrer and associates (l.c.) using trimethylhydroquinone, phytylbromide and zinc chloride. No biological assays were reported. It is probable that the structure they assigned to this substance was in error since they regarded it as a coumaran rather than a chroman. Shortly thereafter Smith, Ungnade and Prichard (l.c.) reported two different syntheses of α-tocopherol, one from trimethylhydroquinone and phytylbromide without a catalyst, and also from the hydroquinone and phytadiene. The evidence suggested that the products were chromans. Thus the chemical investigations of vitamin E, slow at first, but gaining momentum with successive findings, have drawn to a successful climax of rapid discoveries which again illustrate the method of progress in nutrition.

Vitamin E Activity of Synthetic Compounds.—Important preliminary studies of synthetic compounds have been made by Evans, Emerson and Emerson ('38). Vitamin E activity of durohydroquinone, various of its ethers, and certain cyclic ethers was observed when fed at high levels. Synthetic α-tocopherol, prepared by Smith, Ungnade and Prichard, was active in a single dose of 3 mg. The substance prepared from natural sources has the same potency as the synthetic vitamin.

Early Observations on the Nutritional Significance of Vitamin E.—Sterility in animals may be caused by any dietary defects which interfere with growth or which impair health. Thus vitamin A deficiency interferes with ovulation, and hence with reproduction. Deficiency of an essential amino acid, or of any one of the several vitamins, as well as unphysiological relations in the food supply of the indispensable inorganic elements, will lead to failure of normal reproduction. Specific instances of such impairment have been given in previous chapters. In vitamin E we meet a nutrient, a deficiency of which impairs fertility by interference with placental function in the female, and spermatogenesis in the male.

A number of investigators who studied the effects of diets of natural foodstuffs restricted as to source, and also diets composed of more or less purified food substances, observed low fertility or sterility in their animals, but the results could not be interpreted. Evans and Bishop ('22) laid the foundation for all later researches on vitamin E by their study of the precise mechanism of the estrus cycle in the rat. They then turned their attention to the effect of inadequate diets on the ovulatory rhythm and on the several steps in the physiology of reproduction. Evans ('32, l.c.) stated that "to their surprise they found that on supposedly complete dietaries, and with both growth and external appearance normal, animals would also exhibit normal estrus cycles and would breed, ovulate and conceive, yet be unable to experience normal gestation through invariable occurrence of fetal death."

A systematic study was begun in which vitamins A, "B," C, and D were supplied in abundance but without remedying the defect. It was found that addition of certain natural foods quickly corrected the abnormality, and since, in the case of wheat germ or wheat germ oil, the "curative" dose was very small, Evans ('22, l.c.) announced the discovery of a new, and at that time the 5th vitamin, which he and his associates provisionally designated X, and which was later called vitamin E. Confirmatory studies were soon reported by others, notably by Mattill and Stone ('23), Sure ('24), and Mason ('26).

Evans ('32, l.c.) has emphasized that little information concerning vitamin E can be gained by merely noting failure to secure young when the sexes are placed together. It is necessary to observe the occurrence of estrus by means of vaginal smears; to ascertain the occurrence of copulation, by finding of the vaginal plug in the vagina or of residual sperm in the vaginal contents; the cessation of estrus cycles as determined by daily study of the vaginal smear, up to and including implantation as detected by the erythrocyte sign (finding of red blood cells in the smear on the 14th to 17th day) which never occurs unless implantation of the ovum has taken place. It is also necessary to follow the course of pregnancy, to determine whether it is normal or whether resorption occurs (which is characteristic of sterility due to deficiency of E), or impairment of the birth mechanism (which occurs in some stages of sterility due to vitamin A deficiency). Unfortunately, some investigators have attempted to interpret their studies without subjecting themselves to this rigid and laborious task, and as a consequence much of the literature on the various aspects of the vitamin E problem is untrustworthy.

The effects of vitamin E deficiency are remarkably different in the two sexes, the only other parallel case known being a deficiency of manganese.

Vitamin E Deficiency in the Female.—Evans ('32, l.c.) states that "striking proof that the early phases of the reproductive process do not need vitamin E is afforded by the resuscitation and in fact the complete normality of young in gestations when the curative dose of E is not administered until a few hours before implantation of the blastodermic vesicles—an administration as late as the completion of the 5th day after copulation. The vesicles are attached to the uterine mucosa some time early in the sixth day." Stigmas of abnormality do not appear until the 8th day. A sufficiently large single dose of E administered on the 5th day is adequate to enable the female to complete a normal gestation. "Though the fetal rather than the maternal tissue on the placenta shows selective disorder when E is low or absent, there are indications that the maternal mechanism is abnormal in a strange tendency of the uterus in such females to form spontane-

ous nodular deciduomas when pseudopregnancy is provoked by copulation with a vasectonized male." Pseudopregnancy is a remarkable phenomenon described by Long and Evans ('22). It is manifested in a delay in the next estrus events, caused by sterile copulation or stimulation of the cervix. These stimuli do not delay or accelerate an impending ovulation, but delay the next following ovulation. The effect is immediate but the manifestation is deferred.

The integrity of the implantation site, placental tissues, and fetal tissues are dependent on vitamin E. The deficiency state is characterized by many free red blood cells at the site of implantation of the ovum, and hemorrhagic areas are seen in the placenta. The sinusoids are greatly distended. In the fetal tissues the blood islands do not develop normally, and it seems probable that deficient oxygenation, by means of this abnormality, may cause impairment of fetal development.

Urner ('31) described the history of 91 Wistar albino rats, deprived of vitamin E, which exhibited normal growth, estrus rhythm, and breeding behavior. Pregnancy in the entire group of rats was followed by embryonic death and resorption. No first litter fertility was observed. Urner found that from the 10th to the 17th day of pregnancy the embryonic and fetal resorption was more than counterbalanced by continued mammary development, so that the body weight continued to increase, although at a reduced rate. Gross examination of the pregnant uteri (from the 10th day onward) showed a softening of the enlargements, with blue discoloration, due to blood in the amniotic cavity—an indication of abnormal pregnancy. Microscopic examination of serial sections of embryos showed apparently normal embryonic development until the 10th day of pregnancy. At this time there appeared a rarefaction of the mesenchyme, failure of the blood-forming tissues, and death of the embryo. Necrosis began at the fetal surface of the ectoplacenta by the 13th day, and by the 17th or 18th day the mesometrial portion of the placenta was destroyed and cast into the lumen of the uterus. The decidua basalis appeared normal and became converted into tissue which, in the normal course, would become the maternal portion of the normal placenta until it was overtaken by degenerative changes on the 15th day. By the end of the 21st day of pregnancy the uterus had returned to an almost virginal condition, with the exception of the decidual bodies at the sites of recent implantation.

Vitamin E Deficiency in the Male.—Evans described sterility in the male rats as occurring at a later date than in their litter mate sisters. It can be tested for by mating experiments with females of known fertility, but may be predicted by the finding of characteristic "sheaves" or clumps of immotile spermatozoa in the fresh ejaculate.

There is progressive loss of tubular germinal epithelium, and irreparable damage to the basal spermatogonia. Eventually only diminutive tubules, devoid of all cells except the Sertoli elements, remain. Such males still possess normal seminal vesicles, prostate, and coagulating glands and although their germinal epithelium has largely disappeared, continue for a considerable time to manifest sex interest and power to copulate. Later on these sterile males suffer degeneration of the accessory organs and sex interest fades out.

Mason ('33) has reported the most critical study available on the effect of vitamin E deficiency on testicular degeneration. He describes as characteristic a liquefaction of the chromatin material of the spermatozoa and spermatids, and later the less mature cells, followed by the fusion of injured spermatids and spermatocytes into giant cells.

Even when vitamin E is administered at the time of the earliest signs of impairment of spermatozoa, only a few can be restored to fertility. Before these signs have appeared sterility can be invariably prevented by the provision of vitamin E. Evans points out that for the insurance of fertility throughout life in males a higher daily inflow of E is essential than for gestation alone: hence the requirement of the seminiferous epithelium, in proportion to its mass, is greater than that of the placental or fetal tissues. The latter increase much faster in volume than do the germ cells.

Thus, as pointed out by Mattill ('38, l.c.), "The striking difference in response to vitamin E deprivation by male and female animals can be explained, superficially, by the fact that in the male the damage is done to a part of the animal's own tissue and may for this reason be irreversible in contrast to the reparable damage in the female, which is wrought not on her own tissues but on those of the fetus."

Mason ('33, l.c.) has attempted to compare the anatomical effects of vitamin E deficiency with those produced by other dietary defects. Deficiency of vitamin A, in his experience, caused sloughing of the germinal cells into the lumina of the tubules (attributed by Evans to E deficiency). Marked injury to the testes caused by inanition was repaired in 3 to 4 weeks when the diet was increased, but injury caused by A deficiency required from 5 to 13 weeks for its repair. Combined deficiency of A and E caused less severe damage to the testes than did uncomplicated E deficiency, but deficiency of A decreased the time required to cause the changes characteristic of E deficiency. He suggests that vitamin E may be particularly essential for the later stages of maturation of the germinal cells. When the earlier stages of differentiation are slowed down by deficiency of A, vitamin E deficiency produces less profound histological changes in the germinal elements as a whole.

The Effects of Vitamin E Deficiency on Hatchability of Eggs.—A principal effect of vitamin E deficiency in hens is said to be the reduction in hatchability of the eggs and a decrease in the vitality of chicks that are hatched (Card, Mitchell and Hamilton, '30; Adamstone, '31; Barnum, '35). As shown by Adamstone, the first 3 days of development in the egg appear to be normal when the hen has been on an E deficient diet. But the effects of deficiency are precipitous and many chick embryos die on the 3rd to 4th days. Hemorrhages at various sites are regarded by Adamstone as the immediate cause of death. As a rule a "lethal ring" is found completely encircling the embryo at the end of the 5th day. The ring occludes the blood vessels of the blastoderm and thus asphyxiates the embryo. It is produced by extensive cell proliferation in the mesoderm. The nature of this "ring" is obscure. It would seem that the mechanism of chick embryo mortality might have a counterpart in the death and resorption of rat embryos, and perhaps embryos of other Mammalia. The data appear to indicate that deficiency of vitamin E is not infrequently a cause of low hatchability and high chick mortality in poultry production.

Effects of Deficiency on Growth.—For several years it appeared that vitamin E was specifically a reproductive factor but during the past decade evidence has slowly accumulated which prove that this nutrient is indispensable in general bodily functions. The first indication of need for it in other processes besides that of reproduction was furnished by Evans ('28) who noted that vitamin E deficiency caused a failure in the late stages of growth, "the phase intervening after the attainment of maturity." Several years later Blumberg ('35), by means of a highly refined basal ration, observed an earlier failure in growth and, in addition, marked generalized debilitation. Since the effects were quite striking the observations focused attention on the relation of vitamin E to growth. In the same year Ringsted ('35) reported somewhat similar results, but he did not employ curative procedures with sources of vitamin E. Emerson and Evans ('37) insisted that "The defective growth in experiments involving vitamin E deficiency occurs only after the fourth month of life." Olcott and Mattill ('37) also denied the validity of Blumberg's claims that vitamin E, or some related factor, is necessary for normal early growth. However, neither group of workers used basal diets exactly like the one employed by Blumberg. Martin ('37) studied the problem, using Blumberg's basal diet, and noted that slowing of growth occurs at the 10th week, thus essentially confirming the latter's observations. More recently Evans, Emerson and Emerson ('38, l.c.) have stimulated growth in female rats by means of pure α-tocopherol fed to the animals for 50 days after they had plateaued in weight on a vitamin E-low diet. Thus

it is fully established that vitamin E is indispensable for normal growth. Since it is difficult to understand how the growth process can be independent of vitamin E during earlier life but require it later, the logical assumption would seem to be that the nutrient is needed for growth irrespective of any particular period in the span of life. Until it has been demonstrated that young rats are able to grow normally although their tissues are completely devoid of vitamin E, the above claims of Evans and associates and of Olcott and Mattill must be regarded as without full experimental support.

Paralysis and Vitamin E Deficiency.—A curious type of paralysis is associated with marked vitamin E deficiency. As shown by Evans and Burr ('28), young rats from females on a vitamin E-low diet may exhibit a marked paralysis, especially of the hind quarters, about 20 days after birth. Many of the young have difficulty in righting themselves when placed on their backs. By the 21st day about 75 per cent of the animals are paralyzed in the posterior extremities and in part of the body wall musculature. The onset is sudden and some animals die so quickly that there is no loss of weight, showing that starvation is not a complicating factor. Others die after a few days, with some degree of emaciation. At the onset of the disability there is an almost constant exhibition of spasticity of the lower parts of the limbs and a flexor spasm resembling the "claw hand." A few, however, show an initial flaccid paralysis—a dragging of the hind legs—which is succeeded by the spastic condition. The paralysis is always partial. Those which survive 2 or 3 months are said to appear healthy but they remain partially paralyzed, with atrophic skin, and bilateral denudation over the upper thigh and sacrum. Most remarkably, somewhat less than one-fifth of such young recover completely. Also, the animals which survive, but remain partially paralyzed, are able to produce young in spite of the paralysis when a vitamin E concentrate is administered. A single administration of vitamin E, in the form of wheat, yellow corn, or lettuce, to such females, after birth of the young, is sufficient to protect the latter from paralysis. This suggests that the storage capacity for vitamin E is considerable and that the nutrient is secreted in the milk. But vitamin E does not cure the paralysis after it has developed.

Blumberg (l.c.) noted marked stiffness and evidences of muscular disturbance in female rats restricted to a vitamin E deficient diet for periods of 30 to 40 weeks. The animals could be cured by the administration of vitamin E concentrates. Ringsted (l.c.) and Marchesi ('35) also observed paresis in older rats on diets low in E. But Olcott and Mattill ('35, l.c.) were not able to confirm these observations, claiming that E deficiency was not the cause of this peculiar syndrome. However, the evidence undeniably points to the existence of a relationship

between vitamin E and the integrity of muscular or nervous functions concerning locomotion in adult rats. Emerson and Evans ('37, l.c.) found that females, on a vitamin E-low diet for 19 months, developed an unsteady gait and became partially bald. This has been confirmed and amplified by Burr, Brown and Moseley ('37) who restricted young female rats to a purified diet consisting of casein, 12; sucrose, 84.1; salt mixture, 3.9; together with supplements of vitamin A, viosterol, nearly fat-free yeast, pure thiamin, and linseed oil. The growth of the animals was somewhat below normal, as would be expected. At 22 months of age all the rats (5) became paralyzed, with loss of use of their hind limbs. The muscles of the legs and gluteal region were not noticeably atrophic. Electrical stimulation showed that sensibility to painful stimuli was not affected. Isolated muscles and larger nerve trunks of the leg and gluteal region gave normal responses. The sensory ganglia of the brain and spinal cord, as well as the posterior funiculus of the cord, had a yellow color. The paralysis could not be cured by means of vitamin E.

The nature of the paralysis is still obscure. Lipshutz ('36) has attributed the condition in young rats to degeneration of nerve tracts in the spinal cord. Olcott ('38) has made the important suggestion that some relationship exists between this syndrome and that produced in guinea pigs and other Herbivora on certain purified diets, the so-called nutritional muscular dystrophy of Herbivora. (See Chapter XXII.) He has pointed out that the diets which produce the syndrome in Herbivora contain cod liver oil or lard, both autoxidizable substances which quickly destroy E. The symptoms of paralysis in animals on the muscle dystrophy-producing diets bear a striking resemblance to those on E deficient regimens. This is especially indicated by a comparison of the symptoms noted in rabbits (Morgulis and Spencer, '36) with those seen in adult rats deprived of vitamin E (Burr, Brown and Moseley, l.c.). Olcott has concluded that the muscular lesions which occur in the young of vitamin E deficient rats are very similar to those observed in nutritional muscular dystrophy of Herbivora. Since it is believed that the nervous system is not involved in the latter condition, the lesions being primarily muscular, it is possible that the paralysis of vitamin E deficiency is attributable to muscular lesions without involvement of the nervous system.

Whether the "range paralysis" seen in some species of poultry is related to inadequate vitamin E is a subject for investigation. Likewise the various other forms of paresis observed in domestic animals, as well as man, should be investigated with reference to their possible relationship to disturbances in vitamin E metabolism, or inadequacy of this nutrient in the diet.

Vitamin E and Internal Secretions.—It is quite natural that investigators should give considerable attention to the possible relationships between hormones and vitamin E.

As early as 1925 van Wagenen ('25) observed cellular changes in the anterior hypophyses of male rats on an E deficient diet. These changes were further studied by Nelson ('33), who found that there is an increase in the size and number of basophilic cells similar to but less pronounced than that occurring in castrated males. Implantation of these anterior hypophyses into immature female rats showed that they contained more of the gonad-stimulating hormone than did those of normal rats but less than those of castrated rats. In each respect the effect of E deficiency was the same as that of cryptorchidism. The anterior hypophyses of E deficient female rats appeared to be normal. This latter finding is contradicted by Rowland and Singer ('36) who state that hypophyseal suspensions of non-pregnant E deficient rats are only about one-half as potent as those of normal females in promoting ovulation when injected intravenously into the estrus rabbit. They noted that there was a normal amount of gonadotropic substance in the hypophyses of E deficient females, cured by the administration of vitamin E. A third type of response was reported by McQueen-Williams ('34), who transplanted hypophyses of E deficient female rats into immature animals and concluded from the results that there is hyperactivity of the hypophysis in this deficiency, which is somewhat reduced by vitamin E administration. However, the sex-difference noted by Nelson, and others, should be expected on the basis that hypophyseal changes are the direct consequence of gonadal injury which is apparently confined to the male, the injury in females being limited to the placental structures.

Apparently the thyroid also is affected by vitamin E deficiency. Singer ('36) states that the thyroid glands of female rats deprived of E for 12 to 18 months exhibit marked hypoplasia. The condition was not affected by the administration of iodine, but injections of anterior hypophyseal extract caused some improvement. The thyroid became normal when vitamin E was administered. These observations correlate with those of Barrie ('37) who depleted female rats of E until sterility was established. Then the nutrient was administered, but only in very small amounts so that there were only 3 or 4 young per litter in succeeding pregnancies. Although the young appeared to be normal at first they failed to grow and became definitely cretinous. The appearance was characterized by a large head, sparse fur, upturned nose, deformed feet, and fontanelles still widely open. The animals lived 17 to 30 days but whether paralysis occurred was not stated. Singer suggests that E deficiency indirectly affects the thyroid through its influence on the anterior hypophysis.

Vitamin E and the Induction of Tumors.—Since Evans ('28) described the high incidence of deciduomata during pseudopregnancy in E deficient rats there has been some basis for suspecting that vitamin E might play a role in tumor formation. However, there is no clear indication of such a relationship, although several investigators have studied the question. Davidson's ('35) report that a diet rich in vitamin E inhibits the development of tar cancer in mice was not confirmed by Haddow and Russell ('37). However, the latter believes that the apparent discrepancy is due to other factors and is not attributable to E. Adamstone ('36), in a continuation of his earlier studies on the relation of vitamin E to excessive tissue growths in chick embryos, has tentatively concluded that vitamin E sets up some condition favorable to excessive cell proliferation. A complicating factor in his experiments is the use of ethereal ferric chloride to destroy vitamin E in the ration.

The astounding reports of Rowntree and associates ('37) have stimulated interest in this question. They claim that excessive and prolonged feeding of certain types of wheat germ oil, particularly unrefined oil obtained by ether extraction, produced a very high incidence of peritoneal spindle cell sarcomata in rats on a stock diet. McCollum, Becker and Day ('38) have failed to produce a single tumor in rats given large doses of cold-press wheat germ oil refluxed with ether. The oil was administered for 170 days. Hence it does not seem possible that ether, through the oxidizing effect of peroxides, produces cancerogenic substances in wheat germ oil. The relation to tumor formation of substances associated with natural sources of vitamin E requires further investigation.

Vitamin E Assay.—At present there are no satisfactory methods of vitamin E assay. This, of course, constitutes a decided handicap in studies of this group of nutrients. The best methods available are based on the determination of the ability of adult E-deficient females to bear living young following the oral administration of the test substance. At best the data are crude; moreover, they are obtained at considerable expense. Such procedures have permitted the accumulation of considerable information concerning vitamin E but they are in need of refinement for future investigations.

Bacharach and Allchorne ('38) have devised a curve relating dosage and response to vitamin E. They employ virgin rats depleted of E, as ascertained by mating tests. The substance to be tested is divided into 10 equal portions and administered daily for 10 days following positive mating. The results are expressed in terms of "mean fertility dose," the doses that will cause 50 per cent of implanted rats to bear litters (Bacharach, '38).

Palmer ('37) has criticized the procedure of administering the test substances in several divided doses, claiming that a single dose is pref-

erable until more information is obtained concerning the utilization of E at different periods of pregnancy. The criticism appears to be valid. Palmer has described in detail a method of assay applicable to wheat germ and wheat germ oil. Presumably it could be used for general estimations of E in other foodstuffs. The basal ration is: commercial casein, 27; autoclaved tapioca (dextrin), 35; commercial bulk lard, 22; dried brewer's yeast, 10; Hawk and Oser salt mixture, 4; and cod liver oil, U. S. P., mixed in the ration at weekly intervals, 2.

Distribution in Nature.—Since analytical methods for vitamin E have not been developed there are no satisfactory data on its distribution. On the basis of present evidence, however, it appears to occur in many different foodstuffs. The oils from seeds, such as wheat, rice, cotton, and corn are rich sources. Leafy vegetables also contain considerable amounts of the nutrient. Animal tissues and eggs contain it but they are not generally recognized as potent sources. Butterfat is low in E and it is generally believed to be absent from lard and cod liver oil. Commercial hydrogenated fats are good sources. The occurrence of antioxidants (inhibitols) in many foodstuffs is important in determining the vitamin E activity, since in their absence E is rapidly destroyed. Vegetable fats contain them but animal fats do not.

Requirements of Different Species.—Although it seems probable that vitamin E is needed by many different animal species indubitable evidence of its necessity has been established only in the case of rats and mice. These animals, with reproductive ability impaired by restriction to E deficient diets, have been restored to a normal condition by concentrates of vitamin E, i. e., the saponifiable fraction of wheat germ oil. This, in addition to the lack of positive effects from the administration of other known vitamins in generous amounts, constitutes unequivocal evidence of need for vitamin E. In discussing other subjects in this chapter we have presumed, on reasonably valid grounds, that E is required by chicks, but most of the studies of such animals have been based on the use of diets in which E was "destroyed" by treatment with ethereal ferric chloride. Thomas and associates ('38) claim that normal reproduction has been obtained in goats, sheep, and rabbits restricted to a diet prepared in this manner. The claim that reproductive ability is improved in humans, cattle, and pigs, by means of wheat germ oil or other rich sources of E, suggest that they require the nutrient.

Therapeutic Applications of Vitamin E.—There have been several reports that wheat germ oil or other rich sources of vitamin E are efficacious in some cases of sterility. Schioppa ('36) and others have stated that large doses of wheat germ oil increase the size of rabbit litters (cf. Thomas et al., *vide supra*). Bay and Vogt-Møller ('34), in convincing veterinary studies, have claimed that the injection

of sterilized wheat germ oil into apparently sterile, but otherwise normal, cows and sows permitted reproduction in a majority of instances. But many more studies will be necessary to establish its value in animal husbandry. The evidence, however, suggests the importance of extending research in this field.

Several clinical reports have appeared which suggest that wheat germ oil is of value in preventing spontaneous human abortions. But much of the data are rather unconvincing because of the lack of adequate control. Vogt-Møller ('31, '33, '36) has published the results of several studies in which wheat germ oil treatment apparently caused normal pregnancies which otherwise would have terminated in abortion. In these studies structural or functional abnormalities were ruled out as possible causes of previous reproductive failures. Currie ('36) reported a series of 23 women who, between them, had experienced 73 pregnancies, resulting in the birth of 11 living children, five of whom died shortly after birth. He administered 3 minims (0.18 cc.) of wheat germ oil concentrate (5 gm. of oil), and either "adexolin" (presumably a Ca preparation) or calcium gluconate and calciferol, for periods of about 5 months. Following this, 22 of the women were delivered of full term living children and the pregnancy of the remaining one was terminated by abortion. In a later study (Currie, '37) the use of an unsaponifiable wheat germ oil fraction was followed by 31 full term deliveries in 37 women who had previously aborted. Currie states that by means of similar treatment he had success in 14 of 15 cases of threatened abortion.

Watson ('36) has administered daily doses of wheat germ oil to a series of over 40 women, in each of whom abortion had occurred in 60 to 70 per cent of the cases. By means of larger doses threatened abortions were successfully treated in 13 of 19 cases.

Shute ('35, '36, '37) made the interesting observation that the blood serum of 70 per cent or more of women aborting spontaneously displays a characteristic resistance to proteolysis when treated with trypsin solutions. He tested the serum of rats of both sexes, which had been restricted to an E deficient diet, and found it resistant to tryptic digestion. From his description of rough coat and crusts of exudate on ears, nose, and tail, it appears that his animals were also suffering from vitamin B_6 deficiency. In threatened abortion in women he found the presence of the symptoms to coincide with the development of antiproteolytic activity of the serum. By means of large doses of wheat germ oil he has reported marked success in the treatment of a series of 82 cases of threatened abortion.

Shute's studies are of special interest because he believes he has demonstrated that the antiproteolytic substance in serum in cases of threatened abortion is estrin, or estrin-like, since he was able to

reproduce the property of antiproteolytic serum in normal blood by adding estrogenic hormones to normal blood. The presence of estrin, or some similar substance, in the blood is, he suggests, a direct result of vitamin E deficiency. He has been able to produce, for a few hours, blood serum having the characteristic antitryptic property by injection of a potent gonadotrophic preparation of pregnancy urine. He points out that in premature separation of the placenta there has never been a satisfactory explanation offered for the failure of the placental villi to penetrate the uterine wall as far as the serosal coat, since they normally are able to erode their way into the myometrium for a very considerable distance. It has been taken for granted, he says, that there is a certain amount of maternal resistance to such encroachment of the fetal trophoblast throughout every pregnancy. He suggests that when such resistance is excessive, pregnancy ends prematurely. He adduces evidence to indicate that the placental villi, in both normal and abnormal pregnancy, themselves possess, and probably secrete, the antagonist to their own erosive agents, i. e., the antiproteolytic substance. It is further suggested that during pregnancy vitamin E and estrin, or a similar substance, exist in some degree in equilibrium and if there is too much of the estin-like substance, pregnancy is interrupted.

Since the number of clinical trials of vitamin E is still small, and the statistical significance of the data has in no instance been shown, it is too early to assert that the place of this vitamin in therapy has been established.

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CHAPTER

XXIV

Dietary Properties of Foodstuffs

AN OUTSTANDING feature which emerged as nutritional research developed during the present century was the difference in nutritive properties of individual foodstuffs which, according to the chemical procedures for analysis in vogue at the end of the nineteenth century, appeared to be closely similar in composition. Many facts have been recorded in preceding chapters which make clear why foods which yield similar amounts of protein, carbohydrate, and fat often differ greatly in food value. Marked differences in the biological value of proteins, composition of mineral content, and of vitamins, account for these variations in quality of foods from the physiological standpoint. In this chapter is presented a brief summary of the dietary properties of the more important natural and manufactured products that enter into the human diet. These data afford a basis for determining the extent to which generalizations can be safely made as to what foods have supplementary values, and indicate the types of combinations which tend to make diets complete for the support of nutrition.

The Cereal Grains.—The principal cereal grains which are included in human dietaries comprise, in the order of the magnitude of their production and consumption, rice, wheat, maize, rye, barley, millet, and oats. These grains consist of a germ constituting the embryo plant, which is rich in protein and oil, and in the case of wheat at least is devoid of starch; an endosperm, consisting principally of starch but containing some proteins; an aleurone layer surrounding the endosperm, which is closely packed with protein aggregates; and a pericarp which forms a skin about the seed. The pericarp, with adherent particles of aleurone layer and of endosperm, constitute the bran that is separated in milling.

That the cereal grains serve as food for man and animals is a chance occurrence, the purpose of the seed being to produce a new plant. The substance of both germ and endosperm is designed for plant, not animal food. Experiments show that the proteins of cereals have a relatively low biological value, in that they can be utilized for transformation into animal proteins during growth only to the extent of about 25 per cent. The vitamins seem to be largely concentrated in the germ of the cereals. The bran layer is rich in phosphorus, but it is relatively unavailable. The endosperm is relatively poor in inorganic elements except potassium which is fairly prominent. Some of each of the physiologically important mineral elements are present, however, in every part of the grain.

Certain generalizations can be made concerning the dietary properties of these separate parts of the cereal grains. The proteins of the coarser material removed in milling, i. e., the bran and middlings of wheat, being derived largely from the aleurone layer, probably have a somewhat higher biological value than do those of the starchy endosperm which constitutes the highly refined flour. The proteins of the germ are also probably better constituted as respects animal nutrition than are those of the endosperm. We have but little definite knowledge on these points, however, so only general statements are warranted.

Yellow coloring matter in a cereal indicates the presence of some carotenoid, usually carotene, which is a source of vitamin A. In yellow maize, however, the principal yellow pigment is cryptoxanthine, which also is a source of vitamin A. Yellow color also goes along with the distribution of riboflavin. Since this substance has been known less than four years, and methods for its quantitative determination in many kinds of food materials have not been fully developed, we lack any reliable data as to how much of it is present in grains. We do know that it is present in all the cereals named above. White varieties of maize and the white endosperms of rice and oats are essentially lacking in carotene and other vitamin A precursors but it is possible that they contain at least a small quota of riboflavin. Both these pigments are much more abundant in the germs of all cereal grains. Likewise, thiamin and nicotinic acid, and all other vitamins of the B-complex, are much more abundant in the germ than in any other part of cereal grains. The practice of discarding the germ in milling operations employed in preparing cereal products for human consumption is unsound from the dietary standpoint.

Wheat.—From the dawn of history wheat has been the dominant cereal food of the white race. Our earliest ancestors probably pounded the wheat grains in mortars to make cooking easier, and at some

remote period, crude mills were invented which employed the principle of grinding the grain between two stones, the upper one revolving upon the lower. These were grooved radially to carry the particles from the center outward, and during thousands of years almost no advance was made in separating the different parts of the seeds. Stone grinding tended to pulverize all parts of the grain, but the bran layer ground with difficulty, so that it was always possible to sift out some of the coarser particles and thus prepare a flour which had a higher digestibility than had the whole grain product. In the Old Testament "fine" flour is distinguished from the entire wheat flour. It was not until 1879 that the modern roller mill process was invented for milling wheat. In principle this is accomplished by passing the grain between steel rollers which crush the grain by pressure instead of grinding it. The first rollers are corrugated and do not touch, and one revolves faster than the other. The grain is dried to make it brittle; and before milling it is exposed to moisture to enable the outer layer only to absorb moisture. Hence the pericarp becomes tough and flexible and is removed in the form of flakes which are easily separated mechanically. The crushed grain is later passed between smooth rollers which complete the pulverizing process, in which the germs, being rich in oil and protein, and plastic, are also easily separated because of their size and shape. The roller process makes possible, when combined with repeated siftings through screens and bolting cloth, the manufacture of a flour which consists entirely of starchy endosperm with its finely crushed proteins. White flour represents starch, endosperm proteins, gliadin and glutenin, together with some ash substance, principally potassium. White flour is a good source of energy in the form of starch and protein. It is essentially lacking in all the vitamins but in the unbleached state probably contains a little carotene and riboflavin. The widespread practice of bleaching white flour destroys these pigments and makes a vitamin-free flour. Our liking for bread made from white flour is an example of the failure of the appetite to guide us correctly, since coarser flours have higher nutritive value. An important incentive for the manufacture of white flour is the improvement of its keeping properties under modern conditions of food distribution.

Insects which infest grains tend to deposit their eggs in the germ, which is the best part of the seed from the nutritional standpoint. Hence, whole wheat flour, which contains the germ, tends to harbor weevils when it is stored through warm weather. White flour will not support the life of insect larvae. After wheat is milled the oxygen of the air has access to the oil in which the germ is rich; this oil tends to become rancid, spoiling the flavor of the flour. Whole wheat flour

cannot be handled in commerce without danger of deterioration since the wheat-growing regions are generally far from the centers of population. The keeping properties of highly milled flour are a great advantage to the distributor and the baker. Whole, unmilled wheat, can be shipped long distances more safely than can flour for the reasons cited. There are sound reasons why the population should continue to use white flour as a breadstuff; these rest on commercial expediency.

Our liking for the familiar white wheat flour loaf depends on its taste, which is in great measure due to the growth of yeast in the leavening process and the development of aroma during baking together with its peculiarly attractive chewing properties. Wheat flour proteins have the remarkable property of forming, when wetted and kneaded, a dough, which when leavened with a yeast culture entraps the carbon dioxide produced by yeast fermentation of carbohydrate and results in a dough of spongy texture, and hence a spongy loaf after baking.

The tendency in recent years has been to incorporate skim milk instead of water into flour to make dough. This practice is laudable since it makes for a better tasting and more nutritious bread. White bread has been much maligned as a food by unthinking critics. The proliferation of yeast in leavening and the introduction of skim milk both add to the value of bread, and even though we admit that it is still an incomplete food, it is a wholesome component of the diet. When menus are properly planned, bread may be freely eaten without in the least detracting from the quality of the dietary. It should be remembered that nearly all other foods, considered singly, are deficient in certain indispensable nutrients, and that we can secure a highly satisfactory diet only by combining a number of kinds of foods in such proportions that they make good each other's deficiencies and form a "balanced" dietary.

While whole wheat bread is more nearly complete nutritionally than is a bread made from white flour, this is a matter of little practical importance under ordinary conditions where a mixed diet is employed. Whole wheat bread has a distinctive flavor which is preferred by some but not by most people. It, too, is a wholesome food when eaten in moderate amounts in proportion to the entire food supply, but it is by no means satisfactory when eaten as the principal food. This was shown by the experience of certain European peoples during the World War when food shortage necessitated excessive restriction of the diet to bread which was made from nearly whole wheat flour. The nutritive value of the diet largely composed of bread was low and the coarseness of the bread caused intestinal disturbances which were widely complained of.

Bran.—Bran has laxative properties; it increases the bulk of indigestible residue in the intestine and tends to correct certain conditions of faulty bowel elimination. It is questionable whether bran is as desirable a source of indigestible fibre as are leafy vegetables such as cabbage, spinach, lettuce, and numerous others. These, when properly cooked, afford water-holding indigestible residues which are less harsh and less likely to irritate the delicate lining of the digestive tract than is bran.

Wheat Germ.—Wheat germ contains better proteins than does the endosperm of wheat, and its oil is the richest known natural source of vitamin E. The germ is likewise rich in vitamins of the B-complex, being nearly equal to yeast in this respect. Wheat germ is rather difficult to keep long without its becoming rancid, but when much of the oil is pressed out and the germ is packed in vacuum tins it serves as an excellent vitamin-rich supplement to the human diet and might well be more extensively eaten as an adjuvant to most diets.

Rice.—Rice contains less protein than does wheat, but is otherwise very similar to wheat in its dietary properties. Rice is the cereal of the swamp and is produced abundantly in regions where wheat cannot be grown. It is the principal cereal of far more people throughout the world than is wheat. Rice is eaten mostly in the polished state. Polishing is accomplished by abrasion, which removes the pericarp and aleurone layers as well as the germ, leaving the endosperm intact in the form of the familiar white rice of commerce. Polished rice is similar to refined wheat flour in its dietary properties. It is a source of energy and protein but lacks all the vitamins and contains little of any inorganic element with the exception of some potassium.

Rice is polished for the same reason that wheat is converted into refined flour: viz., because it is more appealing to the taste, and polished rice keeps better and does not develop stale flavor. A considerable amount of rice is prepared for consumption by steaming the red rice to gelatinize the starch near the surface of the kernels and subsequent drying in the sun before polishing. Steamed red rice is eaten in a condition which retains some of the outer layer and germ, and thus provides some of each of the vitamins of the B-complex. It is, therefore, superior to polished rice. In addition, some rice is consumed as "under-milled" rice; this also contains some adherent pericarp, aleurone layer, and germ, and contains sufficient of the B-complex to prevent beriberi.

Rice, therefore, requires supplementing with such foods as will make good its deficiencies. These supplements are of the same nature as are those required for wheat flour, viz., foods which provide proteins

abundant in the essential amino acids, foods rich in the essential mineral elements and in the vitamins.

Maize.—Maize culture was restricted to the Americas until the discovery by Columbus, after which it was introduced into Italy, Rumania, Egypt, and other areas where climatic conditions are favorable to its growth. A summer rainfall which is favorable to the development of a maximum yield of maize is ruinous to a wheat crop. The climatic conditions favorable to maize growing are found only in certain areas throughout the world, and these are not the areas where wheat or rice can succeed as farm crops. Climate and soil conditions determine to a remarkable degree the kinds of agricultural products which can be grown, and in earlier times determined the character of the diet of peoples to a much greater extent than under modern conditions of transportation.

The North American Indians prepared hominy from maize by treating the grain with a water extract of wood ashes containing potassium carbonate and hydroxide sufficiently alkaline to soften the hard pericarp. After washing away the alkali the grains were boiled in water, which made them soft and appetizing. Early milling practices for maize involved grinding the entire kernel between millstones, but methods have been refined so as to produce cornmeal free from germ and pericarp.

The dietary properties of refined cornmeal are so similar to those of white flour and polished rice that they need not be enumerated. As respects maize there are greater differences than in rice or wheat flour which are dependent upon color. There are white, yellow, red, and blue varieties of maize but cultivation is in great measure restricted to white and yellow kinds. Yellow maize makes yellow refined meal. There is in the maize kernel a certain amount of riboflavin. White maize is deficient in cryptoxanthine. The potential vitamin A value differs so greatly between yellow and white maize that the feeding value of the former is distinctly superior to the latter, a fact which was observed by swine producers many years before nutritional investigations led to the discovery of vitamin A, and even learned animal husbandrymen long questioned the testimony of practical feeders on this point.

As has been pointed out in the discussion of pellagra, it seems to be demonstrated that a deficiency of nicotinic acid plays an important, if not the determining, role in the prevention of this disease, which has been confined almost entirely to populations subsisting largely upon maize meal. This fact indicates that maize is deficient in nicotinic acid as compared with other grains. It seems remarkable that excessive eating of polished rice and of white wheat flour does not lead to pellagra. The experience available does not offer an

explanation of this problem, which may possibly be that nicotinic acid is present to some extent throughout the endosperms of both wheat and rice; or it may be that wheat and rice eaters almost invariably eat sufficient amounts of other foods containing the pellagra-preventive factor, whereas maize eaters are less likely to do so. The subject invites further study.

Barley and Rye.—These grains serve as bread cereals in many parts of the world. Rye produces about twice the yield per acre as does wheat, and rye and barley varieties yield well under climatic conditions where wheat does not. The dietary properties of rye and barley are very similar to those of wheat, but they produce bread of very different texture from wheat because their proteins possess inferior dough-forming property. These grains are always less highly milled than is wheat and yield reddish or brownish flours which form weak dough and can be leavened only to a small extent, consequently the resulting bread is hard. Most of the rye now goes into the manufacture of beer. Barley is the principal bread grain in semi-arid regions, but in America it is now principally used in soups and in infant foods.

Oats.—Coarse-ground oatmeal was first used as a principal cereal food for man in Scotland, but machinery has been perfected for making rolled oats which is now used extensively as a breakfast food. Most of the oat crop of America is fed to animals, only about 4 per cent being used as human food. Dr. Johnson's famous definition for oats: "A grain fed to horses in England, but eaten by men in Scotland," on which a Scot is said to have commented: "And pray, where do we see such men as in Scotland and such horses as in England?" suggests that the oat is a superior food cereal. This view has not been supported by experimental results.

Rolled oats are deficient in certain of the heat-stable vitamins of the B-complex. Oats are as good a source of thiamin as are other whole cereals, but judging from their appearance are free or nearly free from riboflavin. We know nothing of the content of the oat kernel in nicotinic acid, or vitamin B_6, but it appears that from the nutritional standpoint for the rat one of these three vitamins forms the limiting factor in this grain.

The biological value of oat proteins closely parallels that of the other cereals, but its content of fat is somewhat higher. Like all other cereals, in terms of the nutritional requirement of the rat it is deficient in calcium.

Millet, Sorghum, and Buckwheat.—These grains serve as human food in certain regions, but only to a small extent as compared with wheat, rice, maize, rye, barley, and oats. Millet is eaten in North China, parts of Russia and other regions. It is an inferior grain as

respects its physical properties. The seed coat is extremely hard. Millet is eaten as human food largely where only highly drought-resistant grains can be grown.

Sorghum is another drought-resistant plant of the grass family, and is principally used for feeding animals, especially poultry, because of its unappetizing character for humans. It is eaten by man in some parts of South Africa. Its nutritional properties appear to be like those of other cereals.

Buckwheat is grown on poorly drained and acid soils where other plants of economic value do not thrive, and is widely cultivated in Northern Europe. It is popular in America as a pancake flour when mixed with wheat flour. Few studies have included buckwheat, hence its dietary properties are not exactly known. It has one peculiarity which should be noted, viz., that of containing a fluorescent dye, which, when eaten, sensitizes persons with fair skins to light, causing what is vulgarly called "buckwheat itch." If albino animals (guinea pigs, rabbits) are fed liberally on buckwheat and are then exposed to sunlight, they quickly die. Pigmented animals are not seriously affected. There are several reports in the literature of cattle being injured by feeding on buckwheat as a forage plant when they stand out in the sunlight. When green buckwheat hay is fed to animals kept in the shade no injury results.

Legumes—Peas and Beans.—Peas and beans, including the soy bean, are consumed in enormous amounts by many peoples. They have high protein contents and, with the exception of the soy bean, a low content of fat. Their proteins are without exception of low biological value, but it is not known which amino acids are lacking in these proteins. Pea and bean proteins, including soy bean, do not make good the amino acid deficiencies of the cereal proteins and accordingly are not good supplemental foods in this or other respects.

Qualitatively the vitamin content of these seeds appears to be complete, but it is not high, being apparently of the order of the whole cereals. The mineral content resembles that of seeds in general, being low in calcium but containing some of each of the essential inorganic elements. Like other plant products their mineral content varies greatly with the nature of the soil on which they are grown.

Most, if not all the beans, contain large amounts of certain hemicelluloses which undergo fermentation easily in the alimentary tract with gas formation, principally methane and carbon dioxide. If eaten freely they tend to induce flatulence and for this reason should not be eaten in excess. Rats fed largely on navy beans become greatly distended from ballooning of the stomach and intestines.

Soy beans are used principally by rice-eating peoples, and serve as the principal protein supply when simple dietaries are taken.

There seems to be little supplementary value in soy bean proteins for those of rice; accordingly, their value is independent of any improvement in utilization of rice proteins. Soy beans are rich in an oil and therefore of high caloric value.

Tuber and Root Vegetables.—In this class of vegetables are included the potato, sweet potato, taro, manioc, turnip, radish, carrot, beet, parsnip, and many others. They have in common the property of being fleshy roots or stems specialized as storage organs for starch, to which fact they owe their energy value. All are markedly deficient in protein, and so far as is known such protein as they contain has little supplementary value in improving the utilization of cereal proteins, in the sense of supplying essential amino acids in which cereal proteins are deficient.

Distinction in dietary properties is made in these foods on the basis of color. Yellow varieties contain carotene; accordingly they serve as sources of vitamin A, which is not the case with white varieties. We know nothing as yet concerning the distribution in tuber and root vegetables of riboflavin, nicotinic acid, and vitamin B_6, but it is known that they all contain some thiamin. Likewise, when in the uncooked state, they invariably contain considerable ascorbic acid, hence they are antiscorbutic foods. This substance is largely lost on cooking, the extent depending on the degree to which oxygen of the air has access during the process. The red color of beets, apparently, is without nutritive significance.

Fruits.—The more highly prized fruits have flavors which make them especially attractive to the palate. Otherwise, fruits closely resemble the tuber and root vegetables in their dietary properties. Their antiscorbutic value is outstanding, when eaten uncooked. The citrous fruits are especially rich in ascorbic acid, apples, pears, peaches, apricots, etc., having a lower value in this respect. Grapes are of very low antiscorbutic value. Fruits, as a class, contain more sugar and less starch in proportion to the total carbohydrate than do the tubers and roots. As has been pointed out in the discussion of blood regeneration as affected by diet, apricots possess some still unidentified constituent which makes this fruit unique among the fruits studied.

Berries.—Berries of all kinds appear to be closely similar in their dietary properties to the fruits, and no exceptions worth mentioning are known. Berries differ from the fruits, with the exception of figs, in containing large numbers of small stony seeds which tend to irritate the intestines and cause stimulation of peristalsis. Such seeds have occasionally been accused of causing inflammation in the appendix and in the large mucous glands inside the rectum, but it appears that they have not frequently been the cause of such injury.

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Nuts.—Many people with faddist tendencies as respects foods believe that nuts possess unique nutritive value, and that they are complete in all the essential nutrients. This view finds no support in experimental studies. All of the commonly used nuts, with the exception of the chestnut, which is starch-rich and fat-poor, are extremely rich in proteins and fats, hence they have high caloric value and are good sources of protein. In no instance, however, have their proteins been found to be of high biological value, this being of the general order of the cereal grains. The quantitative studies available are relatively few, consequently no very exact statements are warranted about any of them. Their distinctive and appetizing flavors recommend them as human foods, but their high energy values point to their consumption in moderation by sedentary persons who have a tendency to put on weight. We have at present no data upon which to distinguish in nutritive properties between any of the nuts such as peanuts, walnuts, hickory nuts, hazel nuts, Brazil nuts, almonds, etc. Their vitamin contents are in general practically unknown. Their mineral composition resembles that of cereals, peas, and beans.

The Leafy Vegetables.—One of the most important of the earlier discoveries relating to remarkable differences in the dietary properties of different kinds of vegetable products was that the leaf of the plant is a complete food, whereas none of the storage organs of plants, seeds, tubers, roots or fruits enjoy this distinction. This fact was first noted by McCollum, who pointed out that this difference was associated with the difference in function of the leaf as contrasted with the specialized structures serving as storage organs containing reserve food for the young plant of the next generation. The leaf is the site of synthesis of proteins, carbohydrates, and fats and is rich in actively functioning cells. These cells contain everything which is necessary for the metabolic processes, and they supply all the nutrients which an animal requires.

Familiar examples of the adequacy of the leaves of plants for the nutrition of animals are the subsistence with unimpaired vigor from generation to generation of grazing animals such as the bison of the plains of North America and of the numerous browsing animals which eat the leaves of trees, weeds, and shrubs. Cattle and horses prefer grass to any other leafy food, whereas sheep, goats, moose, giraffe and many others prefer the strongly flavored leaves mentioned. Such animals eat enormous amounts of tannins, glucosides, and other principles which taste bad to grazing animals. Most of the grasses such as make good pastures contain little tannin. The wild grass which covered the plains of the West before the advent of the plough had a pleasant taste to the human palate. The same

is true of the juice of wheat, corn, oat, and other cereal producing plants now cultivated. Pliny mentions the custom of the Ancient Egyptians of chewing the stems of the papyrus rush, swallowing only the juice. In regions where sugar cane is grown it is a common practice of people to chew and swallow the juice. In Mexico the juice of the agave is fermented to make pulque. Other examples of the use of plant juices as food could be mentioned. Before the wild prairie grass was destroyed in the Middle West it was a custom of farmers in raising young calves to supplement the limited supply of skim milk with "hay tea," which was made by steeping well cured hay in water, the extract being added to the milk. This grass infusion was an excellent feed for calves, apparently almost equalling milk, at least, in supplying essential nutrients, especially vitamins and mineral salts in abundance.

It is of interest to note that in 1937, through the courtesy of Prof. J. S. Hughes, the authors had the opportunity to examine a sample of a leaf flour made from young oat leaves which were harvested before stems had developed, dried artificially and ground to a very fine, bright green powder which had a pleasant taste. It would seem that there may be a future in human nutrition for such leaf flours which might be made from any of several of the young cereal plants. Certainly such leaf flours have very high nutritive values as respects all the essentials of an adequate diet.

Kelp and Irish Moss.—Kelp and other marine plants contain much more iodine than do land plants or any other edible products. There is also considerable bromine in kelp. Kelp meal has been promoted as a supplement for farm rations. Kelp meal used to replace a part of a good dairy ration has no beneficial effect on milk production, health, or reproduction of dairy cows consuming a good dairy ration. Dehydrated kelp has no effect on the hemoglobin content of the blood of chickens, or on the composition of the bones as shown by ash determinations of chick bones. The feeding of kelp increased markedly the iodine content of both yolk and white of hen's eggs. It has not been shown that kelp possesses any valuable nutritive properties other than that referable to its iodine content.

Irish moss is frequently eaten by people living on the Atlantic coast. When treated with vinegar it becomes gelatinous and is appetizing. There is no evidence that it is of much value as a food except as a source of iodine. All the evidence available indicates that inorganic iodine, as found in iodized salt, is as satisfactory for nutritive purposes as is iodine in vegetable products.

The Vegetarian Diet.—In all periods in the past of which we have any knowledge there have been people who subsisted upon a strict vegetarian diet, generally for esthetic and moral reasons. The

numerous species of animals which are strictly vegetable feeders afford only presumptive evidence that such diets are satisfactory for humans. Cattle, horses, and many other animals have remained in health and vigor through countless generations when feeding on grass alone, before they were domesticated and were generally given supplements of seed products. The elephant, ox, horse, and buffalo have great strength and endurance, and thrive on vegetable food alone. These and other vegetable feeding animals are leaf eaters. The leaves of all plants appear to be complete from the nutritive standpoint. It is quite otherwise with seed products, tubers, roots, and fruits. Although they contain, apparently without exception, everything that is essential for normal nutrition, they are too imbalanced in their composition to afford a satisfactory diet even when eaten in considerable variety. Thus they are all very deficient in calcium, and in goiterous regions in iodine. They supply less than the optimal amount of protein on account of its relatively low biological value or deficiency of content. All white varieties are deficient or lacking in potential vitamin A value. These deficiencies may be corrected by the inclusion of protein-rich nuts, beans or peas, but since the proteins from these sources are of low value, and do not enhance the proteins of other vegetable foods, it is necessary to eat an abnormally high protein diet in order to supply enough of the essential amino acids. This results in burdening the body with the degradation of excessive protein which cannot be used.

Since the edible leaves are not only complete, nutritionally, in themselves, but also have supplemental values not possessed by other products of vegetable origin, it is possible, by making suitable combinations in which, irrespective of the other foods used, a liberal quota of leafy vegetables is included. It may be questioned whether any diet composed of cereal grains, tubers, roots, fruits, nuts, beans, peas, and sufficient leafy foods, is not more bulky than the human digestive tract can handle to best advantage. Nevertheless, modern nutritional researches make clear why subsistence by man on a strictly vegetarian diet is possible, but it cannot be asserted on any evidence known to the authors that such a diet will prove as satisfactory as does a mixed one.

The lacto-vegetarian diet, or combination of vegetable foods and milk, is, however, easy to plan so as to be highly nutritious, and to promote optimal health. The same can be said of the ovo-vegetarian diet provided the egg moiety is sufficient. We cannot assert with confidence at present just what proportion of milk or eggs in an otherwise vegetarian diet constitutes the minimum for safety or the optimum for quality. Unless a liberal quota of leaf food is included the ovo-vegetarian diet will be poor in calcium. A safe assumption

would seem to be that 25 to 35 per cent of the total calories should come from the ingredient of animal origin. If it were practicable, glandular organs, such as liver, kidney, sweetbreads, could be used as the sole supplements for a vegetable diet, but these, like eggs, contain too high a proportion of phosphorus to calcium and too little absolute amounts of calcium for best results. Muscle meats are less valuable supplements for vegetable foods than are milk, eggs, and glandular organs, since they are less rich in most of the vitamins, contain a poorly constituted mineral mixture that is low in calcium.

Foods of Animal Origin.—*Milk:* Milk and eggs are the only foods which are designed by nature for the nourishment of the young. Experiment shows that they are excellently constituted for this purpose and are equally valuable for adults.

The milk of cows, goats, camels, mares, or sheep has been and is the most prominent article of diet of people living in dry regions where pasturage is the sole or principal agricultural resource. This mode of living is discussed in the chapter on the dietary habits of man.

It is a remarkable fact that the proteins of milk, eggs, glandular organs, and muscle meats are all so constituted as respects their amino acid content that certain of these "building blocks" are present in much higher proportion than they occur in any vegetable foods with the possible exception of leaf proteins. Vegetable proteins, therefore, cannot be efficiently transformed into animal proteins, much of the former being required to make less of the latter. It is on this basis that the value of animal proteins as supplemental proteins rest. They provide in relative abundance certain amino acids which are deficient in proportion to the body's needs in vegetable proteins generally. Milk proteins are of high value in enhancing the value of vegetable proteins.

Milk is the only food of animal origin which is rich in calcium. Since this mineral is generally provided in inadequate amounts by vegetable foods other than leaves, milk occupies a unique position as a source of this element.

Milk contains a full quota of the vitamins, but when pasteurized or otherwise heated it loses ascorbic acid. Its content of vitamin D is far below the nutritive requirements of the young and probably also of the adult. In sunny regions this is not a disadvantage, but in temperate and colder regions a supplement of vitamin D is necessary.

Milk is deficient in iron, copper, and manganese, but apparently provides all other essential inorganic elements in sufficient amounts. Pasteurized milk picks up some copper in the process and is sufficient as a source of this element. These elements are, however, generally provided in sufficient amounts by vegetable foods when a judicious selection of these is made.

Eggs: The egg contains much protein and fat and only about 1 per cent of carbohydrate. It is, therefore, unbalanced from the nutritive standpoint, but it is a complete food qualitatively, especially the yolk. Experimental animals have been fairly successfully raised on egg yolk as the sole source of nutrient. Egg alone does not constitute a satisfactory diet. As a supplemental food it is probably the equivalent of milk except in respect to calcium and ascorbic acid, in which it is deficient. The developing chick absorbs much of its calcium from the shell, which is discarded when we eat eggs. Eggs contain much more vitamin D than does any other ordinary human food. The amount depends greatly on the amount of this substance in the feed of the hen which transfers to her eggs either the natural form of vitamin D as it occurs in fish liver oils or calciferol when these are provided in abundance in her feed.

Eggs are rich in cholesterol in which milk is relatively poor. Since liberal feeding of cholesterol to rabbits and perhaps other herbivorous animals produces changes in the arteries characteristic of arteriosclerosis, the opinion has been voiced from time to time that free eating of eggs may be detrimental to the arteries. This view rests on very slender evidence and it seems highly improbable that the amount of cholesterol ingested when one eats an egg or two a day would do any harm.

Muscle Meats.—Since the edible portion of the carcass consists largely of muscles this form of meat predominates in the omnivorous and carnivorous types of diet. A diet of muscle meats only is wholly inadequate for maintaining adequate nutrition. This is due to its deficiency of calcium, its disproportionately high content of phosphorus, its deficiency of thiamin, and perhaps other vitamins of the B-complex, and of all the fat-soluble vitamins. Muscle meats are good sources of protein of relatively high biological value and meat proteins are good protein supplements for vegetable proteins. Their flavor also recommends them highly.

The Glandular Organs.—The liver, kidney, pancreas, thymus, and other glandular organs, with the exception of the thyroid, have exceptionally high nutritive values. They are complete nutritionally, except for deficiency of calcium. Their content of the vitamins, with the exception of vitamin D, is higher than in any other foods except perhaps the leafy vegetables. Liver and kidney, in particular, contain much of the intrinsic and extrinsic factors which are necessary for blood formation, hence these are largely relied upon (especially liver) as sources of the concentrates which the pernicious anemia patient must take throughout life.

CHAPTER

XXV

Appetite, Normal and Perverted

DISTINCTIONS Between Appetite and Hunger.—Appetite and hunger are distinct physiological manifestations. Hunger is characterized by a peculiar dull ache, referred to the epigastrium, which may pass into a highly uncomfortable pang or gnawing sensation associated with drowsiness, lassitude, faintness, or headache. Cannon of Harvard and Carlson of Chicago have studied extensively the physiology of the empty stomach. The subject is thoroughly discussed by Cannon ('34), Carlson ('19), and Morgulis ('23). It has been shown that in hunger there are painful contractions of the gastric muscles, which may fuse into a continuous cramp. With each period of activity, which may comprise widespread contractions of the digestive canal, there may be a pouring out of bile and of pancreatic and intestinal juices. But generally gastric juice is not secreted at these times. Hunger is usually absent in fever and in infection, with systemic involvement, because of a reduction in movements of the alimentary canal. Boldireff (cited by Cannon) found that when dogs were fatigued, the rhythmic contractions failed to appear. The experience of many people of being "too tired to eat," is thereby given a rational explanation.

Cannon states, "The view has been propounded that appetite is the first degree of hunger, the mild and pleasant stage, agreeable in character; and that hunger itself is a more advanced condition, disagreeable and even painful—the unpleasant result of not satisfying the appetite." He points out, however, that appetite is related to previous sensations of taste and smell of food, and that pleasant or unpleasant tastes and odors, associated with certain edible substances, determine the appetite. There may be pleasure in both anticipation and realization of eating savory foods. Therefore, psychic elements

are involved and perversions of appetite may occur, with consequent ill-effects.

That one may experience hunger without appetite is possible. As Sternberg has pointed out (cited by Cannon), "hunger may be sufficiently insistent to force the taking of food which is so distasteful that it not only fails to rouse appetite, but may even produce nausea." When, for example, a famished soldier can secure no food except stale meat and half-spoiled bread, hunger, but not appetite, impels him to eat the distasteful nourishment. As Carlson (l.c.) has stated, "If only vague or mild hunger is present, the appetite elements occupy the high seat in consciousness; when hunger becomes markedly painful, attention is focused on this element."

Appetite may still persist when hunger is satisfied. We eat with pleasure the tempting dessert, even when we have already satisfied hunger and do not need more food. The age-old custom of serving in succession a series of appetizing courses makes it difficult for the healthy person to limit his eating to the point of comfortable repletion, since the appetite still remains, and invites eating to satiety. The practice of the Roman banqueters in Nero's time of absenting themselves from table to empty their stomachs through emesis, only to return and again enjoy the delights of the palate, is a case in point.

That the appetite of the normal individual calls for eating beyond the immediate needs of the body is a matter of common knowledge and has a survival value under primitive conditions when food is not always available. The body is provided with a mechanism for storing carbohydrate beyond immediate needs in the form of glycogen in the liver and muscles; the energy thus stored is available for slow utilization when the next meal is delayed, or for survival, as the result of excessive exertion in running or fighting in case threatening danger is encountered. A more concentrated form of fuel food is fat which is deposited in depots in the well nourished as a safeguard against an unlucky day when primitive man might not be able to secure food. Yet in our day, the urban dweller, serene in his confidence in the protection of governmental agencies and hence not menaced by a need for flight or combat, still retains his primitive appetite which, in the weak-willed, often leads him to eat as if preparing for a flood. The girl whose admirers keep her supplied with confections, although lolling in comfort throughout a summer vacation, is tempted by her appetite to eat more or less continuously of sweet foods, irrespective of her bodily needs. The idle are notoriously addicted to eating as a pastime and scarcely ever experience really strong hunger sensations.

Extremes in Dietary Practices.—Faddists, on the other hand, develop singularities of appetite. To the ardent vegetarian the flavor

of a juicy beefsteak may be nauseous, while he shows grim satisfaction in masticating his herbs. Such people often exhibit pleasure in denying themselves the more appetizing foods. This savors of acquiring merit through self-denial. Curious views are met with as to the alleged special healthfulness (wholesomeness) of particular foods, such as nuts, or fruits, in persons who, in their zeal to attain perfection of health, tend to restrict their food supply to a few articles. Their appetites are perverted through complex psychic processes. In the light of modern knowledge of the necessity for health of a long list of specific food factors, it is easy to understand that such faddists may come to have actual perversion of appetite. Their case may be comparable to that of the proverbial sternness of the early New England Puritan subsisting perforce upon "the bean and the cod," and probably in a state of chronic malnutrition from several deficiencies. The situation of the Puritans was parallel to that of the Athenian public, referred to in the "Knights" of Aristophanes as "bean-fed surly Demos."

Throughout history, appetite has been an antagonist to be fought against and conquered. The debilitated banqueter has often been the preacher of the virtues of abstemiousness, as others who have sown "wild oats" have been the champions of moderation and grace.

King David and King Solomon lived merry, merry lives,
They had many, many concubines and many, many wives,
But when they both grew older they had many, many qualms
King Solomon wrote the Proverbs, and King David wrote the Psalms.

A modern example of the champion fighting the devil of appetite at close range was Gladstone, who may be taken to typify the middle-aged dyspeptic in failing health. He gave a powerful impulse to mastication in England by attributing his own success in life largely to the fact that he had always made it a rule to give every tooth a chance, and had applied 32 bites to every morsel. Astute statesman that he was, he failed to discern that, with a full dental complement, 16 chews would have served his purpose.

Dietetic theories have often been propounded by those whose metabolism is imperfect. Like Gladstone, Horace Fletcher preached salvation through control of the appetite by excessive mastication. He summed up his philosophy in the meaty statement: "If you eat only when you have earned an appetite, masticate your food thoroughly, and take great care to eat only what your appetite approves, the rest will take care of itself." Mr. Fletcher made of mastication an art and a new theory and discipline of life. The number of his disciples and their enthusiasm for the new system of eating give

one pause who would sweepingly condemn it. The principal claims for Fletcherism, as deliberate and prolonged mastication came to be called, were: increased pleasure derived from eating starchy foods, since they become appreciably sweeter the longer the process of salivary conversion of starch into maltose proceeds; satisfaction of the appetite with a smaller consumption of food; decreased desire for protein because its taste was said not to be improved by prolonged chewing; better utilization of food when thoroughly masticated, because of better digestion and absorption. It is not our concern here to consider the soundness of the statements made by adherents to Fletcherism, but only to call attention to this method of satisfying the appetite without overburdening the digestive and excretory systems with an overplus of food. At a well-known Middle West sanatorium a chewing song was composed, and the patients were said to have taken innocent delight in singing it at their popular munching parties.

Appetite as a Guide to the Selection of Food.—In Chapter VII we have mentioned that phosphorus deficiency in cattle may cause them to develop the habit of bone-chewing, and that the provision of this element in any available form quickly causes the disappearance of the abnormal behavior. Eccles and Palmer state that cows made deficient in calcium will not chew bones in order to get this element. Shelling ('32), experimenting with parathyroidectomized rats, in which blood calcium is unstable and tends to fall rapidly when a high level of phosphorus is absorbed, states that the animals refused to eat diets which were rich in phosphorus, but ate freely of the same food when its content of phosphorus was reduced to a low level. These observations indicate that, as respects certain dietary factors, the appetite calls for what is lacking, and also that a feeling of ill-being, presumably experienced by Shelling's rats, caused by taking a food factor which produces metabolic disturbances, promptly reflects itself in refusal of the offending substance.

Evvard ('29) devised a system of feeding swine which was known as the free-choice, or "cafeteria" system. It involved the provision of several feeding-stuffs having very different composition and nutritive qualities (e. g., shelled corn, tankage, linseed oil meal, and chopped alfalfa) in separate containers, so the swine could eat of each as the appetite directed. Evvard quotes highly favorable comments on this system from many experiment stations throughout the world. However the method has been generally discontinued in animal husbandry since the cost of raising marketable swine is higher than in the system of feeding mixed rations designed to produce a maximum of pork with a minimum of cost for feed and labor.

Osborne and Mendel ('15), in their studies of the nutritive value

of proteins in growth, observed that a diet adequate in other respects but containing only 9 per cent of casein, did not support growth. But when the ration was supplemented with a small addition of the amino acid cystine, growth proceeded at once. They noted that had the rats eaten more of the unsupplemented diet, they would have secured the requisite amount of cystine and could have grown had that been the only required dietary correction. A rat receiving a diet containing 9 per cent casein plus cystine equivalent to 3 per cent of the casein, grew 400 per cent more than did the same animal on the same diet without cystine in a preceding period, although it consumed only 8 per cent more food in the later period. Other rats were observed to grow well on low protein diets when consuming no more calories than those which were not growing because of deficiency of an amino acid. These investigators stated that apparently a marked deficiency in any essential ingredient of diet does not lead to a corresponding compensatory increase in food intake: "They apparently limit their feeding to the amount of food yielding approximately the requisite energy."

In further studies Osborne and Mendel ('18) observed that when rats were given the choice of two diets, one inferior and one superior, the animals had considerable success in choosing the better mixture.

Experiments with Mineral Consumption as Affected by Appetite.—Richter ('36) found that the average length of life of 13 adrenalectomized rats was 11 days on a diet probably containing somewhat less than a normal amount of sodium chloride. A group of 26 adrenalectomized rats fed the same stock diet supplemented with 1 per cent of sodium chloride had an average survival period of 17 days. Thirteen similarly operated animals on the latter stock diet, which were offered a choice between tap water and a 1 per cent sodium chloride solution, showed a survival period 69 per cent greater because of their choice of drinking water containing the salt. Five rats after operation were kept on the diet unsupplemented with sodium chloride and were given a choice between tap water and 3 per cent sodium chloride solution. They drank 6 times as much of the salt solution after adrenalectomy than before, and their survival time was increased by 80 per cent. Although no data are given of the reliability of the surgical procedure it is apparent that after adrenalectomy the appetite for sodium chloride is greatly increased, and that in this circumstance, in rats, it is a guide to the physiological needs.

Richter and Eckert ('37) have sought to learn whether, as in the case of adrenalectomized rats, appetite is generally a guide to nutritive needs created by glandular deficiencies. Inasmuch as an increase in the calcium need of parathyroidectomized rats is well established, a

study was made of the level of calcium appetite in these animals before and after removal of the parathyroid glands. Eighteen rats were used in the experiment. They were kept in individual cages containing a food cup and two sources of water, one containing tap water, the other a 2.4 per cent calcium lactate solution. The animals were given a standard adequate dry diet containing 2.56 gm. of calcium per kilogram of food. During 10 days preceding operation the daily intake of tap water per rat was 19.5 cc., as against 14.7 cc. in the 10-day period 30-40 days after operation. The average calcium lactate solution taken during the 10-day period before operation was 6.4 cc., as against 18.9 cc. daily in the period 30-40 days after operation. During the 10-day period 30-40 days after operation the average consumption of calcium was 3.9 times as great as in the preoperative 10-day period.

When 6 parathyroidectomized rats were treated with parathyroid implants, there was a decrease in the intake of calcium lactate solution, approximately to the original preoperative level. In other experiments parathyroidectomized rats were offered a choice between a calcium lactate and a sodium phosphate solution. It was found that the increase in appetite for the calcium solution was accompanied by a definite decrease in sodium phosphate intake. These results were explained on the assumption that the sense of taste must serve as a guide to the physiological needs of the body.

In their single choice experiments with adrenalectomized rats, Richter and Eckert ('38) report that in these animals, showing greatly increased appetite for sodium in the form of the lactate, there was no voluntary increase in their intake of chlorides of iron, magnesium, calcium, aluminum, potassium, or ammonium. In multiple choice experiments 11 rats were fed a low mineral diet and were given a choice of sodium chloride, sodium lactate, sodium phosphate, potassium chloride, calcium lactate, and water. Adrenalectomy produced an increased intake of the sodium solutions, but also a small increase in potassium chloride. The survival time was greatly increased by offering these salts. In another multiple choice experiment 6 rats were given a choice of a wider assortment of electrolytes: sodium chloride, sodium phosphate, sodium iodide, potassium chloride, ammonium sulfate, calcium lactate, and water. Adrenalectomy produced an increased appetite for all solutions except sodium iodide. The relative proportions of the increased intake of the different ions were practically the same as are found in normal blood serum. Sodium and chloride ions showed the greatest increase, potassium and calcium ions the smallest, and lactate and phosphate ions fell midway between. Daily injections of cortical extract caused the mineral appetite of these animals to return approximately to their normal level. The

latency period of the increase in sodium appetite averaged 3.3 days, with a range of variation of 1 to 9 days. From these results the authors concluded that the adrenal glands affect not only the metabolism of sodium, but of most of the other electrolytes found in normal blood serum.

Barelare and Richter ('38) found that, on the average, rats ingested over twice as much sodium chloride (in a 3 per cent solution) in the 10-day period after conception than in the 10-day period before conception, and over three times as much in the second half of gestation. The sodium chloride intake fell back in the first 10 days postpartum to a level only slightly above the pregestational period. But whether these data are significant as respects appetite during pregnancy is not indicated since the consumption of other specific nutrients is not stated. Since pregnancy requires an increased food consumption, a demonstration of increased sodium chloride intake is not important unless it is shown that the intake is peculiarly large in comparison to that of other nutrients.

Experiments with Thiamin Consumption as Affected by Appetite.—Richter, Holt and Barelare ('37) provided thiamin (a solution of the synthetic chloride) for rats on a diet deficient in this nutrient, and apparently deficient in all components of the vitamin B-complex, and found that the rats showed an overwhelming appetite for it under such circumstances. One thiamin-deficient rat drank 11 cc., or 5,500 International Units, in less than half an hour. The odor as well as the taste of the vitamin aroused great interest in the rats and their craving for it was easily apparent. The animals found the thiamin bottle at once even when there were as many as 12 containers, filled with different foods or solutions, in the cage at the same time. Once the rats had started drinking, efforts to remove the bottle were met with fierce resistance, the bottle being held onto with paws and teeth. When riboflavin was offered under similar experimental conditions the rats showed great interest in drinking the solution, but by no means so intense a craving as for thiamin. In the case of thiamin, the immediate craving shown by the animals, and their consumption of excessive amounts with greediness, seem to show that in this instance, appetite does not depend upon the experience of a beneficial physiological effect upon its ingestion.

The possible relationship between appetite for specific nutrients and the needs for such factors merits particular attention. For this reason we have chosen to discuss some of the present data at some length in this chapter. It is necessary to point out, therefore, that the above report on thiamin craving provides scarcely any information at all on obvious questions relating to specificity of appetite. Obviously the depraved appetite of the deficient rats caused them to ingest

far more thiamin than they could utilize in recovering from the deficiency, had there been only a simple deficiency of thiamin. It is not reported whether, once the animals had ingested a reserve of thiamin, the appetite no longer made them greedy for it. Since several vitamins, including thiamin and riboflavin, are available in pure form it should be easy to quickly determine the extent that appetite, in its broadest sense, may be trusted to direct the ingestion of specific nutrients in amounts required to promote health.

Self-selection of Foods by the Rat.—Richter, Holt and Barelare ('38) reported that rats given a choice of purified casein, olive oil, dextrose, sodium chloride, calcium lactate, dry yeast, and cod liver oil, gained weight more rapidly than animals on a standard diet generally regarded to be adequate. According to their calculations, on the free choice plan the per cent of the total calories consumed in one group were: protein 23, carbohydrate 56, and fat 21 per cent respectively. The animals did not all behave alike, however, since another series selected protein 35, carbohydrate 12, and fat 53 per cent. Both groups appeared to be normal on the basis of growth, activity, and sex cycles. Animals given the above choice without cod liver oil developed symptoms of vitamin A deficiency and when given access to cod liver oil showed appetite for it, resulting in disappearance of the symptoms. When yeast was omitted, they developed a craving for it and ate the supplement with avidity when it was provided. The average free choice consumption of sodium chloride and of calcium was 0.12 and 0.18 gm. per day respectively. The consumption of calcium was at least 3 times greater than has been found to be optimal, as shown by various investigators. In these experiments the only appreciable source of potassium, magnesium, iron, and copper was in the yeast. All of their rats were deficient in vitamin E, as shown by half of a series of 8, which conceived, but did not deliver their young. The results should not be regarded as conclusive until the experiments are repeated using purified materials that have been critically selected.

On the basis of these various studies by Richter and associates, it is difficult to judge whether the data in any instance really demonstrate the development of specific appetite for indispensable nutrients when they are deficient in the diet. However, the indirect evidence on mineral consumption in parathyroidectomized and adrenalectomized rats is rather convincing. The role of appetite in the selection of food is an important field of investigation, but subtle variables are involved which require accuracy in technical procedures and an understanding of present information on nutrition. Moreover, it should be realized that appetite in animals is far less complex than in humans. The latter are constantly assailed by rationalizations based on false

notions, prejudices, and sales propaganda; thus rendering "instinct" in food selection, if it exists, practically without value.

Self-selection of Foods by Children.—Davis ('33, '34) reported experimental studies on self-selection feeding of newly-weaned infants and children. They were offered the choice of 35 articles, viz., beef, lamb, chicken, liver, brain, thymus and thyroid, sea fish, oysters, whole wheat, oatmeal, barley, cornmeal, rye, bone marrow, bone jelly, eggs, certified raw milk, certified raw whole lactic milk, apples, oranges, bananas, pineapple, tomato, lettuce, cabbage, spinach, cauliflower, peas, beets, carrots, turnips, potatoes, sea salt, and table salt. All of these articles were served during each day, in four meals. Both kinds of milk, two kinds of cereal, either fruits or vegetables, and animal protein foods were served at every meal. Cod liver oil was given without considering the child's desire for it.

The results of these studies showed that children selected from the list of simple natural foods a satisfying diet and maintained excellent appetites. They chose both the varieties and quantities of the foods in a manner well within their digestive capacity, as confirmed by the marked gain in weight, their freedom from digestive disturbances, constipation, and anorexia. Analyses of their food records showed that in spite of the apparent chaotic manner of selection their intake of protein, carbohydrate, and fat bore an orderly relation to each other and did not differ widely in the group.

This study would have been of more significance concerning practical dietetics if the list of foods had included products such as sugar, candy, nuts, cake, cookies, preserves, jellies, etc. The data offer no suggestion of children's ability to select food wisely from an array containing the above items which are indeed common in the average household. When the choice must be made between natural foods such as those used by Davis, it is not surprising that children thrive since the palatability of the foods would tend to encourage a wide selection.

Appetite for Tea, Coffee, Alcohol, etc.—There seems to be no evidence that habits such as tea, coffee, mate, and alcohol drinking necessarily depend upon some degree of nutritional want. Certainly many people who have available excellent food supplies form an appetite for one or another of these beverages, and miss them greatly if the supply is interrupted. These dietary habits merge in their nature into appetite for drugs obtained from tobacco, hashish, kat, coca leaf and betel-nut chewing, opium smoking, etc. The physiological incentive for masticating chewing gum should perhaps also merit inquiry. The apparent preference of those used to corn bread over wheat bread, and of the polished rice eater for boiled rice instead of our wheat bread, fall in the same category. The excessive eating of

corn products or of polished rice exposes the individual to pellagra and beriberi. The fact that eating unpolished rice would protect the rice eating Oriental from beriberi has not overcome his distaste for the unmilled product. The Newfoundland fisher folk could readily eat freely of fish livers, and greatly to their advantage as respects health, but experience shows that they do not avail themselves of that advantage. Medical literature is replete with records of persons who voluntarily restrict their diets because of the belief that certain foods, generally found to be wholesome, do not agree with them. The enthusiast meets with embarrassing facts when he tries to push too far the reliability of appetite as a guide to the selection of food. That sugar eating tends to be habit-forming is well known. If, as seems not improbable, it will be established that sugar eating goes far to promote the establishment of *L. acidophilus* as the prevailing mouth flora, with dental decay as the result, the guidance of appetite will be shown to be to our disadvantage.

Clay Eating.—Clay eating is practiced by people in many parts of the world. To most people it appears inconceivable that anyone should like it. Near Clarksville, Texas, the negroes eat a red clay. Some eat it raw, and others bake it hard in the oven. They travel miles to get it (communication from Miss N. P. Morris, through the courtesy of Miss Ruth Van Deman). Possibly this practice points to some inorganic deficiency, yet all people in that region do not form the clay eating habit.

Case (cited by Kellogg, '23) of the Battle Creek Sanatorium, observed that fuller's earth possesses laxative properties. Kellogg cites others who have recommended taking a tablespoonful of washed kaolin before each meal as an excellent laxative. He also cites the report of a traveller in the South Pacific, who said that the natives of certain islands wash down clay with copious drafts of coconut milk. Their reply to the question as to why they ate clay was that it was good for them. Kellogg expresses the belief that clay eating is based upon experience of its laxative properties.

Pica in Animals.—Pica is the general term for perverted appetite. It is common among idiots, the insane, and children infested with worms. Chalk, string, cinders, papers, etc., are eaten. The cause, in many instances, is obscure. In animal husbandry pica is frequently a serious problem. The literature of veterinary medicine and of poultry raising is replete with reference to this form of abnormal appetite. Thus wool eating by sheep may be caused by faulty nutrition, but in some instances it is due to the presence of irritating external parasites. Green ('25) cites a common type of pica. Penned sheep were fed a faulty ration of maize endosperm and a minimal allowance of autoclaved hay. Most of these sheep developed the wool picking

habit, nibbling at each other constantly, and many died of impaction of the rumen with wool.

Green has reviewed the literature on the "licking disease" in cattle, which has been reported from various parts of Germany, Sweden, and France. It became a scourge in areas of reclaimed moorlands in East Prussia. In the beginning there was reduced appetite, some degree of suppression of rumination, and frequent constipation. This was succeeded by depraved appetite, the animals eating mortar, stones, wood, string, clothes, and their own excrement. These animals when kept in stalls licked each other, the mangers, and walls, the licking continuing both day and night. The cause of the licking disease in cattle has never been explained. It occurred so extensively as to be calamitous to farmers in the affected regions. It was noted that hay cut before flowering was harmless, but later cuttings were harmful. The abnormality was confined largely to stall-fed animals, open grazing causing recovery, probably because of the selection of certain plants by the animals. Curiously enough horses did not develop the licking disease when fed the hays which produced it in cattle. In this connection it is of interest to recall that horses seem never to have developed the anemia due to cobalt deficiency, when confined to the same pastures on which cattle or sheep suffered fatal anemia. Comparable with wool picking is hair licking in colts and calves. In general this abnormal habit develops when the animals are confined to food of poor quality. Apparently a counterpart of pica in domestic animals is the licking habit which Orent and McCollum ('38) have observed in rats deprived of potassium, and which thus far has not been described in any other specific deficiency of known nature.

Infantophagia.—Many wild animals, if alarmed over the safety of their young, destroy them soon after birth. That this may be a manifestation of faulty nutrition is clearly shown in the frequent occurrence of cannibalism in rats fed certain faulty diets. The exact cause of this has not been determined. Sows are notoriously given to eating their new-born pigs under conditions of malnutrition, but the idea prevails among swine raisers that this pernicious practice is often due to individual "innate" depravity.

Chick Vices.—One of the most perplexing problems involved in chick raising is that of controlling cannibalism, feather picking, and toe picking. It is not known how far this may be the result of improper feeding, which seems to be one cause. Careful observation shows that too much light in the brooder, especially when the space is overstocked, will tend to cause feather picking. This results in cannibalism. The chicks are attracted by slight abnormalities such as a feather follicle which is congested, or by colored pin feathers, which become highly visible in bright light. They peck at these objects

and wound their companions, then the abnormal appearance of the birds encourages further attacks. Chicks will pick at any bright object, and brightly lighted toes are a temptation to them. Once a toe is injured, chicks will persist in picking at it. Idleness and lack of exercise cause irritability and promote the development of vices. That hunger, and possibly specific hunger for individual nutrients, may cause feather and toe picking, is shown by the fact that withholding food too long from chicks tends to develop this abnormal behavior.

Egg eating is frequently practiced by hens which are fed inadequate diets. McCollum has observed that hens on experimental diets designed to determine whether birds could synthesize lecithin, developed the habit of egg eating so badly that they had to be carefully watched in order to secure the eggs immediately after they were laid, otherwise they were broken and eaten by the hens. Champions of the reliability of appetite as a guide to food consumption should explain why a flock of chicks will fill their crops with grains of rock salt where an ice cream freezer has been emptied and, as a consequence, quickly pass to their avian reward.

Iron Eating by Cattle.—In many parts of the United States cows eat nails and wire. No extensive study seems to have been made to determine the extent of abnormal craving of iron by cattle. A typical experience was reported to McCollum by a physician in Pennsylvania who owned a valuable dairy herd and who had suffered considerable losses as the result of the cows eating nails and pieces of iron wire. Perforations of the third stomach caused peritonitis. The physician made many observations on his cows and found that they would detect bits of wood, porcelain, glass, copper, zinc, pebbles, etc., placed in the mangers and would invariably discard them. On the other hand, when they found a nail or piece of wire, even a foot or more in length, they would treasure it and chew on it, eventually swallowing it. The cows would lick rusty iron objects placed in their way out of doors, but would not lick polished iron. They did not lick copper or other metals which were available. Veterinarians are familiar with the finding of a quart or more of nails in the third stomachs of cattle. It is not known whether this habit is an expression of iron deficiency. Apparently in many instances it is not, since it occurs in cows which are not anemic. Further study of this problem should be made to determine whether fortifying the ration with soluble iron salts would do away with the habit. Such an investigation would be of interest and importance since nail and wire eating cause considerable mortality among cattle in America.

Other Instances of Abnormal Appetite.—Some persons experience an inordinate craving for food (bulimia), as in the case of pregnant women who tend to long for certain foods not easily available.

Also, in certain neurotic conditions there is an abnormal craving for food. A voracious appetite has been noted by Howard ('22) among the symptoms of hyperthyroidism. It is associated with disturbances of the tonic innervation of the alimentary tract. Bulimia is also seen in diabetic patients. In hyperthyroidism and diabetes, excessive craving for food probably represents an expression of actual need for nutriment, since in the latter much carbohydrate food is leaking away unused in the form of sugar in the urine.

Opposed to abnormal craving for food is anorexia, or loss of appetite. In this condition there are no manifestations of hunger although the body may be in dire need of nourishment. Anorexia has been noted by several observers in deficiency of thiamin. It also occurs in gastritis, neurasthenia, and various other conditions. A positive loathing for food is characteristic in certain cases of hysteria.

Whether the arsenic eating practiced by women in Hungary and the Tyrol for whitening their complexions and by men for increasing physical endurance represents a perverted appetite is not clear, since it is done with a definite object in view.

In chlorosis, a form of anemia most common in young women and characterized by marked reduction of the hemoglobin content of the blood, but with a nearly normal red cell count, it is said that not infrequently there develops a craving for sour-tasting foods.

These are only a few of the different manifestations of abnormal appetite. It is possible to conclude that specific dietary deficiencies probably produce cravings for food, which, if allowed to be voluntarily satisfied, would tend, in some instances, to correct the condition. But there is a preponderance of evidence that in humans, appetite generally is not a reliable guide to physiologic needs.

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CHAPTER

XXVI

Dietary Habits of Man in Different Parts of the World

IN THE POPULAR literature relating to healthful living one not infrequently meets with statements which convey the view that uncivilized and primitive peoples generally live more nearly in accord with "Nature's laws," and have better health than do people living under modern conditions, who have more comfortable shelter, a more varied diet, and far greater assurance of a regular supply of foods than was customary in many inhabited regions of the past, or today in the more isolated areas of the world. Careful scrutiny of the facts reveal, however, that peoples living under primitive conditions, now and in the past, have led more or less precarious existences as respects exposure and food supplies. Far more generally their diets have been too restricted as to source and have been too unvarying. As a consequence the people have tended to be malnourished. Under hard conditions of life only the physically best-endowed survive.

That people in regions strongly contrasting as respects climate and soil have survived on diets of widely different natures is not to be construed as indicating that it makes little difference what we eat so long as we have enough to meet our energy needs. As discovery has succeeded discovery in the science of nutrition, attention has been increasingly directed toward appraising the quality of human dietaries in every country. In our present favored position as respects knowledge of the needs of the human body for individual nutrients, it is of great interest and importance to survey the different types of diets which have sustained man at various times and in various places.

The diet of all human kind previous to the modern era of steam transportation by land and sea depended upon what could be secured locally, either by hunting, fishing, foraging for wild products, rearing of domesticated animals, or the cultivation of plants. Climate and

soil determined success in any of these ventures. Even today large numbers of people are entirely dependent upon local sources of food since they have no opportunity to secure other foodstuffs through trade. In this chapter the effects on human nutrition of climate, soil, and natural resources will be briefly considered.

Dietary Habits of Early Egyptians.—It is profitable to begin this discussion with some consideration of the dietary habits of the early Egyptians since agricultural pursuits, obviously of basic importance in the provision of food for large populations, are reputed to have had their origin in the region of the Nile. Ruffer ('21) states that the Egyptians were called "eaters of bread," and to them, as to the Hebrews, bread was synonymous with food. It was made from spelt (which is a kind of wheat), bearded wheat, or barley. Cereals were cultivated from early times. Flour was prepared in the predynastic period by grinding grain between two stones or pounding it in a mortar. Ruffer comments that the teeth of the soldiers must have been severely tried by their daily ration of 4 pounds of bread because of its coarse texture. Lentils, beans, artichokes, asparagus, beet root, and cabbages were doubtless cooked, but onions, cucumbers, garlic, radishes, and turnips were eaten raw. The chief fruits of the Egyptians were grapes, figs, dates, and dûm palm, pomegranates, melons, carobs, olives, apricots, and the seeds of marshy plants. Although the wealthy had large fruit gardens, Egypt has never been an extensive fruit-growing country. Egyptians of the poorer class, like the fellah, probably tasted little fruit except dates and melons.

The diet was not wholly vegetarian, for the predynastic refuse heaps of El Toukh have yielded many remnants of animals which served as food. Cattle were kept as far back as Egyptian civilization can be traced, and many wild animals and birds were kept in captivity and artificially fattened. The Nile and its canals were inexhaustible sources of fish. Salted and pickled fish were favorite articles of food. Pliny states that the ancient Egyptians were accustomed to chew the stems of the papyrus rush, the juice of which was swallowed. This plant has long since been far less abundant than in early times owing to the extension of land utilization.

The Diet in Cold Regions.—Since in the Far North there is little or no exposed soil in most places, and low temperatures prevent any kind of agriculture, man's food is perforce restricted to fish, mammals, and birds which derive nutriment from the water life, together with such land animals as the reindeer, caribou, and musk-ox, that are sustained by the sparsely distributed lichens which they are able to eat. The dwellers in these regions are occupied almost constantly, when weather permits outdoor activity, with the quest for food.

Periods of scarcity and privation are not infrequent and many Eskimos have died of starvation. They work hard when conditions permit and, like hungry animals, eat to satiety when food is abundant. They survive the climate because they wear effective clothing which maintains essentially a subtropical environment about the clothed parts. The only actual stimulation of metabolism is caused by breathing cold air when out of doors in winter and by their consumption of a high-protein diet. Their liking for fats and oils is the result of custom and hunger, and not of climate.

Formerly it was customary to dismiss the Eskimo diet with the statement that it consisted of meat and fat. We now distinguish between muscle meats, incomplete nutritionally in a number of respects, and glandular organs which supply, when eaten raw, those essentials lacking in muscle meats. Eskimos, and the sparse Indian population at the southern end of South America, survive because they eat all parts of the animals which serve them as food. Man could not survive and continue to propagate the race on a diet of muscle meats. Fish, seal, walrus, birds, and eggs, and an occasional whale practically constitute the limit of variety in food for the most isolated Eskimos. Those further south supplement these foods with the fish and organs of reindeer. About the only vegetable food available is the half digested moss of the rumen of the reindeer, which they are said to relish greatly. Like the carnivorous animals which subsist on a similar diet, the carnivorous peoples are healthy and capable of considerable endurance.

That the Eskimo is at some disadvantage because of his carnivorous diet is shown by investigations of Heinbecker ('28, '31, '32), who found the basal metabolism of Polar and Baffin Eskimos to be considerably higher than of persons living in temperate regions, doubtlessly caused by excessive protein consumption. He found their carbohydrate tolerance to be fairly high, and the non-protein nitrogen content of their blood was essentially normal notwithstanding their high-protein diet. Rabinowitch and Smith ('36) also found high basal metabolic rates in Eskimos from Hudson Bay north to Devon and Ellesmere Islands, but their results differ from those of Heinbecker in that they found abnormally high non-protein nitrogen in the blood of these people. Rabinowitch also found some cases of arteriosclerosis. The plasma chloride content was higher than the average for peoples elsewhere, and in some subjects the renal threshold for glucose was above average values. He regards the high non-protein nitrogen of the blood as due to the high protein diet and not to impairment of kidney functions. He cites data collected by Krogh and Krogh, according to which the average daily diet of these Eskimos consists of approximately 54 gm. carbohydrate, 135 gm. fat, and 280

gm. protein. Also, the observation was made that fat is not regarded as a delicacy.

The Diet in Warm Regions.—In large areas of the world's warm regions the principal food is rice, hence our discussion here is concerned largely with this food. But we must distinguish between rice-growing regions and those where it is not grown. Rice is the cereal of the swamp and is grown largely in delta regions in India, China, and certain other areas in the Orient. But in the Philippines, Java, Japan, Ceylon, and several other regions it is produced on terraced hillsides. In both lowlands and terraced lands rice-growing requires an extraordinary amount of human labor. Also, it requires a definite kind of political and social organization. In lands such as India, China, Japan, and Java, the human population presses so hard on the agricultural resources that there is little food available for animals, and food is grown almost entirely by human labor. Smith ('19) gives an absorbing account of this form of agriculture.

Rice is deficient in protein, and since it is generally eaten after polishing, which removes the bran layer and the germ, is far less nutritious than the whole grain, owing to the loss of B-complex vitamins and some minerals as well as protein. In China and Japan the chief dependence for a protein supplement is the soy bean, which contains about three times as much protein as does rice, and twice that of wheat. Its protein has a lower supplementary or improving value for rice proteins than is true of protein of milk, meats, or eggs, but its inclusion in the diet of the rice-eater is extremely important.

Many centuries ago the Japanese and Chinese practiced fish-growing in ponds to supplement the supply from rivers and the ocean. This is one of the easiest and cheapest methods for producing flesh foods. Also, it conserves land and utilizes material in water which otherwise would be wasted. In the densely populated regions of the rice-growing areas of China the only animals produced in considerable numbers are pigs and poultry, since they are foragers and sustain themselves on foods which are not suitable for man. The poor coolie population is nourished largely on rice, soy bean curd, and a little pork and fish. Among those better off economically, eggs, meats, fish, fruits, and cereals other than rice are more prominent in the diet. Since the extremely poor constitute a great majority of the population, simplicity of diet, with excessive rice eating, characterizes all of the congested hot swampy and delta regions, and large hill areas of Oriental countries. Wang ('20) and Wu ('27) have discussed the adequacy of the Chinese diet.

The prevalence of beriberi in rice-eating countries is indisputable evidence of the inadequacy of the diet of scores of millions of people.

Commenting on the nutritional status of the Chinese, Wu (l.c.) states: "The Chinese are small in stature compared with Americans and English. They live shorter lives with higher mortality both among adults and infants. They possess low vital resistance, as evidenced by the prevalence of such diseases as trachoma and tuberculosis. They are over-peaceful, non-persevering, non-progressive, non-enterprising, and are easily contented with the environment in which they find themselves. Are these qualities inherited from the ancient Chinese who came to Eastern Asia thousands of years ago, or are they the result of malnutrition continued from generation to generation? There is considerable evidence that the latter is the case." One is constrained to point out that when a country is grossly over-populated, and the struggle for existence is extremely great, the incentive to effort is lowered, since the prospect of reward for effort sinks almost to zero, except for the favored few. This applies to all the congested regions of Oriental countries. It is stated by a Committee working under the auspices of the Health Section of the League of Nations ('37) that there are large areas in Asia where 90 per cent or more of the extremely small family budget is spent for food. Obviously any attempt to improve the nutrition of people in these regions must begin with an improvement in their economic situation.

The Cassava Eaters of Java.—In Java there is a region inhabited by perhaps 3 to 4 million people, where the soil is almost pure chalk, and the only plant of agricultural value which can be grown is cassava, the starch from which constitutes our tapioca. These people are in the worst extremity economically. They eat excessively of cassava roots and suffer severe malnutrition. The cassava-eater experiences a peculiar craving, a kind of perverted appetite, for the djenkol bean, the product of an indigenous tree. This bean is poisonous, and a single bean causes some degree of illness, but the people persist in eating it because the bean affords a kind of misery differing from and preferable to deficiencies from cassava.

A Problem of Certain Oriental Colonies.—Java affords an excellent example of the plight of certain Oriental peoples. As a colony under Dutch administration the situation of its native inhabitants has undergone great changes. Under primitive conditions it had a considerable population, which was limited to the more favored areas. Under Dutch dominion much land was brought under cultivation to provide sugar, rubber, coffee, tea, and quinine, largely for export. Such trade is the factor that makes it worth while for Europeans to administer colonies in warm countries. They extend to the limit the growing of things which cannot be used as food and ship them away. But such agriculture demands laborers, and a numerous population is desirable. The Dutch are a highly capable people and intro-

duced measures for the control of malaria, dysentery, yaws, beriberi, and other diseases, thus lowering significantly the death rate. The consequence of these health measures, together with considerable improvement in the economic status of a part of the natives, was a seven-fold increase in the population within one century. With the present dense population it is economically impossible to improve the dietary of the Javanese, and were it to be done, it would be at once reflected in a decreased infant mortality, an extension of the child-bearing age of women, and an increase in the span of life. With a population which devotes itself whole-heartedly to procreation, as do the Javanese, the Island would speedily become a liability instead of a colonial asset. In the two chief hospitals in Java, 24 per cent of the 5500 deaths reported during the years 1919 and 1920 were ascribed to starvation. The hospital records represent but a very small fraction of the total illness of natives in this land. The death rate from starvation is appalling.

The Primitive Forest Peoples.—There are said to be about 85 million African people living in the forest area between the Soudan Desert and the Kalahari Desert. These, like the dwellers in Equatorial America, in the jungle areas, and similar groups in the East Indies and Indo-China, all dwell where the rainfall is heavy, the air is hot and moist, and where vegetation grows so rankly that it is almost impossible for man to practice agriculture. In the rice fields of the world much of the work is done by hand, but some aid is secured through the use of oxen and water buffaloes in dragging the ploughs through the muck. In the case of the peoples we are now considering, animals have never been used in agriculture. Water buffaloes withstand tropical heat but they are useful only in rice cultivation since under such conditions the fields are covered with water and mud. The Aryan developed from savagery through the animal-tending or pastoral stage, but these forest peoples developed a hand agriculture. The Panama farmer, who practices this simple form of agriculture has but two implements, the machete to cut brushes and weeds and a sharp stick to make holes for the seed. The natives of Panama depend almost exclusively on such starchy roots as the sweet potato, yam, taro, and manioc (cassava). In addition they eat caladium, bananas, sugar cane, and maize. Apparently the economic and physical condition of such peoples has remained essentially stationary throughout the ages. The climate is enervating, intestinal parasites abound, and insects torment the inhabitants and transmit diseases which impair health. There is no reward for industry, and they work only as they are goaded by necessity. It is not possible to sort out the effects of inadequate diet from other debilitating factors in their environment, as respects their influence on health.

The Diet of the Mambuti, the East Congo Pigmies.—Vanden Bergh ('21) who visited the Pigmies of the eastern edge of the Congo Forest, states that their food consists of a considerable variety including the manioc, and other roots, which they eat raw although these foods are difficult to digest. Nuts, wild fruits, and tender shoots of plants are also eaten. These people are capable hunters, and kill many wild birds and small animals and occasionally an elephant for food. They also eat ants and caterpillars. There is no note of their being frequently short of food and with their varied, mixed diet, it seems strange that their stature should be so small.

Human Food in the Driest Regions.—In the desert borderlands of North and South Africa, Arabia, and Asia, people can subsist only through the conversion of a scanty pasturage into food by means of flocks and herds. These peoples are represented in the extreme north by the Lapps, where cold, mountainous lands compel a similar mode of life, and in more southerly regions by the Mongolian nomad, Kirghiz, Turkoman, Arab, and Taureg. Depending upon conditions they care for reindeer, cattle, sheep, goats, horses, and camels. Although they possess many animals in proportion to the human population, they do not, as a rule, eat much meat. The reason for this is that they cannot afford to kill female animals so long as they are of value for breeding, and such of their wants as are to be obtained only through commerce with neighboring peoples must be purchased generally only by the sale of young male animals and skins. Such peoples subsist principally on milk and milk products. Ordinarily, milk is soured readily by mixing it in containers with a small amount of sour milk, which causes it to undergo lactic acid fermentation promptly. The exceedingly restricted diet is made more tolerable by the use of some cheese. Of course, some other foods are always to be had. The Lapp, for example, takes advantage of any opportunity to secure other foods. Some of the Lapps are fishermen, but they fare less well than the reindeer keepers. All Lapps secure some green food in the form of a few succulent plants found in the mountains and they also buy a little food, mostly cereal, from the neighboring people.

The desert Arab grows tired of his almost constant diet of sour milk, but there is nothing he can do about it. He always gets some barley or wheat which is made into bread, and generally some dates as well as small additions of other vegetable foods, but these foods, supplemented occasionally with a little meat, are all that he has, and they sustain him in health so far as food determines health.

The Kirghiz nomads, who follow their flocks and herds of horses, yaks, and sheep in the lowlands and high plateaus of Central Asia depend more for sustenance on milk, cheese, and butter, than any

other people. They hunger for bread, which is very scarce, and have no means of varying their diet. Yet, with the slight amount of supplementation provided by other foods, their diet is apparently complete, in the sense that it provides everything that is essential for physiological well-being, since the Kirghiz are capable of great endurance.

The Hottentots of the Kalahari Desert region live according to essentially the same plan. They dwell in the region of sparse grass on the border of the desert and raise cattle and sheep. Their food is principally milk and cheese, with some meat, and a small supplement of vegetable foods. Huntington ('27) has provided a fascinating description of the effects of geographic environment on their habits of life. He states that the Hottentot man has been described as indolent, yet he is capable of great exertion. It appears that when people depend for their livelihood on the keeping of flocks and herds, the men must be kept in a rested condition, in order that they may meet successfully the emergencies which not infrequently arise in retrieving animals that have strayed too far, or have been stampeded by a wild animal or bewildered by a storm. Huntington points out that in such emergencies a man, already wearied with other work or weakened by poor diet, would be unequal to the task before him.

These brief examples must suffice as illustrations of the food habits not only of the peoples of the dry regions of the world, but also of the keeper of flocks and herds in colder regions. The point to be emphasized is that these are the great milk users of the world. Their mode of life necessitates reasonably good health and endurance and these their diets sustain despite their monotonous and unappetizing qualities. An appreciation of the mode of life among Pastoral peoples may be obtained from the Old Testament since the conditions described there still exist.

The Diet of Hunting Peoples.—The most typical hunting peoples of today are the Bushmen of the Kalahari Desert, who have been pushed by their neighbors into a region so dry that farming and grazing are impossible, the Samoyedes of Northern Siberia and, in the recent past, the Indians of the Great Plains of Central North America.

The Bushmen live almost entirely by hunting, but their women, by digging, obtain certain succulent herbs which grow in the desert sands. Their chief source of food is the antelope, which, being very timid, requires great intelligence and skill to secure. These they stalk and shoot with arrows, generally poisoned, but at times they chase them on foot, and secure them only after both men and beast are practically exhausted. It is obvious, therefore, that their nearly carnivorous diet sustains good physical development and endurance.

But life is exceedingly hard for the Bushmen and only the very strongest and persevering survive.

The Samoyedes of the lower course of the river Ob have no domestic animals and subsist entirely by hunting and fishing. They have a low order of culture, but, like the Eskimo, their diet, hard as it is to secure in sufficient amount, sustains them under difficult conditions as respects climate and the necessity for hard work. Necessity compels them to eat everything available, and affords an example of the significance of the glandular organs as supplements to a diet of muscle meats and fish. Food is at such a premium that they even eat the flesh of carnivorous animals, including the wolf, although such flesh is of bad flavor.

The Diet of American Indians of the Plains.—The Indians who inhabited the plains east of the Rocky Mountains were in great measure a carnivorous people, since they subsisted mainly on the flesh of the bison, which roamed in enormous herds from Texas to Southern Canada. Catlin (1861) visited a Mandan tribe in 1832, and states that in addition to buffalo meat, which they secured in sumptuous supplies in their hunts, these Indians ate some wild fruits and berries; and some tribes cultivated corn, pumpkins, and squashes. A kind of wild turnip, called "pomme blanche" by the French, was much sought after by migratory Indians and was eaten raw. So highly was this plant valued it is said that the route taken in following the buffalo was often determined by the localities where this turnip was to be found. Wild plants and trees furnished some variety in season, of plums, choke cherries, grapes, elderberries, raspberries, strawberries, etc. "Indian potato" and falcata beans were also eaten. The latter produced two products: pods containing small beans, to which little attention was given and large underground beans which were sought after because of their agreeable taste. These beans were collected by meadow mice into hoards of a pint or more, and were secured by the Indians from this source. For winter use the Plains Indians dried buffalo meat in strips over a smoke fire to preserve it during warm weather and to keep off the flies, the maggots from which would spoil the meat. This meat was pounded into a coarse powder and mixed with fat to form pemican. The fat most highly prized was that from marrow bones, but the body fat was also used for this purpose. Pemmican was stored in bladders, or in skin bags.

Wherever conditions made agriculture possible, the primitive Indians grew corn, beans, pumpkins, and squashes, to supplement the game on which they principally depended. This was especially true of the Indians of the Southwest, and certain parts of the Southern States, where some tribes were more settled agriculturalists. Although

statistics are wanting, there is abundant testimony that the teeth of the hunting Indians were essentially free from caries. Catlin (l.c.) examined a large number of skulls of Sioux Indians, which it was their custom to collect and preserve in circles on the ground after the burial scaffolds gave way. He says: "I was forcibly struck with the almost incredibly small proportion of crania of children; and even more so, in the exceptional completeness and soundness of their beautiful sets of teeth, of all ages, which were scrupulously kept together, by the lower jaws being attached to the other bones of the head. The teeth arrange themselves very regularly as the keys of a piano. Their teeth do not decay but preserve their soundness and enamel and powers of mastication to old age." Catlin, when in company with a party of 14 Iowa Indians visiting England, states: "The Chief informed me that one of the most striking peculiarities which all Indian tribes discovered amongst the white people was the derangement and absence of their teeth which they believed were destroyed by the number of lies passing over them." Needless to say this attractive hypothesis is not supported by modern research, but no one can deny that, for American Indians living under the conditions of that period, it was a plausible deduction.

The Indians of the Southwest of today differ much in respect to certain articles which they eat, for, in general, they all subsist too largely upon maize, beans, and coffee. They generally have access to meat of some kind: beef, mutton, and wild game. Some eat field mice and prairie-dogs, and others, as the Apache, eat considerable amounts of cactus fruits. At the present time some of the Apache move in the spring to areas where the giant cacti (Cereus giganteos) grow abundantly and remain there for two or three months until the last of the fruit has been matured and gathered. Numerous seeds of wild plants are also gathered and eaten. It seems unwarranted to attempt any appraisal of the adequacy of the diets of these Indians in relation to their health, since they are generally poor and are sometimes in want. Hrdlička ('08) has written an extensive account of the foods of Indians in that region 30 years ago. He states that, on the whole, the health of the Southwestern and North Mexico non-civilized Indians is superior to that of whites living in larger communities. He regards the advantage of these Indians to lie principally in their greater freedom from those various morbid conditions that arise through poor inheritance. The only genetic disadvantage of the Indian, he states, consists in a possibly weaker resistance to a few of the contagions.

The Diet of the Reservation Indian.—As wards of the Government the reservation Indians have suffered greatly from tuberculosis, and from epidemics of various kinds, to a greater extent than has the

general population. That their diet has been inadequate seems highly probable from their health record and also because of the marked increase in the incidence of dental caries since they became dependent. In the absence of knowledge concerning the properties of foods and of the nutritive requirements for health, it is not surprising that in the past the rations supplied to reservation Indians provided excessive amounts of milled cereal products, syrup, molasses, sugar, and canned goods such as peas, corn, and tomatoes, which had little supplemental value as respects each other. These Indians require almost above all other needs a better food supply.

The Diet of Primitive Pacific Islanders.—We have an excellent account of primitive life of the Fiji Islanders from the book written in 1859 by the Rev. Mr. William Cross and the Rev. Mr. David Cargill (1859), English missionaries, who, with their families went to Fiji in 1834 and remained there for many years. The natives raised large quantities of taro, a variety of the elephant's ear or dasheen. In composition it does not differ much from such tubers as the potato, sweet potato, yam or cassava. They also grew sweet potatoes, bananas, plantains, and sugar cane. Wild trees furnished breadfruit and coconuts. They made bread of taro, yam, arrowroot, breadfruit, or bananas. An inferior bread was made with the fruit of the mangrove and arum, and the seed pits of dawa and kaveka. The bread was baked in excavated pits. The less desirable foods were eaten following gales which swept away the plants from cultivated land. Other wild products available in abundance were the bulou, which resembles an old potato, weighing from 1 to 8 pounds; the yaka, a creeper with a root very like licorice; wild tumeric; the root and fruit of kaili, a climbing plant; two kinds of tomatoes; the leaves of bele which were eaten as greens; two kinds of nuts, agolago and vutu. The latter tastes not unlike the peanut. There were also wild plums, wild figs, and the fruit of the pandanus. There were men who devoted themselves to fishing, and an abundance of fish were obtained. Turtle fishing was a regular practice and turtles and their eggs formed a staple article of diet. Bicho-de-mar, which is the sea slug (also sea cucumber) were abundant and were eaten frequently. They were also collected for barter. Almost every kind of inhabitant of a coral reef, including molluscs, shrimps, and other creatures were eaten. They also made several kinds of warm infusions from aromatic grasses and leaves for drinking and they drank the juices of the ti-root, sugar cane, and coconut. The chiefs and other dignitaries were addicted to a drink called yaqona, which was made from the root of *Piper methysticum*. It had narcotic properties. The drink was prepared by chewing the root, an operation performed by young men. Water was added to the chewed root, the solid particles were squeezed out, and the liquid

strained. The drink was stupefying. As a rule women did not drink yaqona.

It will be seen that the primitive Fijians had a remarkably varied and excellent diet. All who have written of them have remarked upon their physical perfection. Their teeth were of excellent structure, and generally remained free from decay. This is of particular interest because of their custom of eating freely of cooked starches, capable of fermentation to acid, and their consumption of the sweet juice of sugar cane. It is obvious that cooked starch, though pasty and capable of adhering to the teeth in sheltered parts, may be eaten daily without predisposing to tooth decay.

The Sequelae of Foreign Invasion of Pacific Islands on the Health of the Natives.—Cilento ('28) states that the Western Islands of the Territory of New Guinea formerly supported a numerous population of vigorous people, but commercial grants made to foreign companies during the past fifty years have deprived the natives of the best agricultural lands and have forced them to subsist mainly upon swamp taro, yams, crude sago, bread fruit, and bananas. The dispiriting influence of pure food, malaria, and tuberculosis have reduced their efforts in fishing, and although some fish, shark flesh, pigeons, parrots and lizards are eaten, their diet is very poor as compared with that before foreign invasion. It is deficient as respects proteins, vitamins, and mineral elements. Dental defects are very common, and Cilento found the degree of dental deficiency to vary in different islands directly with the insufficiency of food as judged by nutritional quality. Loose, blackened, and filthy teeth are extremely common among these islanders. It is not possible to determine how far the prevalence of disease as opposed to poor diet is responsible for present conditions.

The Island of Tristan da Cunha.—The Island of Tristan da Cunha lies in the South Atlantic, about 2000 miles west of the Cape of Good Hope, and about 4000 miles northeast of Cape Horn. Marshall ('26), surgeon of the *Discovery,* visited the Island in 1926. The population consisted of 75 males and 66 females, of whom 69 were adults, 21 were aged 14-21, and 51 were under 14 years of age. Three women were over 80, and the oldest man was 68 years old. The island is visited by ships only at long intervals, and the natives are dependent upon their local food supply. The islanders possess cattle, sheep, pigs, donkeys, chickens, and geese. Potatoes are the principal source of carbohydrates. Onions, pumpkins, turnips, carrots, beets, and leeks are grown in lesser quantities. Fish, although plentiful, are not popular as food. The natives have an abundance of mollymauk eggs, and young mollymauks are consumed in great numbers. Milk is always abundant and is a staple article of diet.

The islanders do not have cereals and hence do not eat bread. They have only insignificant amounts of sugar, tea, coffee, butter, jam, and marmalade. Of special interest is the high degree of freedom of the islanders from dental defects. Marshall examined the teeth of more than one-third of the population and reported results as presented in Chapter XXVII.

It was Marshall's view that, had the entire population been examined, the incidence of dental disease would have been found still lower. The teeth examined were almost without exception regular and well shaped. Although in no case did anyone admit cleaning his or her teeth oftener than once a week, and then with finger and soap, the teeth were clean and remarkably free from salivary calculi. Tristan islanders afford another example, therefore, of a people who practically never clean their teeth, who subsist in large measure on milk, potatoes, eggs, and meats, and who remain remarkably free from tooth decay. The islanders, being isolated most of the time, have few epidemics of disease, but complain that after ships call there epidemics of colds run through the island, lasting about three weeks.

The Diet of the Japanese.—The food supplies of the Japanese have been most thoroughly described by Grey ('28). As is well known, polished rice constitutes the principal food. This product, which is lacking in all vitamins, and is extremely poor in mineral elements and low in proteins, may be regarded as an energy-yielding food only. The Japanese also eat considerable amounts of potatoes, sweet potatoes, yams, taro, arrowhead, kikyo root, lily root, lotus, and other root vegetables, which, while of greater value as sources of vitamins and minerals, are poor protein supplementary foods when combined with polished rice. The people are saved from the more serious forms of malnutrition, except beriberi, by their liberal consumption of soy bean products, which are very rich in proteins, but unfortunately of relatively low biological value, and by their fairly high consumption of fish, and of green vegetables. Soy beans, however, are not to be rated as an excellent supplementary food for rice, tubers, and root vegetables. Fruit eating is rather general. The outstanding shortage of the more important foods in Japan are milk and flesh foods.

In considerable measure the small stature of the Japanese is probably referable to inadequacy of their diet. Kanzaki ('21) found Japanese children of all ages, born and reared on the American Pacific Coast, to be larger than children of corresponding age in Japan.

The Diet of the Chinese.—Wang (l.c.) and Wu (l.c.) have described the diet of the Chinese. They have a wide variety of foods, including eggs, meat, fish, fruit, cereals, and numerous vegetables. In the southern part of China rice is the staple cereal, but in the north-

ern part there are many people who never eat rice, but eat wheat instead. Pork is almost universally eaten, and is so common that the more well-to-do refrain from eating it. Lamb is said to be abundant, but beef is seldom eaten. The quantity of meat consumed is, on the whole, small, being used largely for adding flavor to vegetable foods. Fish, crabs, shrimps, lobsters, and oysters are extensively used. The Chinese are less fastidious about eating brain, blood, spinal cord, and skin, than are Americans. Chickens, ducks, and geese are produced for food in large numbers, and pigeons, turkeys, and pheasants are also commonly available. Eggs of hens, ducks, and pigeons, enter in one form or another into many Chinese dishes. Eggs are preserved in at least three ways: hulidan, or salted eggs, dsaudan or fermented eggs, and pidan or Chinese old eggs.

Vegetables are eaten more freely by the Chinese than by Americans. Spinach, cabbage, potatoes, onions, radishes, radish leaves, shepherd's purse, bamboo sprouts, and several kinds of sea weeds, are common. The late Mr. Holden, a missionary, told one of the authors (E. V. McC.) of the interest shown by the Chinese in young alfalfa leaves as human food, when he introduced the plant on the Mission farm.

The Chinese cultivate no less than 20 varieties of soy beans. Adolph ('25a, '25b) has discussed the Chinese diet, and particularly the uses made of soy beans. This bean is perhaps their main reliance for protein. In the northern parts of China wheat, maize, and millet are extensively eaten. Of special importance is the practice of sprouting grains and beans before using them as food. During germination ascorbic acid is formed in relatively large amounts, and there is reason to believe that sprouting increases the availability of nutrients in dried seeds. Buck ('37) has given an extensive account of food production in China.

On the whole, Chinese peoples have a satisfactory diet as respects quality but even in the best times food is scanty in China because of overpopulation. As in other countries, the very poor, who are numerous, subsist too largely upon cereal products and beans. Hawks ('31-32) has reviewed the literature relating to growth of Chinese children in China, Hawaii, and Chicago. It appears from data available that Chinese children are taller for their weights than American children. Chinese peoples represent a number of different racial strains, rather than a homogeneous stock. It is unprofitable to attempt an interpretation of the adequacy of the Chinese diet as compared with that of other countries on the basis of size of children or adults. It is evident that when the economic situation of the individual is such as to make possible the provision of an adequate diet, the composition of the Chinese diet approaches full adequacy. But the frequent famines,

especially in the Yellow River Valley, have from time immemorial been accompanied by epidemics of cholera and typhus, and not infrequently diseases due to dietary deficiencies have been a sore affliction.

The Nutrition Problem of Young Children in Oriental Countries.—From what has been said it is apparent that the milk supply of Oriental countries generally is quite inadequate. This is particularly important in the nutrition of infants and young children. In all geographic areas where the milk of animals is available it has always been the custom to transfer the child after weaning to a diet in which milk is prominent. The Oriental types of diet are not suitable for young children, and there is a period following cessation of breast-feeding when the child's health is jeopardized by feeding it on what the adult population eats with impunity. A diet of cereals, vegetables, and flesh foods will not support optimum growth in the very young, and needs to be supplemented with milk. If this is not done the child tends to suffer a period of retarded development, and may experience considerable digestive disturbance. To meet this problem it has long been the custom in Oriental countries to breast-feed infants and children much longer than in Western countries where an abundant milk supply is available. Appleton (private communication) stated that she could easily recognize in a group of Chinese children any who had been given canned milk.

The Diet of Labrador.—Appleton ('21) described the diet of the natives of Labrador. Its approximate composition is set forth in the following table:

Bolted wheat flour	1.25 to 1.5 bbls.	per person per year
Salt meats, pork or beef	1 to 2 bbls.	for a family of eight
Salt codfish	200 to 400 lbs.	
Salt herring	1 to 3 bbls.	
Molasses	160 gals.	
Potatoes	1 to 2 bbls.	
Rutabagas	1 to 2 bbls.	
Dried peas	20 to 40 gals.	
Raisins	10 to 20 lbs.	
Butter substitute	80 to 160 lbs.	
Condensed or evap. milk	from a few tins to 2 cases	
Tea	20 to 40 lbs.	

Fresh trout, cod, and salmon are caught only during the summer. In favorable seasons enough cabbage is grown to last until November. Rice, onions, and dried beans are used to a very limited extent. The only fresh fruits eaten are partridge berries, and a little wild berry called baked apple. Dock and Alexander plant, which resemble spinach and parsley, respectively, could be used as greens to

considerable advantage, but they are little eaten. Thus the appetite does not guide in that favorable direction. Many individuals drink 10 to 18 cups of tea a day which is sweetened with molasses. Toward spring they prepare spruce beer from spruce tips, a popular beverage that has antiscorbutic value.

In the light of our present knowledge it is easy to see why these people suffer from obstinate constipation, gastrointestinal disorders, night blindness, scurvy, edema, and occasionally beriberi. The children, although practically all undernourished, do not have rickets, since their supply of fish provides vitamin D. Tuberculosis is very common. The women suffer to an unusual degree from amenorrhea. Nervous instability increases suddenly in March or April, 4 or 5 months after fresh food is lacking in the diet. Psychoses develop in persons with a predisposition or under unusual strain, such as lactation or worry. The teeth of these people are badly diseased. Improvement or recovery from the effects of the winter diet regularly follows transition to the summer diet when more fresh foods become available.

The Diet in Newfoundland.—Appleton (l.c.) found a much higher incidence of beriberi in Newfoundland than across the Straits of Belle Isle in Labrador. Xerophthalmia was frequently encountered there. On the Newfoundland side, she found the people to have no canned milk and no vegetables. She states that the Newfoundlanders are considered much more prosperous than people in Labrador, since they have many cows. Many of the Newfoundlanders eat some butter, which in the Labrador region is a rarity. But the cows are not milked later than October to December of each winter. The hay had been cut late that year (1921) after the grass had become dry, and many of the cows perished before the end of winter. Those which survived were in such poor condition that they did not recover until the middle of summer. Cows were fed hay only and were never given salt. Only with respect to the seasonal availability of milk is the food supply of the people of Newfoundland different from that in Labrador. It is not uncommon, as pointed out by Aykroyd ('33), for a fisherman who has subsisted for six months in winter largely on tea and white bread, to develop beriberi which incapacitates him for fishing during the next season. This places him at a still greater disadvantage in the purchase of food for his family, and the next winter's supply will contain less of the better class foods. Thus the chief cause of the insufficient dietary of these people is their poor economic status.

The Diet of Italian People.—The Italian people may be taken as representatives of the Mediterranean region. Mariotti ('19) has discussed the diet of the better class of Italians. Many kinds of cheeses

are used in their cookery, and extreme care is taken to develop special flavors in foods; for example, strawberries are never eaten with cream, they are not washed in water but in wine, and they are served with claret or lemon juice and sugar. Peaches are soaked in white wine at the table. Sliced ham is served with fresh figs to bring out its flavor.

The peasantry live largely on cornmeal made into a mush or into a flat cake without yeast. The Tuscan and Umbrian peasantry are said to live almost exclusively upon a vegetable soup. Beet root, cabbage, broccoli, or potatoes are cut into small pieces and boiled in water to which is added a small slice of pork chopped very fine with an onion and some parsley. Tomato paste is added to this mixture. When served it has grated cheese sprinkled over it. It is said that only at Christmas and during the haying season do the peasantry drink wine or eat meat. No discussion of Italian food would be complete without mention of macaroni. Macaroni is only one kind of what is called "pasta." There are many varieties of this foodstuff, of which the more familiar examples are spaghetti and vermicelli.

Davis and Wood ('21) also reported on the dietary of the Italians. They state that wheat, corn, and other cereals, vegetables, fruits, chickens, pigs, and the milk of the goat constitute the food of the farmers. Fruits and vegetables are produced in great quantities in Italy and even the poorest people have them in abundance. Children drink goat's milk, since every peasant has his goat, or else milk is obtained from his neighbor. The people consume little meat, and that which is used is made into stews with macaroni and tomato sauce. Eggs are used more abundantly than meat. Desserts of the Italian are frequently fruits. The Italians make their own cheese from goat's milk. They are very fond of green vegetables, and in the large American cities Italians select in the markets a larger proportion of these when they can afford them than do other nationalities. They find milk and green vegetables relatively expensive and they substitute coffee for milk. Since they have been accustomed to liberal amounts of Italian cheese, they buy this cheese at a dollar and a quarter a pound rather than American cheese at fifty cents, or less, a pound. A taste for sweets is soon acquired in this country, and they also come to use the refined cereal products, since they are one of the cheapest foods. Gillett ('22) states, "No milk and too much coffee and candy are the greatest factors of the nutritional problem of the malnourished Italian children in New York City." Mudge ('23) has also described her studies on the Italian dietary.

Sherman and Gillett found in a study of 92 families in New York City in 1917 that 48.9 per cent of the families were receiving less than the standard allowance of 1.44 gm. of phosphorus per man

per day; 53.2 per cent were receiving less than 0.68 gm. of calcium and 41.3 per cent were receiving less than 15 mg. of iron. The study in the winter of 1918 showed the following increased deficiency:

	1918	*1917*
Families receiving less than the standard allowance of phosphorus	61.8	48.9
Families receiving less than the standard allowance of calcium	57.3	53.2
Families receiving less than the standard allowance of iron	51.2	41.3

Phillips and Howell ('20) in commenting on these studies, say that doubtless this deficiency in mineral content was one of the causes for the undersize and lack of vigor noted among the children of the families studied. Among the Italian children there were many cases of rickets,—in fact, one would seldom see a child between 1 and 8 years of age who was not extremely bow-legged. Sherman and Gillett found that in many homes no milk was bought because it was considered too expensive, but at the same time a small piece of Italian cheese was purchased at a dollar and a quarter a pound, or more.

Dietary Study in Poland.—The Polish diet may be regarded as representative of the diet of many Slavic peoples. Morzkowska and McLaughlin ('28) have reported studies made in the fall of 1926 in Poland on Polish food habits. A Pole is more likely to begin the day with tea than with coffee. The staff of life among the peasants is rye bread. This is a coarse black bread depending upon wild yeasts for fermentation. In the cities a finer flour and cultivated yeasts are used. Baking powder is almost unknown. Several varieties of cereals play a large part in the diet, but not in the form of prepared breakfast foods. Barley, buckwheat, millet or wheat grists are made into thick porridges and sour soups, or are baked in the oven with salt pork and served with meats or milk.

A Pole eats about one-half as much meat as an American, but it must be remembered that peasants make up about 40 per cent of the population, and that they eat meat, usually pork sausage, only on special occasions, such as weddings or holidays. In the country, game, especially partridges and wild hares, and smoked meats jerked over aromatic fumes, are favorite delicacies; in the cities, brain, liver, tripe, lungs, and tongue are preferred to other meats.

Potatoes are eaten extensively. They form part of almost every meal. Cabbage, especially as sauerkraut, is popular. Beets are used mostly for making soups. Barscz, made from fermented beets with the juice of fresh beets added to give a red color, is a favorite soup. Vegetables are cooked for a long time and are usually served in thick

sauce. Fresh fruits, though less abundant, are the same as in the United States. Milk and eggs are used freely in the country, but the milk supply for the cities is not well organized. Salt pork, more than butter and lard, is used for fat.

The Effect of Modern Transportation on Food Supplies and Dietary Habits.—With the development of the steam engine, early in the 19th century, transportation of foods over long distances became more and more common. This is what brought about the enormous development of the sugar cane industry in the West Indies and elsewhere, and the distribution of highly milled wheat flour wherever a market was available. In the not very distant past no peoples had a generous supply of sugar; and honey and sweet fruits were the only sources of sugars. Today we witness the spectacle of nations such as Great Britain, the United States, and many others, consuming 100 pounds and upwards of sugar per capita per year. Sugar provides no proteins, vitamins or mineral elements. It tends to crowd out of the diet foods which are better constituted for the maintenance of health.

Most of the wheat growing regions of the world are far from the more densely populated areas. This is one of the important reasons for the milling of refined cereal flours. From time immemorial people sifted the flour ground between stones and made a somewhat refined product. But before 1879, when the roller mill process for flour making was invented, milling was done in the same manner as in olden times—between the upper and the nether millstones. This was a grinding process, and the different parts of the grain could not be separated very well after grinding. The roller process does not grind, but crushes the grain by pressure. When the wheat kernels are dried to the right degree, and the bran layer then allowed to take up moisture, the latter toughens so it does not break up and can be removed easily by sifting, as can also the germ which is not disintegrated in the process. We have had thoroughly refined wheat flour only about 60 years. The principal incentive for extreme refining of flour is to improve its keeping qualities so it can be shipped anywhere in any climate, and stored without deterioration. One of the most important factors in lowering the quality of the diet of many millions of Western peoples is the excessive use of refined flour and other cereals, and sugar.

There is no valid objection to the use of these foods in certain quantities, provided they are supplemented with sufficient amounts of the protective foods, namely, milk, eggs, leafy vegetables, and meats. The trouble is that this is not done. Here lies the principal problem of dietary reform in Europe, America, and other parts of the world where economic conditions will permit such changes.

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CHAPTER

XXVII

Diet in Relation to the Teeth

DENTAL CARIES is the most widespread of human diseases. Decayed teeth not only cause pain and the early loss of teeth when dental attention is not available, but are a menace to health. If a carious cavity is properly filled before the pulp cavity is invaded a tooth may be saved and be useful for years. If the pulp cavity has been opened by decay it is necessary to devitalize the tooth and fill the root canal as an alternative to its extraction. This practice was common years ago, but has fallen into abeyance because it was observed that a tooth with a filled root canal generally developed an abscess at the root end. Bacteria or their poisons found their way into the blood stream from such abscesses and set up secondary infections in such sites as the heart valves, joints, and kidney. In the aggregate, infected teeth have been and are a source of an immense amount of ill health, discomfort, and expense as well as early loss of teeth. Any considerable improvement in the health of the teeth would, therefore, prove to be a great contribution to public health. This subject has attracted much attention in recent years and many facts of importance have been established which have a bearing on the control of dental disease. As these facts are considered, it will become apparent that there are several schools of thought which are in conflict as to the cause of dental disease and the measures which should be taken to combat it.

Bunting ('36) points out that "The disease (caries of the teeth) is most unique. There is no other pathologic process which even remotely resembles it. Dental caries is not comparable with caries of the bone. It is not a true necrotic process, nor is it attended by inflammatory reactions in the affected tissues. It is characterized by the formation of progressive lesions in the teeth, simple decalcifica-

tions by acids formed locally as a result of fermentation of carbohydrates by certain aciduric types of bacteria. The process is dependent on the infestation of the mouth by specific types of bacteria, capable of producing acids by fermentation of residual carbohydrate food materials in the mouth, and capable of living in their own acid products." Bunting agrees with the fundamental concept of Miller (1889) that caries of the teeth always begins from the surface of the tooth and advances inward, a view now universally held. He goes beyond Miller in showing that not the number alone, but the kind of microorganisms, is the determining factor. He holds that *Lactobacillus acidophilus* is the specific agent which causes decay of the teeth because it is capable of producing a high concentration of lactic acid by fermentation of sugar and can live and thrive in a strong acid medium.

M. Mellanby ('29-'34), on the other hand, supports the thesis, on the basis of the most extensive and critical study of tooth development and susceptibility to dental caries which has been made, that tooth structure, together with those nutritional factors which play a role in calcification, are the most significant causes which operate to determine whether an individual will be caries-susceptible or caries-immune. Her conception of the etiology of dental caries includes, therefore, consideration of the importance of irregularities in the enamel surface (pits and fissures), the physiological state of the tissues in the pulp cavity, which nourish the living tooth, and the character of the saliva which bathes their surfaces, as well as the degree of perfection of body chemistry as respects many factors, especially those conducive to normal calcification processes. It would seem that her views provide some explanation for the fact that *L. acidophilus* attacks the teeth of some individuals and leaves intact those of other individuals.

Hyatt ('33) has approached the problem essentially from the point of view of the dentist whose purpose is to prevent as far as possible the development of caries of the teeth. He points out that decay always begins in pits or fissures in the enamel. These are potential food traps and areas of potential stagnation which determine the surface liability of the tooth to decay. Into these receptacles food debris is lodged, and fermentative organisms decompose the carbohydrate moiety. At the bottom of such pits or fissures the nutrient material remains long undisturbed, and each affords a harbor, where acid accumulates and remains in contact with the tooth substance which it gradually dissolves, forming a cavity. As the food debris in such pits becomes liquified, a new deposit takes its place and so the process of etching is more or less continuous. Hyatt proposed, therefore, that the most effective method of preventing tooth decay

is for the dentist to search with unusual care for pits and fissures, and when these are found, to drill a small hole in the tooth, enlarging the enamel defect sufficiently to permit the insertion of a filling. The surface is then polished, and the potential food trap is thereby obliterated. An extensive clinical experience by Hyatt and others would seem to have proven the efficacy of this procedure, which merely involves finding the site at which decay will eventually occur, and mechanically repairing the anatomical defect, thus preventing decay.

That the problem is not so simple as it would seem to be is shown by the study carried out by Palmer and his associates (Franzen '30) to determine whether it is possible to prescribe a definite manner of finding those occlusal surfaces of first molars which are liable to caries and those which are not. In this study of the first molars of 1636 eleven-year-old boys and girls, they state that every effort was expended to make the best use of training, supervision, and consultation with dentists in making the observations. Nevertheless, they were unable to distinguish first molars which were liable to caries, from others not liable to decay. First molars which appeared to their observers to have no enamel defects were more liable to decay than were those which could be described by them in terms of pits and fissures. First molars which had escaped caries at the age of 12 were found to have as much enamel defect as non-carious first molars in the mouths of six-year-old children. These experimenters concluded that it is not possible to plan an effective program for caries-prevention on the basis of detecting by examination the tooth which is liable to decay. They believe that tooth decay is periodic and that it is a function of the individual organism and not of the tooth.

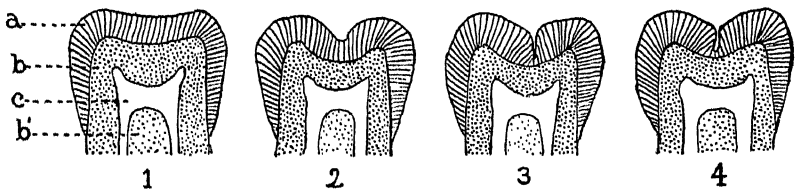
Hanke ('33) presents an imposing series of observations which substantiate his view that tooth decay is, in general, due to an insufficient supply of ascorbic acid (and possibly other substances provided by citrus fruit juices). He reports an arrest of about 50 per cent of dental caries in children by the administration of a pint of orange or lemon juice daily in addition to an otherwise nearly sufficient diet. The frequently observed gingivitis in children is reported to clear up under this regimen.

A consideration of the course of development of the teeth and the influence of certain dietary factors upon tooth structure is necessary in order to visualize the biological processes involved in the etiology of dental caries, and to appraise the experimental and philosophic observations presented by the several schools of thought on the subject under review.

Tooth Development.—For our present purpose it may suffice to state that the tooth consists of a crown of enamel overlying a structure of dentin to the neck of the tooth. The root consists mainly of dentin.

There is within the tooth a pulp cavity which contains the nerves, blood vessels, lymph vessels, and connective tissue.

Rosebury, Hastings and Morse ('31) arrived at a formula for enamel represented by $CaCO_3n(Ca_3(PO_4)_2)$, where n has a value of not less than 2 or more than 3. There are other constituents, such as magnesium and fluorine which are in much smaller quantities than calcium and phosphorus. Qualitatively dentin has a similar composition but a different histological structure and it is not as hard as enamel. The deposition of minerals, therefore, requires that the state of the body in general be suitable for calcification processes. There are specialized cells for the formation of the enamel and dentin, viz., the ameloblasts and the odontoblasts. With few exceptions all the teeth begin to calcify from 4 centers, which eventually coalesce and become a tooth. It is frequently observed that these lobes where they coalesce do not join in a perfect manner, thus leaving the tooth with

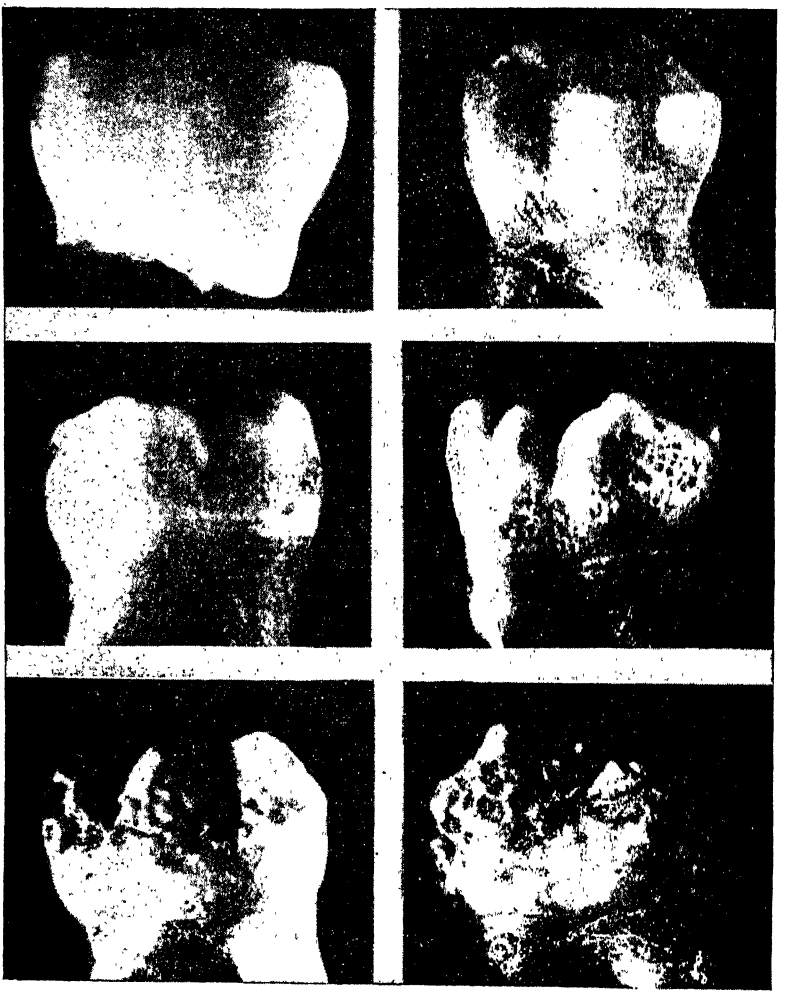


Diagrammatic illustration of structural flaws in the occlusal surfaces of molars and bicuspids: 1, enamel has no fissure; 2, normal fissure, can be readily cleansed; 3, fissure is so narrow that food particles may lodge and evade removal; and 4, fissure forms a "bottle-neck" in which debris will ferment undisturbed by ordinary cleansing. a, enamel; b, b', dentin; and c, pulp cavity.

structural flaws. This is illustrated by the accompanying figure which indicates how food particles may be lodged in fissures and evade removal by ordinary means.

Enamel Formation.—The enamel forming cells (ameloblasts) are derived from the same embryonic tissue (ectoderm) from which gum epithelium has its origin, and although they are specialized for calcification they are, like epithelium in general, extremely sensitive to deficiency of vitamin A. The ameloblasts form a cap-like structure on the inner surface of which enamel rods are laid down in a mosaic of six-sided prisms, each cell forming a prism. In perfect enamel these are laid together so precisely that, when finished, the surface is smooth to molecular dimensions. The first part of the enamel prism to be formed is the innermost end. As the enamel prisms lengthen the ameloblasts retreat. The enamel is, therefore, formed from within outward.

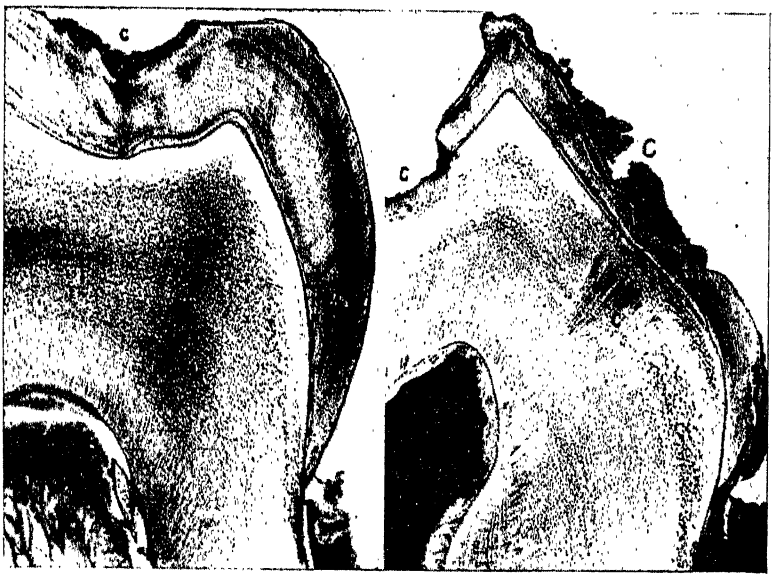
In deficiency of vitamin A the enamel forming cells become abnormal in appearance and instead of forming an even plane or curved surface they tend to buckle so that when the enamel is



Photographs of human deciduous teeth illustrating various degrees of hypoplasia. (Courtesy of M. Mellanby.)

finished the surface presents an uneven contour like a landscape of hills and valleys. These cells, when inadequately nourished as respects vitamin A, do not lay down enamel of the normal density, but rather

an incompletely calcified product with interstices. M. Mellanby has described these abnormal processes in detail, and has shown that incompletely calcified (hypoplastic) enamel permits dyes to permeate it to considerable depths. One can visualize the permeation of such hypoplastic enamel by water or saliva, carrying into the enamel material which might serve as food for bacteria, and in severely hypoplastic enamel, microorganisms may be capable of finding their way below the surface of the enamel.



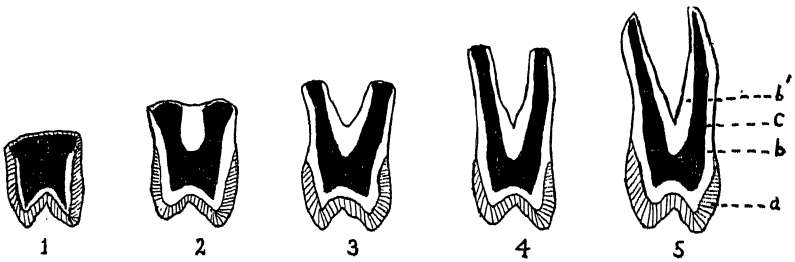
Photomicrograph of human deciduous teeth illustrating carious processes (c) in enamel and dentin. (Courtesy of M. Mellanby.)

In case of localized buckling of the ameloblastic layer, or changes in the individual cells so severe that each fails to form its enamel prism, localized areas on the completed tooth may exhibit fine tubes penetrating the enamel to varying depths depending upon the stage of development at which the injury to the ameloblasts occurred.

On the occlusal, or chewing surface of the teeth, the lines along which the separate lobes of the developing tooth coalesce, as the tooth nears completion, there is considerable likelihood that the enamel areas will not join perfectly when vitamin A is deficient in the diet. In that case there will be a deep pit or groove in the enamel which may penetrate to the dentin. When food is forced into this pit or groove it tends to remain until bacterial action digests and liquifies it.

Such pits form nests of bacterial growth. Hypoplastic areas may occur anywhere on the enamel or within it. Such developmental defects as are here considered are well illustrated in the accompanying photographs supplied by the courtesy of M. Mellanby (l.c.).

Dentin Development.—Enamel develops from within outward; dentin develops from the outer surface inward. After the earliest deposit of enamel is formed, the odontoblastic layer of cells which lie in contact with it begin to lay down dentin and retreat inward as the process extends. Thus, in a young tooth the pulp cavity is large and becomes smaller as more dentin is laid down. The uppermost portions of dentin are first formed, and the tooth lengthens as it grows, the root tip being the last part to be constructed. These processes are illustrated in the accompanying figure.



Diagrammatic illustrations of structural relationships in development of the human upper first molar: 1, at 5th year; 2, at 6th year or the approximate time of eruption; 3, at 7th year; 4, at the 8th year; and 5, at the 9th year when calcification of the external parts of the tooth have been completed. a, enamel; b, b^1, dentin; and c, pulp cavity.

Dentin is derived from the mesoblastic tissue and is, therefore, embryologically related to bone. The odontoblasts, which form dentin, are extremely sensitive to deficiency of ascorbic acid. If the tissues become depleted in this substance while the teeth are in process of growth, defective dentin will result.

But since all calcification processes are dependent upon a normal concentration of calcium and phosphate ions in the blood, both enamel and dentin will be hypoplastic when the supply of the vitamin D is deficient, even though the health of the ameloblasts and odontoblasts may be normal. It will be seen, therefore, that the dietary factors which most directly influence tooth structure are vitamins A and D, ascorbic acid, and calcium and phosphorus. It should be emphasized, however, that just as severe illness of any kind causes interruption in the growth of hair and nails, producing weak spots in the former and grooves in the latter, so such illnesses will cause

interruption in the development of the teeth, resulting in hypoplastic areas in enamel and dentin irrespective of the character of the diet.

The tooth is a living structure possessing a blood and lymph supply in the pulp cavity. It contains processes, the Tomes fibrils, extending from the odontoblasts into the canals which permeate the dentin to the base of the enamel. These carry lymph throughout the dentin.

The circulation of lymph throughout the dentin by way of dentinal tubules and the presence in these canals of dentinal fibers would seem not to be without purpose. The most obvious purpose would seem to be nourishment of the dentin. That there is a circulation of lymph in the dentin is indicated by the fact that the organic material is more abundant in proportion to the mineral substance in youth than in later life. A circulatory medium is necessary for the transport of the calcium, phosphate, carbonate, and other ions, which are deposited after the maturity of the tooth. The matrix of dentin is said to be structureless but it is derived from connective tissue.

Dentin is capable of reacting to stimuli from without by the formation of secondary dentin, which seems to represent a sclerosis with dense calcification of the matrix. Thus, after injury or irritation there may be formed immediately beneath the peripheral lesion a calcified barrier which seals off an area of injured or dead tubules. It is certain that this method of protecting the tooth against destruction does not serve effectively in man to arrest invasion of the dentin by carious processes. But when caries-producing agencies cease to act it appears that there is some attempt by the dentin to increase the density of the mineral deposit in the region bordering the cavity.

It is of extraordinary interest that in hyperparathyroidism, which results in mobilization and excretion of calcium salts from the bones, the dentin of the teeth is not decalcified. The teeth may fall out owing to resorption of alveolar bone which destroys the sockets but, as shown by Albright, Aub and Bauer ('34), and others, rarefaction of the jaw bones stands out strikingly against the well-calcified teeth in hyperparathyroidism. Schour and associates ('34) produced experimental hyperparathyroidism by injecting an extract of the gland, and found rarefaction of the alveolar bone but not of the teeth. Repeated pregnancies and lactations do not decalcify the teeth as they do the bones, as pointed out by Schour ('38) who has reviewed the subject.

Enamel, on the other hand, appears to be too dense to admit a flow of fluid through the extremely tenuous organic matrix which binds the enamel prism. It affords an outer fortification which represents nature's best effort to produce a protective covering. If there

are breeches in this fortification, or the walls are crumbling in places, the tooth is more vulnerable than when it is of good construction.

Enamel is susceptible to dissolution by chemical agencies such as the presence of acids or other substances which are capable of dissolving calcium phosphates. The teeth are constantly bathed by saliva, a fluid containing about 99.42 per cent water, and 0.58 per cent of solids. Of the latter about one-third represents inorganic constituents, and the remainder organic substances, including mucin, enzymes, and debris. Saliva is charged with carbon dioxide, which accounts for its tendency to froth, and is so well buffered that it is approximately neutral in reaction. Extremes of pH values reported are 5.75 to 7.05. Most samples fall within the range pH 6.35-6.85. It is capable of promptly neutralizing acids taken into the mouth or formed there by fermentation of sugar, except when the acid is shielded from contact with saliva; as in the case of a pit or fissure plugged with food debris or under a mucinous plaque on the enamel surface. Saliva is a somewhat variable product, but from the standpoint of its function as a protective coating of the teeth the essential qualities would seem to be its approximate neutrality, its capacity to neutralize acids, and its content of calcium and phosphate ions, which, according to the laws of solubility, should prevent it from exerting a solvent action on the calcium phosphate complex constituting the enamel.

Variation in Incidence of Dental Caries.—Ruffer ('21) records many observations on the teeth of prehistoric peoples of Europe and of Egypt which make it certain that from the earliest times caries of the teeth have afflicted mankind in various parts of the world. There have been peoples at various times and places, however, who were relatively or entirely free from this affliction. Weld (1795) stated that American-born men and women of the 18th century had decayed teeth at an early age. We refer to this observation because the consumption of sugar at that time was very small and, as we shall point out, sugar *per se* is regarded by some investigators as the most important factor in the production of dental caries. Catlin (1861) noted the extraordinary perfection of the teeth of Sioux Indians, both in the living and in skulls of past generations. Hrdlička ('08) stated that at the time of his observations dental caries was not rare among the Indians of the Southwest, though much less common than among the white people of the region. Various observers are agreed that the primitive Eskimos were free from dental caries, as are those who today subsist upon their primitive diet of meats, fish, and other foods principally of marine origin. Price ('34) and others have commented upon the rampant caries of the teeth of Eskimos and Northwest Indians who have changed their diet to one of refined cereals and sweets. Ottofy ('08) found 68 per cent of Igorot children to have

perfect teeth, and the remaining ones to exhibit imperfections so slight that they would have escaped notice if casually inspected.

Marshall ('26) visited the Island of Tristan da Cunha and examined the teeth of more than one-third of the 141 inhabitants, 69 of whom were adults; 21 were between the ages 14 to 21; and 51 were under 14 years of age. The results of his examination were tabulated as follows:

	Age Period			
	3–14	15–20	21–44	45–90
Number examined	13	8	21	12
Number of teeth	290	235	662	337
Number of carious teeth	2	0	11	21
Number of carious mouths	1	0	5	8
Number of teeth missing	0	0	6	45
Percentage of carious teeth	0.68	0	1.6	6.2
Percentage of carious mouths	7.6	0	23.8	66.6
Chronic general peridontitis	0	3	14	7

The food of the Islanders is entirely home-grown. Fish are plentiful but are not eaten extensively. Hen's eggs are scarce, but molly-mauk's eggs are eaten in large quantity. Milk is nearly always plentiful, and potatoes are the main source of carbohydrate. Onions, pumpkins, turnips, carrots, beet-root, and leeks are grown, but in small quantities. Butter, jam, and marmalade are rarely eaten. No bread is eaten since cereals cannot be grown. The outstanding shortages are flour, sugar, jam, and tea and coffee.

The data of Steggerda and Hill ('35-36), as well as those of other investigators in recent years, confirm the high incidence of caries in certain populations. The table on page 610 by Steggerda and Hill was compiled from data from the mouths of 1358 Dutch in a Dutch community (Holland, Michigan), 944 Maya Indians, 1861 Navajo Indians, and 599 Jamaica colored persons.

That the very high incidence of caries in the Dutch group is probably typical of dental conditions in the white youth of America is supported by the data of Brekhus ('31) who examined 10,445 students at the University of Minnesota whose average age was 18. Ninety-seven and one-half per cent were found to have caries and there was an average of over 10 cavities, or fillings, per mouth. Although the following table shows practically no differences between Maya and Navajo Indians, it is important to point out that the diet of the former contained a high percentage of carbohydrate and the cavities

FIVE-YEAR GROUPINGS OF INDICATED RACES, SHOWING PERCENTAGE OF POPULATION HAVING CARIES, PERCENTAGE OF TEETH AFFECTED, AND AVERAGE NUMBER OF CAVITIES PER MOUTH

AGE	DUTCH (WHITE)				MAYA (INDIAN)				NAVAJO (INDIAN)				JAMAICA (COLORED)			
	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities	No. Examined	Percentage Having Caries	Percentage of Carious Teeth	Average Cavities per Person Having Cavities
6–10	702	46.9	4.2	2.4	373	3.2	0.2	1.8	647	5.3	0.3	1.6	3	...	...	..
11–15	533	88.2	12.8	4.3	319	10.7	1.9	1.5	634	17.0	1.0	1.7	304	69.4	10.2	4.1
16–20	123	96.7	24.1	6.9	106	37.7	2.5	2.0	491	32.6	3.3	2.8	144	71.5	18.6	7.2
21–25	...	...	...	..	76	60.5	6.1	3.33	60	58.3	7.4	3.5	86	80.2	24.0	8.4
26–30	...	...	...	..	43	65.1	7.2	3.7	21	66.7	7.0	3.2	44	90.0	26.6	9.4
30–35	...	...	...	..	25	72.0	8.9	4.4	8	50.0	9.4	6.4	21	95.2	28.3	9.0

tended to appear on the teeth with smooth surfaces. The diet of the latter contained much protein and little carbohydrate and the cavities were almost entirely confined to teeth having pits and fissures.

From the observations recorded it seems clearly apparent that the diet plays an important role in the etiology of dental caries, but how? The studies of Hyatt (l.c.) deserve first consideration. He points out that of about 400,000 teeth of 12,753 persons between the ages 16 to 60 years, 52.44 per cent of all the carious cavities were found on the occlusal surfaces of the bicuspids and molars; 5.90 per cent were on the buccal surface of the lower molars; 4.44 per cent were on the lingual surfaces of the upper lateral incisors, while the remainder of 37.22 per cent were distributed over all the other surfaces.

Hyatt, as already mentioned, incriminates the pits and fissures on the occlusal surface as the pre-carious defect, since these are food traps in which lactic acid is formed from carbohydrate food remaining undisturbed there because of the food pack, thus affording time for enamel and dentin decomposition and resulting in the formation of a cavity. His view is in accord with the fact that people tend to be caries-free who subsist largely upon food of animal origin, and hence have scarcely any fermentable food capable of forming acid.

On this basis we should have to account for the relative absence of dental caries among peoples who eat much cooked starch, which would be fermentable if packed in a pit in the enamel by assuming that their diet was such as to induce the formation of teeth which had no pits or fissures in their enamel. On this point we have no data sufficiently exact to be of value. Hyatt points out that the pits and fissures on the occlusal surface of teeth are frequently so small that a bristle of the smallest diameter found in tooth brushes cannot enter; hence the futility of relying upon cleaning the teeth by brushing, or other mechanical means. There is, however, much evidence that cleaning the teeth does afford protection to some extent on those parts of the surface which can be reached by mechanical means.

Nutritional Investigations of the Cause of Dental Caries.—The researches of Mellanby, which are confirmed by the work of several others, have conclusively demonstrated that the character of the teeth and jaws, as regards their general development and their microscopic structure, can be greatly influenced by diet. If the diet of a puppy is changed from one poor in those dietary factors concerned with tooth formation to one rich in these substances, there is an immediate response shown by improved calcification of the developing enamel and dentin. In her experiments, improper feeding of the mother during pregnancy had a deleterious influence on the tooth structure of the pups. Obviously this is a matter of degree since Klein, McCollum, Buckley and Howe ('30) found that pregnant sows confined

to diets which, when fed to pigs after weaning, promptly induced rickets, made sufficient sacrifice of their own nutrient reserves to insure essentially normal development of the teeth of the young at birth. The mother also made sufficient self-sacrifice during lactation to afford great protection to the suckling pigs. In both cases her health may become impaired.

M. Mellanby also found that, notwithstanding hypoplasia, caries of the teeth developed in but 2 of 28 dogs, and in these there were pre-carious cavities in the enamel. The presence in the food of those microorganisms said to be responsible for dental caries in man, *i.e. Streptococcus mutans* and *L. acidophilus,* did not in her experiments induce caries, whether the diet possessed high or low calcifying properties. Likewise, the addition of even large amounts of glucose, a fermentable sugar, did not induce caries.

Bunting and his associates, starting with full confidence in the theory of Miller (l.c.) that acid decomposition of the teeth is the cause of caries, have carried out an extremely illuminating series of studies on the etiology of dental caries. Hubbell and Bunting ('32), examined the saliva of 125 children of varying caries-susceptibility, of ages 7 to 16 years. Some of the children received supplements of milk, viosterol, and tomato juice. The calcium and phosphorus values varied widely from day to day in the same individual and no relationship to the degree of activity of dental caries could be found. In those children receiving the supplements there appeared to be a slight decrease in the incidence of dental caries but no accompanying change in the salivary calcium or phosphorus could be found.

Hubbell ('33) made a more detailed study of 32 children, 9 to 16 years of age, 15 of whom were caries-free and 17 distinctly caries-susceptible. Both blood and saliva were analyzed chemically. The results showed that the values for calcium, inorganic acid-soluble phosphorus, carbon dioxide capacity, and pH were the same in both groups. In the saliva the values for total solids, ash, calcium, phosphorus, chloride, diastatic activity, and pH showed no consistent difference in the two groups. A difference in the salivary acid neutralizing power was noted in that the average carbon dioxide capacity of the caries-free group was about 44 per cent higher than in the caries-susceptible group, and the average titratable alkalinity was 24 per cent higher in the caries-free children. There was a wide range of variation in each group, however, and the values were not sufficiently constant for each individual to be of significance.

Koehne, Bunting and Morrell ('34) found that the carbon dioxide capacity and the alkalinity of the saliva of children in whom caries had been arrested by dietary control, were not consistently higher than during the earlier period preceding dietary control. The alkalinity

of the saliva could not be raised by increasing the basic constituents of the diet.

Hubbell and Koehne ('34) increased the caloric intake of children 16 to 18 per cent by added sugar, and found a tendency to increased nitrogen and phosphorus retention, but a slightly decreased retention of calcium. White and Bunting ('35) observed no significant differences in the pH and ammonia content of saliva in caries-susceptible and caries-immune children. Bunting and his coworkers conclude, therefore, that the diet does not affect the composition of saliva in any known way which may influence susceptibility to caries of the teeth.

On account of the wide variations in morphology and cultural characteristics, some investigators have questioned the identity of the organisms of dental caries with the *L. acidophilus* group commonly present in the intestine. Hadley, Bunting and Delves ('30) studied these variants obtained under laboratory conditions from oral and intestinal strains of *L. acidophilus* and found that the cultural differences between oral and intestinal strains also exist between intestinal strains as a group, and that there is no justification for separating these into distinct groups. They described three forms of colonies, viz., smooth, intermediate, and rough forms of oral strains and produced many dissociation variations between them. The organisms associated with dental caries vary widely in type and characteristics, but belong to the general group of *L. acidophilus,* and are related to those occurring in the intestine. Hadley and Bunting ('32), by means of a rough quantitative method, found an average of 60,000 *L. acidophilus* organisms per cc. in the saliva of 14 caries-susceptible children, as against an average of 600 in 10 caries-immune children; many counts being negative and in 81 per cent of the counts the number was less than 100 per cc.

Jay, Crowley and Bunting ('32) prepared a vaccine from 40 oral strains of *L. acidophilus* isolated from cases of dental caries. When this was introduced intradermally into caries-susceptible individuals, definite cutaneous reactions were produced in nearly all cases. Similar inoculation of caries-free individuals produced no reaction in the majority of cases. This finding indicates the existence of some form of sensitization to the organism. In two children who were caries-susceptible and gave positive reactions to the vaccine, the *L. acidophilus* agglutinins were increased in the blood and the skin reactions to the vaccine became negative. In a later study Jay, Crowley, Hadley and Bunting ('33) observed positive skin reactions in 90 per cent of 83 caries-susceptible persons. In 40 caries-free individuals similarly treated, a positive reaction was observed in 12, or 30 per cent. The local reactions to the vaccine were so severe that further study of this

problem was discontinued until means become available to prevent the unfavorable sequellae.

These important researches appear to have established that organisms of the *L. acidophilus* group are present and growing in large numbers in practically all mouths in which caries is active, and are either absent or sporadically present in small numbers in practically all caries-free mouths. The organism is similar to the *L. acidophilus* commonly found in the intestine. The researches have also brought to light the existence of some form of immunologic principle which influences the growth of oral *L. acidophilus*. Bunting and his associates have found it impossible to implant the organism in many caries-free mouths, and in such individuals the presence in the blood serum of an agglutinin for the organism can usually be demonstrated. They have been able in a few individuals to raise the agglutinin titre against the organism, thus suggesting the possibility of development of a vaccine therapy against dental caries.

In the nutritional studies of Bunting's group the conclusion is reached that in a large percentage of cases the feeding of an adequate diet, in which the content of sugar is reduced to a low level, reduces the *L. acidophilus* count of the saliva, and decreases or arrests, dental caries. Furthermore, they found that the addition of much sugar to an otherwise normal diet will, as a rule, greatly increase the growth of *L. acidophilus* in the mouth and will stimulate the activity of dental caries.

A nutritional study was made by Koehne and Bunting ('34) of a group of 169 orphanage children of 7 to 16 years of age. These children had been observed over four and a half years during which time repeated bacteriological examinations were made and the institutional diet was checked at intervals. They had a remarkably low incidence of active dental caries. Seventy to 80 per cent of them were known to have had no new caries over a period of several years, although many of the children had open cavities which had been formed in earlier years, or previous to their coming to the institution. The salivary *L. acidophilus* content was also remarkably low, the great majority of cultures being negative. The correlation of these counts to the degree of caries activity in the whole group was 88 per cent.

An appraisal of the diet showed that children over 8 years of age received milk and oleomargarine routinely for breakfast, and occasionally for supper. No butter was provided. A high proportion of bread and other cereal foods was used regularly. Many vegetables and some fruits were raised on the institution farm. Apples, carrots, tomatoes, onions, potatoes, cabbage, beets, and turnips were abundant at all times. Celery, spinach, peas, string beans, corn, berries, etc.,

were given to the children in season. Carrots, onions, cabbage, turnips, and tomatoes were frequently served raw. Oranges were provided only during the Christmas holidays, except when sent to individual children at other times by relatives. Canned berries, peaches, and apples, together with dried prunes and peaches were purchased. Fresh bananas were occasionally provided. Eggs were available to the children during the spring when they were produced on the farm in considerable numbers. The only desserts given regularly were fruit gelatin for Sunday dinner and cookies served once a week. Raw apple was used in place of dessert for dinner and supper at practically all times. Fresh and smoked meats were included in the menus. Meat was served almost every day but the portions were quite small. Candy sent to the children was not given to them. Relatives and friends were urged to limit food gifts to fresh fruit. No sugar was placed on the table and but a minimum was used to sweeten foods.

Most of the children were below the average (Woodbury tables) in both height and weight, and their caloric intake was estimated to be lower than the minimum requirement. Their vitamin D supply was derived essentially from eggs and from sunlight.

In order to test the effect of sugar in the diet 51 of these children were each given approximately three pounds of candy each week over a period of five months and the activity of the oral *L. acidophilus* and dental caries was observed. Previous to this experiment all of these children had been restricted to the regular institutional diet, which was uniform and low in sugar. During the previous year only 7 showed any evidence of active dental caries. Throughout the 5 months in which the candy was provided, and was eaten in addition to the regular diet, the oral acidophilus counts were markedly increased in 80 per cent of the children, and at the completion of the period 44 per cent showed evidence of active dental caries. Three months after discontinuance of the sugar feeding and return to the low sugar ration, a subsequent examination showed that in practically all cases the acidophilus counts had dropped to their former levels and that there was no further extension of caries in any case.

Koehne and Bunting ('34, l.c.) state that observations on a group of public school children living in their homes in Ann Arbor, Michigan, seemed to indicate that irregularities in meals and in type of food eaten day by day, associated in most cases with liberal use of artificially sweetened food, encouraged cavity formation. They state that they have observed several orphanages in which it is the practice to accept all gifts of confectionery which are frequently made by well-meaning business firms and to dispense such food liberally to the children. Among such children the incidence of dental caries corresponds to that found in public school children.

Koehne and Bunting suggest that the secret of controlling caries activity through diet may, for the majority of persons, lie more in what is withheld from the diet than on the nutritional adequacy of what is permitted. They make an important comment relative to the significance of acquiring a knowledge of the previous history of caries incidence among children before beginning observations on the effect of any prophylactic measures designed to test their efficiency in the control of dental caries. Only 32 of the 169 children in their orphanage study were free from caries when examined in October, 1931. The remaining 137 had teeth showing varying amounts of decay, and open cavities occurred in many instances. If they had begun to give viosterol or other treatment at that time, without any previous knowledge of caries incidence among the children, and had based their conclusions on clinical findings in October, 1932, they would have been able to present striking evidence for the beneficial effect of any treatment which might have been applied.

In the light of Bunting's conclusions the consumption of sugar in several countries is of interest. The following figures were compiled by the International Institute of Agriculture ('36):

PER HEAD CONSUMPTION OF SUGAR PER YEAR (PERIOD 1930–1934)

	Pounds		*Pounds*
Austria	61	Norway	70
Belgium	62	Poland	23
Bulgaria	10	Roumania	12
Czechoslovakia	56	Sweden	95
Denmark	120	Switzerland	98
Finland	51	United Kingdom	110
France	57	Canada	95
Germany	52	United States	103
Italy	18	Australia	107
Netherlands	68		

Tooth Decalcification in Relation to Candy "Sucking."—Miller and Neuwirth ('35) are in agreement with Hyatt on the point that dental caries, or the pre-carious pit or fissure, when properly treated mechanically, can be arrested, often never to recur at the original site. Decalcification of the enamel, they point out, presents an entirely different situation. They have studied many patients whose teeth were undergoing rapid dissolution, and have discovered that this is traceable to exposure of the enamel to frequent baths of strong sugar solution in the saliva. This occurs when the subject is addicted to sucking or biting hard candies, lozenges, flavored wafers, or cough drops. Any type of candy eating which involves slow dissolving would accomplish this end, of subjecting the teeth for considerable periods to a strong sugar solution. Persons who smoke large numbers of cigarettes were frequently noted to form the habit of holding a

peppermint, or other candy, between the teeth between smokes or during smoking. Such patients were found to develop large numbers of cavities in all the teeth. Such teeth become sensitive and the cavities become carious. It is pointed out that this habit spoils the appetite, and that the eating habits become such as to bring on malnutrition from imbalanced diet.

Discontinuing the use of candy in all forms, reducing the amount of smoking, adoption of a daily tooth brush drill, as well as a good diet, relieved the patients of the progressive decalcification. The teeth assumed increased luster and sensitiveness disappeared.

It is not certain at present whether a strong sugar solution will decalcify enamel, or whether acid formation through the agency of microorganisms is a necessary factor in this process. It seems very probable that excessive candy eating would result in the flowering out of a profuse flora of *L. acidophilus* organisms in the mouth with consequent acid formation. In this connection it is interesting to note that hard candies, in addition to flavoring, usually contain a considerable amount of citric acid. Dissolution of enamel may be referable to this acid.

The Natural Protective Coating of Enamel.—All dentists seem to agree that in many mouths the teeth fail to decay although they are hypoplastic, the enamel presenting the rough, stained appearance illustrated by photographs in M. Mellanby's reports on experimental studies of dental caries. Such teeth are covered by a glary film that gives the mouth a filthy appearance and yet the teeth fail to decay. This fact has been brought forward as an argument against the view that the character of the enamel has anything to do with susceptibility to dental caries. Of interest in this connection is the work of several investigators with regard to the "protective film" found on many teeth. Chase ('26) gives a thorough review of the theories concerning the nature of this film. Bibby and Van Huysen ('33) have discussed its nature. Several investigators are agreed that when a tooth is treated with acids, a membrane-like pigmented structure can be separated from the surface of the crown. This membrane is shown to consist of a bacterial mat composed of gram-positive thread forms, together with other bacterial types. The amount of mucin in this film is negligible. As a result of the formation of this superficial film the surface becomes resistant to the destructive action of acids and bacteria, and the enamel is protected from decalcification and hence against dental caries.

This view is interesting since it has been reported by Enright and Friesell ('33) that simple lactate or citrate buffer solutions will dissolve enamel slowly. Saliva is generally said to be saturated with calcium and phosphate ions, and if this is so it could not dissolve

enamel. We do not know that such saturation always exists. Enright and Friesell state that lactate or citrate buffer solutions saturated with tricalcium phosphate dissolve enamel only at hydrogen ion concentrations more acid than pH 5.0. They found that the only microorganism which is ordinarily found in the food debris on the teeth which can tolerate and grow in an environment more acid than pH 5.0 is *L. acidophilus*. Their conclusions concerning the role of this organism in tooth decay are in harmony with those of Bunting. Apparently the protective covering on normal enamel is due to the growth of some calcium-fixing organism, probably *Leptothrix,* which has been shown to form calcareous concrements and are probably the cause of the deposit of tartar on the teeth.

These findings are of interest in connection with certain observations of Simonton and Jones ('27) who described a destructive type of deposit on teeth. In experimental studies on dogs fed deficient diets, stained areas which were white, cream, gray, yellow, green, brown, or black, occurred as spots or broad masses, sometimes affecting the incisal tips of the teeth, or as bands around the teeth occurring on various areas of the crown. Disintegration of the enamel occurs coextensive with the areas of stain. In some cases the affected areas were soft, in others, dry and chalky. The lighter colored stains seemed to be associated with the more active processes. On scraping these areas it was found that the enamel was softened so that it could be easily removed, thus exposing the dentin. In other cases, after a surface layer of soft enamel was scraped off, hard, vitreous enamel was encountered. In some the entire crown was soft and could easily be cut away leaving merely stumps of dentin. They found that such areas might be microscopic as well as macroscopic in size. The condition was associated with rickets-like symptoms which occurred in young dogs, or in dogs restricted to a diet containing an excess of basic ions. Simonton and Jones never observed this condition in unerupted teeth and concluded that it does not represent a developmental fault. They showed clearly that the softening starts from without and advances into the enamel. This condition was attributed to an oral bacterio-chemical complex related to some systemic condition associated with the ricketic state. These investigators pointed out that the conditions in the teeth in rickets are the opposite of those in scurvy. In rickets there is progressive failure of calcification: first incomplete calcification, then the formation of organic matrix alone, and finally, cessation of development. In scurvy it is the formation of the organic matrix which is first affected. In rickets the tooth may present a large pulp and shell-like walls; in scurvy the pulp tends to become solidified by the formation of pulp bone. Since this condition, leading to disintegration of the enamel, is in no way

associated with caries of the teeth, and develops after the enamel is formed, it seems probable that it is due to some peculiar oral flora which is enabled to flower out when the systemic condition of the animals becomes altered as respects certain inorganic elements, notably calcium and phosphorus, and an excess alkalinity of the diet. It would appear that the stained areas described by Simonton and Jones repreent microbial growth of a kind or kinds not yet identified. In this instance we see the formation of a tooth coating which is destructive rather than protective.

Arrest of Dental Caries by a Diabetic Diet.—Boyd and Drain ('26, '28) made the important observation, based upon repeated routine examination of patients in the dental division of their pediatric clinic, that many instances of definitely arrested caries of the teeth were encountered. Large cavities, which ordinarily would have an area of softened dentin surrounding the zone of destruction, were found instead to be walled off with very dense dentin. In these mouths, in which there was evidence of rapid and extensive involvement of many teeth, subsequent examinations showed that further invasion had been checked, and open cavities did not show signs of progress months after they had been first observed. The occurrence of salivary calculi on these teeth was almost universal, and it recurred rapidly after its thorough removal. Salivary calculus is not uncommon in the adult, but is unusual in the child. Otherwise these children did not show anything noteworthy from the dental standpoint. Shedding and eruption were about normal in most cases. The degree of oral hygiene varied greatly as in any unselected group of similar size. Some teeth showing unquestionable arrest of caries were found in the most poorly kept mouths.

Upon correlation of these observations with the medical histories of the children it was found that without exception the ones with arrested caries were diabetic patients who had been under careful management for 6 months or more. That diabetes itself affords no protection against dental caries was indicated by the fact that a review of the earlier examinations showed that the carious lesions were active at the time of admission of the children to the hospital. Since arrest of caries was not found in any other disease or condition, it appeared that it must depend on some factor in dietetic management. Further study established a parallelism between the establishment of diabetic control and the quiescence of caries.

The diet for the control of diabetes differs from the usual concept of an ideal diet for a normal child in that fat, rather than carbohydrate, is used as the chief source of energy, the fatty acid: dextrose ratio being 1.5:1. All the children were maintained on the same ratio of protein:carbohydrate:fat, viz., 7:9:21. Adequate insulin dosages

kept the blood sugar concentration within normal limits, and glycosuria was infrequent. Boyd and Drain point out that when diabetes is well under control the diabetic child may be regarded as essentially a normal individual. Insulin administration is regarded as having no effect other than the maintenance of normal sugar metabolism. If these premises are granted the only factor in the arrest of caries is the character of the diet. The diet was adequate in all respects for the normal development of the child. It provided an abundance of dairy products, fruits, and vegetables, and was supplemented with cod liver oil. The provision of an adequate supply of mineral salts and vitamins was assured. They suggest that the preponderantly basic ash content of the diet was of significance. The management of these children differed from that of normal children in that the diabetic child was not allowed to use his appetite as an index to his food needs, his menus being determined by the choice of foods offered him. Their conclusion that these diabetic children ate regularly a diet more in accordance with the needs of a normal child than the average child receives, seems justified. They conclude that dental caries is the sequel to recent dietary inadequacies, such as may be associated with the usual food intake of the average child.

Boyd, Drain and Nelson ('29) recommend the following basic formula for the diet of normal children in the control of dental caries:

Daily Intake

One quart of milk
One serving of meat or fish
One orange or tomato
One additional fruit
One teaspoonful cod liver oil*
Six teaspoons butter
One portion (½ cup) leafy vegetables (spinach, chard, greens, cabbage, etc.)
One portion (½ cup) meaty green vegetables (peas, beans, carrots, etc.)

* Other standard sources of vitamin D would be satisfactory.

They describe a typical day's menu, which includes the basic formula components, as follows:

Breakfast	*Lunch*	*Dinner*
Orange juice	Cream of tomato soup	Beefsteak
Wheatena with milk	Crackers	Baked potato
Poached egg	Salmon salad	String beans
Whole wheat toast	Bran muffin	Spinach
Butter	Butter	Tomato and lettuce salad
Bacon	Apple	Whole wheat bread
Cocoa	Milk	Chocolate pudding
		Milk

The above menu for the day in the amounts used provided: protein 92.1, fat 123.1, carbohydrate 194.8, calcium 1.337, phosphorus 1.573, and iron 0.0212 grams, respectively.

Boyd and Drain have postulated on the basis of their observations, supplemented by extensive studies on normal children placed on supervised dietaries, that dental decay is dependent upon some dietary deficiencies, and that any diet which contains an abundance of all the recognized essentials for normal nutrition should be adequate to arrest dental decay. They noted that if the child actually eats the foods of the character and in the amounts prescribed, the bases of the cavities harden within two or three months through deposition of secondary dentin, and no further progress is noted. Starches and sweets, they emphasize, should not be permitted to displace the more valuable fruits and vegetables.

Boyd, Zentmire and Drain ('33) made a bacteriological study of 45 children kept under strict dietary control along the lines indicated, and concluded that the nature of the oral flora and its ability to produce acid, cannot be correlated with incidence of dental caries. They observed that *L. acidophilus,* and other organisms of equivalently high acid-producing power, may exist in large numbers in mouths persistently free from active caries. Children receiving diets of such a nature that caries becomes inactivated and remains so, show no characteristic change in the morphological nature of the oral flora or in acid-producing capacity of the organisms. The incidence of *L. acidophilus,* and of other organisms having similar acid-producing ability, appeared as great in frequency and numbers in the mouths of children receiving continuously such protective diets as in the mouths of those whose diets were not supervised. They conclude, therefore, that no available facts demonstrate that bacteria can of themselves induce caries of the teeth of individuals whose diet is adequate.

Boyd, Drain and Stearns ('33) carried out extensive metabolic studies of children with dental caries. They concluded that the levels of serum calcium and phosphorus, and the acid-base relationships are not primarily of significance in the production of dental caries, and the same is true of the chief constituents of the saliva. They found a definite and, they believe, a very significant relation to exist between adequacy of retention of calcium and phosphorus, and resistance to tooth decay. Their data offer no evidence that moderate dietary deficiency of phosphorus is an outstanding factor in the incidence of dental caries, or that degrees of acid-base imbalance induced by any ordinary diet can be held responsible. They indicate that a close correlation exists between metabolic efficiency of the organism as a whole and the resistance of the teeth to decay. According to their views, resistance to decay of the teeth is dependent primarily upon factors operating within the tooth.

McBeath ('32) made a study in three orphanages, employing the

FOOD ANALYSIS

Weight in Grams per "Man" per Day

INSTITUTION	MILK	MEAT	FISH	ANIMAL FAT	VEGETABLE FAT	CEREALS	GREEN VEGETABLES	ROOT VEGETABLES	LEGUMES		FRUITS	
									Fresh	Dried	Fresh	Dried
I	706	110	24	45	Very little	417	104	353	2	5	27	4
II	772	98	22	45	Very little	433	89	316	18	6	31	10
III . . .	640	130	20	48	Very little	467	86	384	23	4	30	11

Vitamin Content per "Man" per Day

INSTITUTION	VITAMIN D	VITAMIN A	VITAMIN C
	I. U.	*I. U.*	*I. U.*
I	410	1,290	1,480
II	450	1,280	1,480
III	380	1,340	1,460

Supplements added to these diets:

I Daily addition of 28 to 42 grams of "Golden syrup"
II Daily addition of 14 to 21 cc. of olive oil
III Daily addition of 14 to 21 cc. of cod liver oil

ordinary diet of the homes. For the control groups he used the same diets modified by supplements of eggs, vegetables, fruits, and cod liver oil. The studies covered 6 to 7 months, and the incidence of new cavities during the experimental period was compared. The experimental groups all showed a smaller increase of caries than the control groups. Analyses of the diets failed definitely to correlate the protective action with any one factor, though vitamin D was the most markedly increased factor in all the protective diets. In a fourth study the effect of adding ascorbic acid in the form of bananas, oranges, or fresh tomato juice was observed. The results did not support the view that ascorbic acid is of primary significance in preventing dental caries.

M. Mellanby (l.c.) studied children in 3 institutions at Sheffield and Birmingham to determine the effect of a supplement of vitamin D on the incidence of tooth decay. The diets are described in the table on page 622.

Space does not permit a detailed presentation of the extensive data secured and analyzed in these institutional studies. The original report should be consulted. Her results show that the percentage of caries in these children was lowest when cod liver oil was added as a supplement and was highest when a sugar addition, as syrup, replaced cod liver oil. A supplement of olive oil instead of cod liver oil protected against caries to some extent, the incidence being lower than when syrup was given. Olive oil contains no known vitamin. It would appear, therefore, that raising the fat content of the diet, apart from its vitamin content, exerts a protective effect against dental caries. This is of interest in connection with the effects of the high-fat low-carbohydrate diet employed by Boyd and Drain with diabetic children on which caries became arrested. Does this mean that protection against caries may roughly parallel the replacement of carbohydrate by fat in the diet?

BOYD AND DRAIN DIET FOR DIABETIC CHILDREN

(Boyd and Nelson, '26)

PROTEIN	CARBOHYDRATE	FAT	POTENTIAL		RATIO	CALORIES
			Glucose	Fatty Acid		
Grams	*Grams*	*Grams*				
1.0	1.28	3.0	2.165	3.16	1:1½	36.1
7.0	9.0	21.0	15.16	22.12	1:1½	253.0
2.76	3.55	8.3	5.95	8.74	1:1½	100.0

The foregoing table shows the relationship between the various factors of the diet when the protein, carbohydrate, and fat are in the ratio of 7:9:21 respectively.

In the diet employed by Mellanby, the CHO:Fat ratios were

I. 1.0:0.201;
II. 1.0:0.18; and
III. 1.0:0.25

These represent a fat to carbohydrate ratio of about one-seventh that used by Boyd and Drain in the treatment of diabetes.

In a more recent study McBeath and Zucker ('38) reported an investigation of over 800 children, most of whom were under observation during 4 years, to determine the influence of a supplement of vitamin D on the incidence of dental caries. Their data clearly show that dental caries is seasonal, the highest incidence being in late winter and early spring after the effects of the winter diet had become manifest, and was lowest during the summer. Their study points to the beneficial effects of vitamin D in preventing caries. The administration of graded amounts of vitamin D in the form of vitamin D milk resulted in graded caries prevention. Of the three levels given (250, 400, and 800 I. U. daily) only the last named was adequate to prevent an increase during the height of the caries season above that of the previous period.

Fortifying the diet with "protective foods" or simply increasing the allowance of milk led to a moderate reduction in caries when no appreciable vitamin D was added. The change was clear cut in the autumn-winter period but not so definite in the winter-spring period. A reversal of "control" and "experimental" regimens during two successive years in over 100 cases showed that individual susceptibility to caries was negligible compared to the effect of nutritional factors. These investigators also found that irradiation of children with ultraviolet light had a favorable effect in reducing the incidence of caries, which is in accord with the lower incidence during summer. It appears from their data that the administration of vitamin D in the form of vitamin D milk is the full equivalent of summer sunlight in reducing susceptibility to tooth decay. McBeath and Zucker's study fully substantiates the findings of M. Mellanby.

Ascorbic Acid and Dental Caries.—Hanke ('31) discussed the various theories concerning the etiology of dental caries and concluded that the prevention of this disease cannot be attributed to any one factor of the diet but a liberal ingestion of all the essentials. In an extensive study he found that the provision of a pint or more daily of orange juice to a diet otherwise nearly normal not only arrested caries but brought about marked improvement in the health of the gingival tissues. His theory of the effectiveness of a high intake of

ascorbic acid in preventing dental caries, or in arresting it, is based upon the findings of Höjer ('24), which have been set forth in detail in Chapter XVII. These relate particularly to the impairment of the health of the blood capillaries in ascorbic acid deficiency. The daily diet regimen prescribed to patients by the dentists associated with Hanke's study, and which is reported to have given excellent results in practice consists of the following:

8 ounces of milk for adults, 32 ounces for children.
1 egg a day, except for adolescent children and pregnant women, who should have two eggs a day.
One-fourth to one-half head of lettuce.
Half a pound of fruit a day besides the orange juice.
Four-fifths to one pound of vegetables.
Meat once a day.
One pint of orange juice plus the juice of one lemon.

Other than these requirements the subjects were left to eat what they liked.

Hanke attempted to correlate the incidence of dental caries with the occurrence of some one type of acid-producing organism, but in this he was unsuccessful. He found some relationship between the persistence of *L. acidophilus* and cavities in the teeth, but in his study this organism was not always found, even in mouths highly susceptible to dental caries, and it occurred rather frequently and consistently in the mouths of children who had never had any dental caries, or in whom no new carious lesions developed during a period of two years.

Hanke presented the results of his entire studies in book form ('33, l.c.). The illustrations afford impressive evidence of the effectiveness of dietary treatment in these conditions but, for purposes of interpretation, it would have been of great value had he conducted a series of experiments in which boiled orange juice was employed, to rule out the possible effects of factors in orange juice other than ascorbic acid. The data available at present seem to indicate that the maximum content of ascorbic acid in blood and tissues can be secured by a daily intake of citrous fruits, or other raw vegetable foods, considerably below what he considered necessary. It has been pointed out that the data of McBeath (l.c.) are not in agreement with those of Hanke.

The Experimental Production of Dental Caries in Animals by Diet.—M. Mellanby (l.c.) failed with few exceptions to produce experimental caries in the teeth of dogs. Various diets were employed. Some of these contained large quantities of glucose and other sticky fermentable foods. Others consisted of diets including either large or

small amounts of vitamins A and D. Administration of *Streptococcus mutans* or *L. acidophilus* also failed. In some cases she removed enamel to assist lodgment of debris. The almost complete absence of caries under such conditions made it obvious that the dog is practically immune to dental caries.

McCollum, Simmonds, Kinney and Grieves ('22) reported a study of the lesions in the teeth of rats fed deficient diets varying primarily in the amount of vitamin A, calcium, phosphorus, and protein. They observed "caries-like" lesions in over 5 per cent of rats fed a diet low in calcium and vitamin A, and less than 2 per cent in those fed a comparable diet rich in vitamin A, whether the calcium was high or low, but none was found in their stock animals fed a good mixed diet. At that time vitamin D was unknown and their diets were deficient in this substance. Similar results were reported by Toverud ('36).

Howe ('27), working with monkeys, states that the most rapidly occurring and pronounced caries in these animals occurred when the diet was largely composed of cereals and gelatin; that a deficiency of ascorbic acid played a part in the production of caries; that excess of sugars and starches did not cause caries in 9 months; and that monkeys fed milk, vegetables, and fruit had excellent teeth.

Hoppert, Webber and Canniff ('32) reported that rats fed a diet containing coarsely ground cornmeal developed caries of the teeth, but when the cornmeal was sifted and only that portion was fed which passed a 60-mesh sieve, no caries was produced. They suggested that caries was the result of impaction of the deep grooves, which are natural in rats' teeth, with coarse cereal particles. The plug of cereal underwent dissolution and fermentation produced acid, which was held *in situ* by the plug, so the saliva could not neutralize it. This is the old story of acid dissolution of enamel and dentin. There is every reason to accept their explanation. The rat's molars are particularly designed to make them susceptible to dental disease under these conditions of feeding.

Klein and McCollum ('31) examined 750 rat skulls with the purpose of determining the relation between the age of the animal and the incidence of caries of the teeth. They concluded that rats fed a caries-producing diet until 60 days of age do not show dental caries. On the same diet, at ages 81 and 165 days, caries was observed in 88 per cent of the animals. Rats fed the stock diet to 60 days of age did not have carious teeth. Between 300 to 500 days of age a certain number exhibited caries. The most probable explanation for these facts would seem to be that in rats on a caries-producing diet the enamel is defective, whereas in those on a non-caries producing diet there is enamel in the deep grooves, which is thicker, more dense

and free from fissures; hence periodical plugging of the grooves, with consequent acid action, requires a longer period to etch away the enamel.

The view has been held by several investigators that the content and ratio of calcium and phosphorus in the diet, and more especially a deficiency of phosphorus, may be the determining factor in the etiology of dental caries. Failure of salivary analyses to reveal significant differences in the phosphorus or calcium content of the saliva leaves this theory with little support. It seems clear, however, that such relationships play an important role in determining the structure of the teeth. The evidence that many hypoplastic teeth remain free from caries seems to point clearly to other influences as of more significance.

The Prevention of Tooth Decay.—A fair appraisal of the evidence available seems to lead to the conclusion that the view of Bunting and his associates rests upon stronger evidence than does any other theory. It is their opinion that an acid-forming mouth flora, flourishing in great numbers, is the principal cause of dental caries. According to this view the structure of the teeth is of little importance in relation to caries susceptibility.

There are certain puzzling problems which remain to be solved. One of these is why certain individuals harbor an aciduric flora while others do not, even when the organisms are persistently implanted in the mouth. The observations of Bunting's group suggest a possible immunity to these organisms due to bactericidal or bacteriostatic substances in the saliva or mucus in caries-immune persons which hold these organisms in abeyance. If such immunity is possible one of the most pressing research problems relating to public health is the discovery of a vaccine which will immunize people against *L. acidophilus*.

If this theory of the etiology of dental caries is sound one would expect to find a high incidence of caries in pastoral peoples who subsist largely upon sour milk. There is an extensive literature supporting the view that sour milk drinking tends to the establishment of an acidophilus flora in the colon, and as pointed out above, Hadley and associates ('30, l.c.) could find no cultural characteristics which warrant separation of the intestinal and oral acidophilus organisms into 2 groups. Why do not these milk drinkers develop a profuse oral flora of the strong acid formers? Such evidence as is available indicates that excessive dependence upon milk as a food does not predispose to excessive caries of the teeth. The excessive milk users eat relatively little cereal foods and do not, therefore, take a diet which would favor the formation of deposits of fermentable material in stagnation areas. This is probably of importance.

Orr and Gilks ('31) found dental caries to be less prevalent among the Masai, who drink milk freely, than among the Kikuyu, who are

largely vegetarian. One is reminded of the finding of Smith, Persons and Harvey ('37) that dogs suffering from multiple deficiencies, but primarily a deficiency of vitamin A, and dogs deficient primarily in the blacktongue factor, showed profuse overgrowth of the mouth by fusospirochetal organisms, and that recovery from these deficiency states was attended by clearing up of mouth conditions (Chapter XXI). Presumably this result was determined by humoral factors residing in the mucus, or saliva, or in the superficial epithelial cells of the mouth, which in health are capable of holding in check forms of organisms which may overgrow the debilitated membranes.

Another puzzling problem is why sugar eating is so effective in promoting the growth of aciduric organisms in the mouth in comparison with cooked starch eating. Cooked starch is pasty and adherent to the teeth and the diastatic ferments of the saliva would certainly form from retained starchy particles in a stagnant area a constant supply of fermentable sugar. One would expect cooked starch eating to be more caries-producing than sugar, since sugar is so soluble that it can hardly be expected to remain long in the mouth. It is of course easy to visualize how sugar-saturated saliva would remain in pits and fissures, and in stagnant areas, but it should do so to a lesser extent than cooked starch. Yet the present available evidence indicates that sugar is far more effective in inducing caries of the teeth than starch.

Of peculiar interest is the effect of a high-fat, low-carbohydrate diet, such as is employed in the control of diabetes, in reducing susceptibility to dental caries. When lodged in areas of stagnation this type of therapeutic diet, while low in carbohydrate, must still provide an abundance of this substance for acid formation in pits, fissures, or incipient cavities formed by acid erosion. We suggest that another role of fat in the diet, viz., that of greasing the tooth surface and water-proofing it so that acid is prevented from actually coming into contact with enamel, or dentin when the latter is exposed, may be important in protecting the tooth against even a high concentration of acid at a site of decay or potential decay. It is well known to chemists that many substances become coated with oil in preference to water when both liquids are simultaneously present. One may visualize the saturation of the debris lining a carious cavity, or a pit or fissure, with oil derived from food fat. This would tend to remain indefinitely on the caries-susceptible surface, completely water-proofing it and shielding it from contact with acid arising from fermentation of food carbohydrate. This view seems not to have been previously recorded. Investigations should be made to test its validity.

Researches are now needed to test thoroughly the imposing array of evidence in support of the view that vitamin D (or its equivalent in sunlight) reduces susceptibility to caries of the teeth through im-

proving the effectiveness of the body in an immunological sense by militating against the profuse growth of an oral acid-forming and acid-tolerating flora. If such an effect could be demonstrated it would go far toward reconciling the conflicting data of Bunting and his associates and those of M. Mellanby, McBeath, and others.

The weakest point in the theory of Bunting lies in the attribution to sugar of a greater caries-inducing power than cooked starch, and in the assertion that hypoplastic enamel, with its rough surface and pits, which from any viewpoint should afford numerous areas of stagnation of food residues, is not more likely to favor decay than does normal enamel. The present writers are able to suggest only one possible way in which starch might exert a protective action against acid erosion. It is on the basis that starch might be able to absorb considerable amounts of fermentation acid, thus holding it away from contact with the enamel surface. There is at present no experimental evidence bearing on this point.

It is clear that if we were all to turn to a carnivorous diet, which is impracticable or impossible in most countries, tooth decay would disappear. It seems that were we to turn to a low-sugar, high-fat type of diet, such as is prescribed for diabetic patients, we might expect a prompt and marked reduction in caries-susceptibility. This type of diet is practicable in many countries, but fats are in many regions considerably more expensive to produce than are starches and sugars. At any rate we now know how to produce good teeth as respects structure and how to preserve them in considerable measure from decay. We may confidently expect that further researches will within a few years see complete unanimity of opinion as to the factors which operate to cause caries-susceptibility. Nutritional research has scored a great achievement in the field of dental science.

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CHAPTER

XXVIII

Diet in Relation to Healthful Longevity

IN PAST AGES, as well as the present, thoughtful essays have been written which diligently discussed the problem of prolonging human life (Minot, '08; Metchnikoff, '10; Rolleston, '22; Pearl, '22; Robertson, '23; Warthin, '29; Dublin and Lotka, '36). Man's interest in longevity is indicated also by the constant occurrence of news items in the daily newspapers which report panaceas for aging readily furnished to eager news-gatherers by men and women who happen to reach a ripe old age. Some factors commonly regarded in these accounts as important in the prolongation of life are diet, alcoholic beverages, tobacco, coffee, work habits, and sleep. But isolated reports have scarcely any scientific validation. In order to assess the role of any factor in prolonging life it is necessary to make a large number of authentic observations in which other factors are statistically constant, i. e., have no influence on the variable being measured. Obviously, therefore, it is practically impossible to learn the probable effect of important factors through personal inquiries directed to old persons. Moreover, there are no available records on human beings from which statistical analyses can be relied to answer many of the significant dietary questions regarding human longevity. We are able, from reference to life tables, to predict approximately how many persons born in a given year will be alive at some given time in the future. But we are unable to state definitely, on the basis of any data, what influence several different dietary factors will have on the prolongation of life. For example, we do not know with certainty whether it favors longevity to retard a child's growth through food restriction, as suggested by some data to be discussed subsequently, or whether the growth of children should be made to proceed at a rapid rate.

The evidence unequivocally suggests that dietary practices do influence the span of man's healthful existence, but our preoccupation with this factor in longevity should not be construed as an indication that we regard other factors as without much importance. Space, and the general scope of this book, preclude references to other significant factors in determining the length of life. In the discussion to follow it shall be our purpose simply to review the most pertinent data from animal experimentation which we regard as having sufficient bearing on human longevity to warrant consideration and to present our interpretation of the present evidence. Since most of us are more interested in healthful longevity than in mere extension of the life span through a long period of immaturity and senescence, the emphasis shall be on the role of diet in extending the number of years of well-being during maturity.

Prolongation of Life Through Retardation of Growth Rate.— As shown by Osborne and Mendel ('15), the capacity of rats to grow is not necessarily lost at the end of the period at which growth ordinarily ceases. Young rats restricted for months to diets low in certain essential amino acids grew at a very slow rate, but when the diet was made to yield a generous amount of these amino acids rapid growth occurred. Also, resumption of growth was accompanied by the capacity to reproduce. At the time of these studies it was believed that such retardation of growth occasioned no impairment of the individual. But Jackson's ('36) investigations indicated that prolonged stunting, by means of protein deficiency, causes permanent injury. In a later study Jackson ('37) investigated the effect on growth of calorie restriction and noted that refeeding did not cause a full recovery since there was permanent stunting. Unfortunately it is impossible to clearly interpret his data owing to the use of a stock diet composed of natural foods for the control animals, while the experimental animals were restricted to low-protein or low-calorie diets composed of purified ingredients.

There are, of course, various ways in which growth may be retarded by dietary means. Although some writers of past generations believed that slow growth promoted longevity only a few attempts have been made to test this hypothesis. The more recent investigations made by McCay and associates ('35, '38) require considerable discussion. In studies of the nutritional requirements of brook trout they (McCay and Crowell, '34) were led to postulate that "an individual in a species that grows to maturity slowly will have a greater life span than one that grows rapidly. . . ." On this basis they questioned the wisdom of inducing rapid growth in infants and children. It is little wonder, therefore, that their investigations have aroused the interest of biochemists and pediatricians.

They have completed two investigations, but at this date (early summer of 1938) only the first one has been published in full. In this study 106 rats were divided into 3 groups at weaning. The basal ration was fed to Group I *ad libitum*. From weaning Group II was allowed to have only enough to permit a gain of approximately 10 gm. per rat at intervals of 2 to 3 months. Group III was given the basal ration *ad libitum* for 2 weeks after weaning and then restricted in the same manner as Group II. After 766 days on the diet one-half of the animals in Groups II and III were given the food *ad libitum* and after 911 days the remainder were allowed to eat all they desired.

In such investigations the composition of the diets obviously is very important. Unfortunately it appears that the dietary procedure used here was not entirely suitable for the purpose. For instance, the ration was entirely devoid of vitamin E and the protein level was two times higher than was necessary to promote rapid growth in young animals. The composition of the diet was: starch 22, cellulose 2, lard 10, sucrose 10, salt mixture 6, dried yeast 5, cod liver oil 5, and unpurified casein 40. Although this same diet was furnished to each group of rats, those receiving it in limited amount were given an additional 3 drops of cod liver oil and 0.5 gm. of yeast per rat daily "in order to further compensate for the possible shortage of vitamins in the retarded growth groups." Thus complicating factors were introduced which made it difficult to fully interpret the data.

The average life span in days was as follows:

	Group I	Group II	Group III
Males	483	820	894
Females	801	775	826

Twelve animals in the retarded groups were still alive after all of those in Group I had died. The greatest life span attained by any animal was 1421 days in Group II. Although the data have not been treated statistically it appears evident that food restriction did promote longevity.

But when the data are examined it becomes evident that the lengthened life span was accompanied by a decided lack of well-being. In fact the restricted animals were practically in a state of chronic invalidism throughout their unhappy lives. Observations on the sexual development, by Asdell and Crowell ('35), showed that in the more severely restricted group the age at first estrus was 357 days, whereas in the unrestricted females it was at the 55th day of life. It is difficult

to decide on the basis of the data whether food restriction caused a prolongation of the period of regular estrus cycles; but whether it did or did not, it is evident that the females allowed to eat *ad libitum* enjoyed a longer period of sexual maturity since they got started 302 days ahead of their food-restricted sisters. Moreover, as life waned the restricted animals were in an abject state since senility was prolonged. It was estimated that at least one-half of the animals became blind after they had lived more than 2 years. The bones of the food-restricted groups were extremely fragile and "Some crumbled in the course of dissection."

Thus the data indicate that food restriction during the greater part of a rat's normal life span promotes longevity, but the existence is colorless and in no respect, as yet revealed, does the evidence suggest the desirability of human food restriction, except in ordinary moderation, as a means of promoting well-being, and particularly healthful longevity.

Healthful Longevity Through Improvement of the Dietary.— Although innumerable observations during the past 30 years of nutritional investigations have suggested the necessity for well-being of dietaries abundant in all the various essential nutrients, striking direct evidence that healthful longevity is promoted by such regimens has come during the past few years from the studies of Sherman and Campbell ('24, '28, '30, '35a, '35b, '37). Many generations of rats have been raised with success, as judged by moderate standards, in Sherman's laboratory, using a diet of 1 part dried whole milk powder, 5 parts of ground whole wheat, and a little sodium chloride. But when Sherman and Campbell ('37, l.c.) began to "improve" the diet by adding calcium carbonate or more milk they obtained "better results as judged by observations upon growth, adult vitality, and length of life."

The percentage composition of the diets investigated was:

INGREDIENT	DIET 16	DIET 162	DIET 165	DIET 166	DIET 167
Ground whole wheat . .	81.97	81.66	77.44	77.14	65.79
Whole milk powder . . .	16.39	16.33	16.41	16.35	16.45
Calcium carbonate . . .	...	0.39	...	0.38	...
Butter fat	...	...	4.59	4.58	...
Skim milk powder . . .	...	...	...	...	16.45
Sodium chloride	1.64	1.63	1.59	1.55	1.31

The following table, constructed from Sherman and Campbell's data, summarizes most of the significant findings we wish to emphasize:

Variable Observed	No. 16 No Enrichment	No. 162 Ca as Carbonate	No. 165 Butter Fat	No. 166 Ca and Butter Fat	No. 167 Skimmed Milk Powder
Gain in gm. during 28th to 56th day of age { male	56.3	71.0	51.0	66.1	96.4
{ female	47.0	60.2	45.2	57.9	73.5
Days age at birth of first young	132	111	134	107	110
Days duration of capacity to reproduce	213	279	286	241	234
Young born per female	24.1	32.6	34.2	27.7	27.6
Young reared per female	12.7	20.4	19.0	15.3	16.1
Average weight of young at 28 days	38.9	42.4	38.9	40.4	44.6
Length of life in days { male	658	703	667	689	681
{ female	723	746	818	739	754

Although we have omitted the statistical analyses of these data, in the interest of brevity, it is clearly indicated that supplementation of the basal wheat-milk-sodium chloride ration improved the well-being and longevity of rats restricted to such diets. The basal diet was apparently deficient in calcium, containing only 0.195 per cent of that element. In addition, the content of protein was only 13.1 per cent, and not more than one-third of this was furnished by milk.

As stated by Sherman and Campbell ('37, l.c.), "Throughout youth and early adult life the appearance of animals on diet 167 (skimmed milk powder replacing part of the wheat in the basal ration) as judged by the condition of the coats, and firmness of body was superior to all others." They noted that in these animals senility began to appear at about the same age as in animals receiving the basal ration fortified with calcium carbonate alone, butter fat alone, or calcium carbonate butter fat. However it progressed more rapidly than in the latter groups of animals. Moreover, it was observed that in the three groups receiving calcium carbonate and/or butter fat a youthful appearance lasted longer than in the animals receiving the unfortified basal ration.

In view of the thesis championed by McCay and associates (McCay and Crowell, '34, l.c.), as well as others, that the well-being of individuals is promoted by slow growth during youth, the data of Sherman and Campbell offer definite contradiction. During the 28th to 56th days of life rats receiving the basal ration fortified with skim milk powder grew about 40 per cent faster than those receiving the unsupplemented diet. The supplemented animals lived longer, were sexually mature much earlier, reared more young per female,

the young weighed more at weaning, and the duration of their capacity to reproduce was considerably longer. The findings of Sherman and Campbell confirm the early views of Robertson and Ray ('20) that animals (mice) which grow rapidly live a longer time than slowly growing animals (mice).

The evidence at present suggests indubitably that general well-being and healthful longevity are promoted, not by food restriction during youth, but by the generous provision of a well-balanced diet.

Some Problems of Diet in Relation to Healthful Longevity in Humans.—It is apparent to the thoughtful person that each experiment, in investigations of the nature being discussed, require several years for completion and is therefore exceedingly expensive. Moreover, the time element renders more likely the introduction of variables which tend to cause subtle changes in the experimental conditions, thus making it difficult to interpret the data. For this reason alone it is necessary to be particularly cautious about inferring the desirable dietary practices for humans on the basis of observations on animals.

In none of the investigations to date have attempts been made to determine the most suitable dietary regimens for different periods of life. For instance, it seems rather evident that the amount of protein required during the growth period is different from that needed in adulthood. What are the optimum levels of protein required during different periods of life? Also, what dietary modifications are beneficial in old age to promote clarity of mental processes and freedom from crippling physical handicaps? It seems improbable that a diet which is advantageous to a young person is equally suitable when he is old. Aged persons, with years of experience and accumulated knowledge, personally can enjoy their attainments and perform valuable services to society if their regimen of living can be reasonably regulated in such manner as to favor full utilization of the powers which the inevitableness of senility gradually throttle to extinction. Investigations of the future will attempt to determine more precisely the potentialities of improved dietary practices in promoting healthful longevity.

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APPENDIX

TABLE I

NUTRITIVE VALUE OF PROTEINS OF CEREALS, LEGUMES, AND VEGETABLES FOR GROWTH OF RATS

* Names of investigators are listed on page 660. † Letters in parentheses refer to notes on page 658.

PROTEIN OR PROTEIN-FOOD	METHOD OF PREPARATION	ASSAY METHOD	INVESTIGATOR*	Level of protein in test diet *Per cent*	NUTRITIONAL VALUE: Per cent of absorbed N not eliminated in urine	NUTRITIONAL VALUE: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Corn	Presumably whole corn. Method of preparation not stated.	Mitchell N bal.	(15)	10	61.3	
	" " "	" " "	(1)	10	59.6	
	Ground South American yellow corn	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	8	67	
	" " "	" " "	(3)	5	84	
	Presumably whole corn. Method of preparation not stated.	Mitchell N bal.	(1)	5	72	
	Whole yellow corn finely ground	Mitchell paired feeding	(10)	8		1.58 (d)†
	" " "	" " "	(10)	8		1.55 (d)
	" " "	" " "	(10)	8		1.80 (d)

	Ground South American yellow corn	Osborne and Mendel	(16)	9.9		1.29 (s)
	" " "	" " "	(16)	9.9		1.38 (s)
	Ground South African white corn	" " "	(16)	9.0		1.30 (s)
Wheat	Whole wheat ground and dried at low temp.	Mitchell N bal.	(9)	8	67 (a)	
	Whole wheat ground	Mitchell paired feeding	(10)	8		1.99 (d)
	" " "	" " "	(10)	8		1.64 (d)
	" " "	" " "	(10)	10		1.70 (d, f)
	" " "	Osborne and Mendel	(11)	10		2.08 (s)
	" " "	" " "	(11)	10		1.58 (s)
	" " "	" " "	(17)	10		1.4 (f, s)
	" " "	" " "	(17)	8		1.2 (s)
	" " "	" " "	(17)	5		0.6 (t)
	Soft English wheat ground	" " "	(16)	9.7		1.36
	Soft English wheat ground and cooked	" " "	(16)	9.7		1.51
Oats	Not stated	Mitchell N bal.	(1)	5	78.6	
	" "	" " "	(1)	10	64.9	
	"Precooked"	" " "	(7)	5	82	

TABLE I—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Oats (*cont'd*)	Standard brand of rolled oats—hulled	Mitchelled paired feeding	(10)	8		2.52 (d)
	Oatmeal	Osborne and Mendel	(11)	10		2.19 (s)
	"	" " "	(11)	10		1.89 (s)
	Oat groats (hulled)	" " "	(17)	10		1.2 (s, t)
	" " "	" " "	(17)	8		1.4 (s, t)
	" " "	" " "	(17)	5		1.8 (s, t)
Barley	Whole grain ground	" " "	(17)	10		1.6 (s, t)
	" " "	" " "	(17)	10		1.2 (s)
	" " "	" " "	(17)	8		1.9 (s, t)
	" " "	" " "	(17)	8		1.6 (s)
	" " "	" " "	(17)	5		1.7 (s, t)
	" " "	" " "	(17)	5		1.4 (s, t)

Rye	Not stated	Mitchell paired feeding	(13)	10		1.98
	" "	Osborne and Mendel	(13)	10.1		2.51 (s)
	" "	" " "	(13)	10.0		2.05 (s)
	Whole grain finely ground	" " "	(17)	10		1.3 (s)
	" " "	" " "	(17)	8		1.5 (s)
	" " "	" " "	(17)	5		1.4 (s)
	Whole grain finely ground	" " "	(17)	10		1.2 (s)
	" " "	" " "	(17)	8		1.3 (s)
	" " "	" " "	(17)	5		1.3 (s)
Rice	Not stated	Mitchell N bal.	(1)	5	86.1	
Rice bran	Not stated	Osborne and Mendel	(18)	9.02		1.47 (s)
	" " "	Mitchell N bal.	(31)	10	67	
Patent wheat flour	Standard commercial brand	" " "	(8)	8	52	
	Prepared from soft English wheat	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	61	
	" " "	" " "	(3)	6	68	
	Source of flour not stated	Mitchell N bal.	(7)	5	72	
	Commercial brand	" " "	(19)	10		0.88 (s)
	" "	" " "	(11)	10		1.27 (s)
	" "	" " "	(11)	10		1.16 (s)

TABLE I—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value	
					Per cent of absorbed N not eliminated in urine	Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
"Whole wheat" flour	Not stated	" " "	(7)	5	73	
Wheat bran	" " "	Modified Mitchell paired feeding	(20)	6.6		1.05 (d)
	" "	" " "	(20)	5.3		1.23 (d)
	Commercial brand	Osborne and Mendel	(19)	10		1.71 (s)
	" "	" " "	(19)	10		1.96 (s)
Wheat germ	Not stated	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	69	
Cornmeal	"Endosperm" prepared from South American yellow corn	" " "	(3)	7	70	
	Not stated	Osborne and Mendel	(18)	9.02		1.18 (s)
Corn gluten	Prepared from South American yellow corn	" " "	(16)	9		0.0± (s)
Edestin	Not stated	" " "	(30)	8		1.49
	" "	" " "	(30)	8		1.41

Peanuts	Roasted	Mitchell N bal.	(21)	9.2	57.9	
	"	" " "	(21)	9.2	55.8	
	Fried Chinese peanuts	" " "	(4)	10.4	59	
Peanut oil meal	Not given	Osborne and Mendel	(18)	9.02		1.45 (s)
Coconut meal	Not stated	Mitchell N bal.	(31)	5	77	
	" "	" " "	(31)	10	58	
Soy bean meal	Beans ground, ether extracted and dried	Mitchell paired feeding	(10)	10		0.19 (d)
Soy bean flour	Commercial preparation called "Edelsoja"	Osborne and Mendel	(22)	8.5		1.64 (f)
	" " "	" " "	(22)	10.6		1.57 (f, s)
	" " "	" " "	(22)	15.3		1.58 (f, s)
Linseed meal	Flaxseed ground, dried at low temp. and ether extracted	Mitchell N bal.	(23)	8.7	78 (b)	
	" " "	Mitchell paired feeding	(23)	8.7		1.55 (e)
Cottonseed meal	Cottonseed ground, dried at low temp. and ether extracted	Mitchell N bal.	(23)	8.4	78 (b)	
	" " "	Mitchell paired feeding	(23)	8.4		1.33 (c)
Navy beans	Beans were steam-cooked	Mitchell N bal.	(1)	10	38.4	
	Beans were soaked, boiled until soft, ground and dried at low temp.	Osborne and Mendel	(11)	10		1.48 (s)
	" " "	" " "	(11)	10		1.32 (s)

TABLE I—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Potatoes	Whole potato used. Method of preparation not stated	Mitchell N bal.	(1)	5	68.5	
	" " "	" " "	(1)	10	66.7	
	Potatoes were boiled, peeled, riced, dried at low temp. and ground	Mitchell paired feeding	(24)	7.9		1.34 (d)
	Guberin used. Prepared by heat coagulation of potato juice	Mitchell N bal.	(25)	8.1	71 (t)	
	" " "	Osborne and Mendel	(25)	8.5		2.0 (f, s)
	" " "	" " "	(25)	10.5		1.66 (f, s)
Sweet potatoes (Ipomea batatas)	Protein prepared by heat coagulation of potato juice and drying of purified preparation at low temp.	Mitchell N bal.	(5)	10	93	
	" " "	Osborne and Mendel	(5)	10		1.5 (s)

Alfalfa leaf meal	Source and method of preparing meal not stated	Modified Mitchell paired feeding	(20)	11.0		0.40 (d)
	" " "	" " "	(20)	11.7		0.69 (d)
Cabbage (Brassica Pekinensis) .	Protein isolated according to method of Kao (26)	Mitchell N bal.	(5)	10	76	
	" " "	Osborne and Mendel	(5)	10		0.9 (s)
Yeast	Whole yeast used	Mitchell N bal.	(1)	5	85.5	
	Leading brand of baker's yeast in Poland	Osborne and Mendel	(22)	8.4		1.48 (f, s)
Coco	Ether-extracted Hershey's coco	Mitchell N bal.	(27)	8	37	

TABLE II

NUTRITIONAL VALUE OF PROTEINS OF MILK, EGGS, AND MEATS FOR GROWTH OF RATS

* Names of investigators are listed on page 660. † Letters in parentheses refer to notes on page 658.

PROTEIN OR PROTEIN-FOOD	METHOD OF PREPARATION	ASSAY METHOD	INVESTIGATOR*	Level of protein in test diet *Per cent*	NUTRITIONAL VALUE	
					Per cent of absorbed N not eliminated in urine	Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Casein	Not stated	Mitchell N bal.	(1)	5	70.8	
	Purified and heated for 3 days	Chick and Roscoe modified Mitchell N bal.	(2)	4.4–15	47	
	Purified without heat	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	6	76	
	Not stated	Osborne and Mendel	(29)	9.3		1.45 (f, s)†
	" "	" " "	(5)	10		2.6 (s)
	" "	" " "	(5)	10		1.9 (s)
	" "	" " "	(6)	10		1.98 (s)
Lactalbumin	" "	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	65	
	" "	Osborne and Mendel	(29)	7.9		3.01 (f, s)

Whole milk	" "	Mitchell N bal.	(1)	5	93.4	
	Commercial whole milk powder	" " "	(7)	5	89	
	Roller dried winter milk	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	86	
	Not stated	Mitchell N bal.	(1)	10	84.7	
Skimmed milk powder	" "	" " "	(8)	8	85	
	Commercial brand containing some fat	Osborne and Mendel	(6)	10		2.89 (s)
Whole egg	Dried	Mitchell N bal.	(9)	8	93	
	"	" " "	(8)	8	94	
Egg albumin	"	" " "	(8)	8.9	83	
	Not stated	" " "	(5)	10	93	
Beef muscle	Beef was ground, dried, reground, ether extracted and sieved	" " "	(8)	9.4	69	
	Trimmed round steak, ground, dried at low temp., ground and ether extracted	Mitchell paired feeding	(10)	8		2.77 (d)
	" " "	" " "	(10)	20		1.64 (d)
	Trimmed round steak, ground and dried at low temp.	Osborne and Mendel	(11)	10		3.15 (s)
	" " "	" " "	(11)	10		2.55 (s)

TABLE II—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Veal muscle	Ground, dried, reground and sieved	Mitchell N bal.	(8)	7.5	62	
	Trimmed, ground and dried at low temp.	Osborne and Mendel	(6)	10		2.88 (s)
Beef liver	Trimmed, free of visible fat and dried at low temp.	Mitchell N bal.	(12)	8	77	
	" " "	" " "	(12)	16	58	
Liver meal	Not stated	Osborne and Mendel	(13)	10.1		1.97 (s)
Beef spleen	Trimmed, ground and dried at low temp.	" " "	(6)	10		2.75 (s)
Beef brain	" " "	" " "	(6)	10		2.97 (s)
Hog brain	" " "	" " "	(6)	10		2.89 (s)
Calf thymus	" " "	" " "	(6)	10		1.81 (s)
Tripe	" " "	" " "	(6)	10		1.73 (s)
Cracklings (pork)	Fat back rendered at 100° C. Cracklings ground and ether extracted	Mitchell N bal.	(14)	8	25	

Rat tissues	Lungs, livers, hearts, kidneys, and muscle tissue of adults boiled 10 min., minced and dried at low temp.	Boas, Fixsen and Jackson modified Mitchell N bal.	(3)	7	55	
Beef lips	Trimmed, ground and dried at low temp.	Osborne and Mendel	(6)	10		2.29 (s)
Beef cheek meat	" " "	" " "	(6)	10		2.36 (s)
Beef tongue	" " "	" " "	(6)	10		2.85 (s)
Hog tongue	" " "	" " "	(6)	10		2.86 (s)
Tankage	Commercial method of prep. not stated	Mitchell N bal.	(1)	10	31.5	
Beef heart	Trimmed, ground and dried at low temp.	" " "	(12)	8	74	
Beef kidney	" " "	" " "	(12)	8	77	
Pork muscle	Dried at low temp. and partially ether extracted	" " "	(9)	8	74	
Pork tenderloin	" " "	" " "	(14)	8	79	
Pork muscle	Not stated	Osborne and Mendel	(11)	10		2.96 (s)
	" "	" " "	(11)	10		2.46 (s)
Lamb muscle	" "	" " "	(11)	10		3.12 (s)
	" "	" " "	(11)	10		2.48 (s)

TABLE III

SUPPLEMENTARY RELATIONSHIPS BETWEEN PROTEINS, AMINO ACIDS, AND PROTEIN-FOODS FOR GROWTH OF RATS

* Names of investigators are listed on page 660. † Letters in parentheses refer to notes on page 658.

PROTEIN OR PROTEIN-FOOD	METHOD OF PREPARATION	ASSAY METHOD	INVESTIGATOR*	Level of protein in test diet *Per cent*	NUTRITIONAL VALUE	
					Per cent of absorbed N not eliminated in urine	Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Corn	Presumably ground whole corn	Mitchell N bal.	(15)	10	61.3	
Milk	Presumably dried whole milk	" " "	(15)	10	84.7	
Corn and milk (protein ratio 3 to 1)		" " "	(15)	10	75.7 (g)	
Tankage	Commercial product	" " "	(1)	10	31.5	
Corn and tankage (protein ratio 1 to 1)		" " "	(15)	10	60.0 (h)	
Corn	Whole yellow corn ground	Mitchell paired feeding	(10)	8		1.58 (d)†
	" " "	" " "	(10)	8		1.55 (d)
Corn and lysine (0.25%)	" " "	" " "	(10)	8		1.78 (d)
Corn and lysine (0.15%)	" " "	" " "	(10)	8		1.82 (d)

Corn and tryptophane (0.13%) .	" " "	" " "	(10)	8		1.44 (d, q)
Corn, lysine (0.15%), and tryptophane (0.13%)	" " "	" " "	(10)	8		2.07 (d)
Cornmeal	Source of cornmeal not stated	Osborne and Mendel	(18)	9.02		1.18 (s)
Peanut oil meal	Source of peanut oil meal not stated	" " "	(18)	9.02		1.45 (s)
Cornmeal and peanut oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.21 (s)
Cornmeal and peanut oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.46 (s)
Cornmeal and cottonseed oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.23 (s)
Cornmeal and soybean oil meal (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.76 (s)
Cornmeal and rice bran (protein ratio 2 to 1)	" " "	" " "	(18)	9.02		1.63 (s)
Rice bran	" " "	" " "	(18)	9.02		1.47 (s)
Cornmeal and beef muscle (protein ratio 1 to 1)	Apparently commercial cornmeal. Beef was round steak trimmed, ground and dried at low temp.	Osborne and Mendel	(11)	10		3.16 (s)
Beef muscle		" " "	(11)	10		3.15 (s)
Wheat	Hard spring wheat ground	" " "	(11)	10		2.08 (s)

TABLE III—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Wheat and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		3.22 (s)
Wheat and beef muscle (protein ratio 2 to 1)		" " "	(11)	10		2.68 (s)
Wheat	Wheat was ground	Mitchell paired feeding	(10)	8		1.99 (d)
Wheat and cystine (0.25%)	" " "	" " "	(10)	8		1.91 (d)
Wheat	" " "	" " "	(10)	8		1.64 (d)
Wheat and lysine (0.25%)	" " "	" " "	(10)	8		2.05 (d)
	" " "	" " "	(10)	10		2.16 (f, d)
Wheat	" " "	" " "	(10)	10		1.70 (f, d)
Patent wheat flour and beef muscle (protein ratio 2 to 1)		Osborne and Mendel	(11)	10		2.67 (s)
Patent wheat flour and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		2.95 (s)
White wheat flour and dried lean beef muscle (protein ratio 2 to 1)		Mitchell N bal.	(8)	8.8	73 (o)	

White wheat flour and dried veal muscle (protein ratio 2 to 1)		" " "	(8)	8	62 (n)	
White wheat flour and skim milk powder (protein ratio 2 to 1)		" " "	(8)	8	71 (m)	
White wheat flour and dried egg albumin (protein ratio 2 to 1)		" " "	(8)	9.25	66 (l)	
White wheat flour and whole dried egg (protein ratio 2 to 1)		" " "	(8)	8	75 (j, k)	
Oatmeal	Standard brand of rolled oats	Mitchell paired feeding	(10)	8		2.52 (d)
Oatmeal and lysine (0.25%) . .	" " "	" " "	(10)	8		2.66 (d)
Oatmeal and beef muscle (protein ratio 1 to 1)	Round steak was ground and dried at low temp.	Osborne and Mendel	(11)	10		2.52 (s)
Oatmeal		" " "	(11)	10		1.89 (s)
		" " "	(11)	10		2.19 (s)
Oatmeal and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		3.13 (s)
Oatmeal and beef muscle (protein ratio 2 to 1)		" " "	(11)	10		3.25 (s)
Oats and gelatin (protein ratio 1 to 3)	Method of preparing oats and gelatin not stated	Mitchell N bal.	(15)	10	42.6 (i)	
Rye	Presumably whole ground rye	Osborne and Mendel	(13)	10.1		2.51 (s)

TABLE III—*Continued*

Protein or Protein-food	Method of Preparation	Assay Method	Investigator*	Level of protein in test diet *Per cent*	Nutritional Value: Per cent of absorbed N not eliminated in urine	Nutritional Value: Gain in body weight per gm. protein ingested (N × 6.25) *Grams*
Liver meal		" " "	(13)	10.1		1.97 (s)
Rye and liver meal (protein ratio 2 to 1)		" " "	(13)	11.4		2.41 (s)
Rice	Not stated	Mitchell N bal.	(1)	5	86.1	
Beef muscle	Round steak trimmed, ground and dried at low temp.	Osborne and Mendel	(11)	10		2.55 (s)
Rice and beef muscle (protein ratio 1 to 1)		" " "	(11)	10		2.50 (u)
Soy bean meal	Beans ground, ether extracted and dried	Mitchell paired feeding	(10)	10		0.19 (d)
Soy bean meal and cystine (0.25%)	" " "	" " "	(10)	10		0.90 (d)
Navy beans	Soaked, boiled, ground and dried at low temp.	Osborne and Mendel	(11)	10		1.48 (s)
Navy beans and beef muscle (protein ratio 1 to 1)	" " "	" " "	(11)	10		2.32 (s)

Garden peas	Peas were shelled, ground, and dried at low temp.	Mitchell paired feeding	(24)	8.7		1.18 (d)
Garden peas and cystine (0.24%)	" " "	" " "	(24)	8.7		1.62 (d)
Potato (Ohio red)	Boiled, peeled, riced, dried at low temp., and ground	" " "	(24)	7.9		1.34 (d)
Potato and cystine (0.24%)	" " "	" " "	(24)	8.1		2.00 (d)
Potato and beef muscle (protein ratio 1 to 1)	Potatoes were peeled, boiled, ground and dried at low temp.	Osborne and Mendel	(11)	10		1.84 (s)
Beef muscle	Round steak trimmed, ground and dried at low temp.	" " "	(11)	10		2.55 (s)
Pork crackling	Fat back rendered at 100° C. Cracklings ground and ether extracted	Mitchell N bal.	(14)	8	25	
Pork tenderloin	Meat was ground, dried at low temp. and ether extracted	" " "	(14)	8	79	
Pork crackling-tenderloin (protein ratio 1 to 3)		" " "	(14)	8.4	72 (p)	

FOOTNOTES TO TABLES ON NUTRITIONAL VALUE OF PROTEINS AND PROTEIN-FOODS FOR GROWTH OF RATS

(a) The practice of discarding unconsumed food residues as was employed here may introduce errors of varying magnitude in the assay of protein of complex food materials such as cereals unless they are very finely ground. There is considerable difference in the quality and quantity of protein in various parts of cereal grains. Hence, when the test animal is allowed to select the part it likes best, variability is introduced which may cause erroneous results. This has been critically discussed by Osborne and Mendel (*J. Biol. Chem.*, *34*, 521) and Mitchell (*J. Biol. Chem.*, *58*, 873).

(b) In the interpretation of these results it may be of significance that the test diet apparently was not sufficiently adequate with respect to the vitamin B complex for, as the author states "In the next to the last transition period, each rat was given daily 0.5 gm. of Northwestern Yeast Company's dried yeast up to within one day of the start of the following collection period, in a further attempt to stimulate food consumption."

(c) Calculated from data given in paper. It should be noted that these data, secured by a paired feeding procedure, indicate a higher biological value for linseed meal than is given for cottonseed meal. However, data secured by the nitrogen balance method (b) suggest no difference between these proteins. The explanation is that a greater percentage of the linseed meal protein is digestible. The nitrogen balance method is concerned only with the nutritional value of absorbed nitrogen. Appreciation of this fact is important in evaluating the protein feeding value of foods on the basis of values given here.

(d) Calculated from data given in paper.

(f) The significance of these average values is somewhat in doubt owing to the lack of adequate numbers of test animals.

(g) According to Mitchell's calculations the biological value of this mixture of protein foods would be 67.2 if there were no supplementary relationships. Since the actual value is 75.7, by determination, it is evident that a supplementary action occurs when corn and milk are fed together.

(h) Since the biological value of a mixture of corn and tankage (1 to 1) is scarcely different from that of corn alone (61.3), while that of tankage is only 31.5, a supplementary relationship exists between the proteins of these feeds.

(i) Since gelatin is an incomplete protein it is not possible to decide, on the basis of these and other available data, whether a supplementary relationship exists.

(j) There is considerable variation between individual values, ranging from 62 to 92.

(k) It appears that a distinct supplementary relationship exists, since the computed value, if no supplementing effect exists, is 66.

(l) A supplementary action is scarcely indicated, since the computed value, if no supplementary effect exists, is 64.

(m) The value to be expected if no supplementary relation exists is 62, in contrast to the actual value of 71.

(n) The value to be expected if no supplementary relation exists is 53, in contrast to the actual value of 62.

(o) The value to be expected if no supplementary relation exists is 64, in contrast to the actual value of 73.

(p) Since the nutritional value of pork tenderloin is approximately 79 and that of pork cracklings is about 25, it is indicated that a supplementary relationship exists.

(q) It may be of considerable significance that corn supplemented with only tryptophane appears to be less capable of promoting growth than unsupplemented corn. Mitchell and Smuts note this apparent growth-depressing effect

of tryptophane but suggest no explanation of it. Perhaps the growth relationships between corn and tryptophane should be further investigated.

(r) Although these data on the supplementary relationships between alfalfa leaf meal and wheat bran appear to indicate a supplementary effect, it should be noted that the protein levels of the various diets compared were not comparable. For example, in one series wheat bran is fed at a level of 6.6% protein in the diet while the mixture of alfalfa leaf meal and wheat is fed at a level of 8.8% protein and that of alfalfa leaf meal alone is 11.0% protein.

(s) This value may not represent the actual biological value of the protein, as compared with that of protein in other foods, owing to the lack of comparability in food intake. The test animals were allowed to eat the diet *ad libitum*. Since there is bound to be differences in the palatability of rations, it is possible that differences in apparent nutritional value may be attributable, in part, to differences in food intake. It would seem that the only way to rule out palatability as a factor in protein assays is to restrict the food intake of test animals to that of animals given a standard protein, perhaps casein or egg protein. That would necessitate also the use of a standard basal diet. In this way the nutritional value of all proteins would be determined with reference to a standard arbitrarily selected protein.

(t) The significance of this average value is somewhat in doubt owing to the large differences between individual values.

(u) The nutritive value of rice protein has not been determined under comparable conditions.

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TABLE IV

Distribution of Vitamins A, B_1, and D in Foodstuffs*

	International Units per 100 Grams		
	A	B_1	D
Cereal and cereal products			
Alfalfa, leaf meal	16,800–17,000	..	..
Barley	0	50–100	..
Bread — Graham	0	75	..
whole wheat	0	60	..
white	0	10	..
Buckwheat	..	110	..
Cornmeal — white	0	65	..
yellow	350–1,000	65	..
Flour — Graham	..	110	..
white patent	0	4–25	..
whole wheat	0–20	110	..
Hominy — white	0	70	..
yellow	1,200	70	..
Oatmeal — whole grain	0–25	325	..
prepared breakfast	0	60–140	..
Rice — brown	..	50–100	..
polished	0	0	..
polishings	0	300–760	..
Rye — whole	..	100; 136	..
germ	..	300; 750	..
Soybean	200–500	200–400	..
Wheat — whole	0–20	75–250	..
bran	..	100–500	..
germ	400	400–2,200	..
Fruits			
Apples	100	5–40	..
Apricots — fresh	7,500	10–15	..
commercial — dried	9,800	..	..
Avocados	150–700	40–140	..
Bananas — fresh	385	15–50	..
powder	1,400	50	..
Cantaloupe	595	10	..
Cherries	35–1,150	..	..
Cranberries	28	..	..
Dates, commercial	150–196	10–30	0
Figs — fresh	80	18	..
dried	50–100	25–100	..
Grapes	15–90	10	..
Grapefruit	0	10	..
Olives — canned, green	392	..	..
Manzanillo, ripe	350	2	0
Mission, ripe	140	..	..
Oranges	300–400	10	..

* Various sources of these data have been used, the compilations of Daniel and Munsell (U. S. Dept. Agric., Misc. Pub. No. 275, 1937) and that of Boas-Fixsen and Roscoe (Nutr. Abstr. & Rev., 7, 823, 1937–38) being especially helpful.

TABLE IV.— *Continued*

	International Units per 100 Grams		
	A	B_1	D
Fruits (cont'd)			
Peaches, fresh, white	7	..	..
fresh, yellow	250–2,800	..	..
dried, yellow	2,000–6,300	10–15	..
Pears	8–15	15–30	..
Pineapple	100–115	25	..
Plums	..	40	..
Prunes — dried	1,000–3,500	90	..
Raisins	95	0–50	..
Raspberries	..	..	..
Strawberries	..	..	..
Tangerines	..	..	..
Watermelon	126	9	..
Juices			
Grape, commercial	0	0	0
Grapefruit	0	10	..
Lemon	0	15	..
Orange	95	30	..
Pineapple	50	..	..
Sauerkraut — commercial	0	..	..
Tomato	825	..	..
Nuts			
Almond	..	40–80	..
Chestnut	..	55–90	..
Filbert	..	220	..
Hazelnut	..	110–220	..
Peanut	..	220–325	..
Pecan	250–500	50	..
Walnut	..	60–150	..
Oils			
Black sea bass liver	52,000,000	..	1,000,000
Cod liver	100,000–250,000	0	8,500–25,000
Corn — refined	0	0	0
Cottonseed	0	0	0
Halibut liver	1,920,000–36,000,000 (varies greatly)	..	120,000
Herring — entire body	4,860–6,400	..	100,000
Olive — refined	140	..	..
Lamprey — liver — river	..	..	5,300
sea	..	..	4,700
Salmon liver — (Chinock)	2,800,000	..	140,000
Sardine — entire body	24,240–270,000	..	8,000
Swordfish, liver	25,000,000	..	1,100,000
Tuna liver (Bluefin)	512,000–8,400,000	..	4,000,000
(Yellowfin)	4,800,000	..	1,000,000
Dairy products			
Butter	1,000–8,500	..	40–150
Cheese — Cheddar	3,500	0	..
cottage	110	..	..
cream	4,850	..	..
Parmesan	1,750	..	..

TABLE IV — *Continued*

	International Units per 100 Grams		
	A	B_1	D
Dairy products (cont'd)			
Cream (20%)	1,050	..	..
(40%)	2,400	..	..
Eggs — Hen's whole	1,400–4,500	25–60	15–175
yolks	1,700–8,800	60–125	45–500
Milk, Cow's fresh, whole, market	110–600	10–25	0–10
pasteurized	252	..	..
dried, whole	750–2,800	60–75	0
dried, skim	25	75–100	0
evaporated	775	..	..
condensed	775	..	..
malted	6,750	165–1,010	..
Human, whole	200–500	..	..
Vegetables			
Artichoke	392	28	..
Asparagus — green	980	100–135	..
bleached	0	40	..
Beans — string, fresh	580–1,400	20	..
lima, fresh	..	50–75	..
kidney, dried	0	125	..
navy, canned	75	40	..
Beets	0	5–10	..
Broccoli — flower	10,000	30	..
leaf	40,000	30	..
stem	2,000	..	..
Brussels sprouts	400	60	..
Cabbage — green	90	25–35	..
bleached	0	25–60	..
red, Chinese	2,800–7,000	10–15	..
Carrots — young	1,800–4,200	25	..
mature	7,700	20–60	..
Cauliflower	70	60–110	..
Celery — green	2,100	..	..
bleached	20	..	..
Chard	34,000	100–200	..
Collard	6,300	30	..
Corn — sweet	..	..	..
Cucumber	35	30	..
Dandelion greens	35,000	..	..
Eggplant	70	20	..
Escarole	28,000	..	..
Kale	42,000	25	..
Kohlrabi	..	15	..
Lentils	..	125	..
Lettuce — green	6,000	10–20	..
Iceberg	175	10–20	..
Romaine	750	..	..
Mushrooms	0	50	..
Okra	840	5	..

TABLE IV — *Continued*

	International Units Per 100 Grams		
	A	B_1	D
Vegetables (cont'd)			
Onions	0	10–40	..
Parsley	105,000	..	..
Parsnips	..	35	..
Peas — fresh	500–3,000	25–60	..
dried	1,750–8,500	70	..
Peppers — green	1,000–1,300	5–10	..
red (pimentos)	7,700	5–10	..
red (chili)	12,500	..	..
Potato	50	10–40	..
Pumpkin	..	10	..
Radish	..	16–60	..
Spinach	35,000	20–70	..
Squash — fresh	8,000–40,000	20–50	..
Hubbard	7,000	..	..
summer	400–1,400	175	..
Sweet potato	1,000–7,000	10–35	..
Tomato — mature, green	1,100	15	..
ripe, red	1,000–2,100	10–40	..
Turnips — white	0	15–40	..
yellow	22	15–40	..
Turnip greens	8,000	25–30	..
Watercress	..	30	..
Meat and meat products			
Bacon	22	90	..
Beef, lean	20–105	25–100	..
Chicken — dark meat	..	300	..
light meat	..	50–150	..
Ham	..	120–190	..
Heart — beef	..	75	..
pork	..	36	..
mutton	..	40	..
Kidney — beef	800–900	40	..
mutton	1,155	40	..
veal	1,150	40	..
pig	..	340	..
sheep	..	190	..
Liver — beef	10,000–41,000	35–150	15–45
calf	52,600–159,800	45	10
chicken	60,150	35–50	45
lamb	6,700–113,100	..	17
pork	11,200–36,700	45	44
Pork — lean	0	25–185	..
salt	0	25	..
Fish			
Clams	28	6	5
Cod	7	40	..
Haddock	7	20–45	..
Herring	50	15–45	..
Oysters	50–280	75	5

TABLE IV — *Continued*

	International Units Per 100 Grams		
	A	B_1	D
Fish (Continued)			
Roe	4,200	70	..
Salmon — canned	28,800	14–50	200–800
Sardines — tinned	..	30	..
Miscellaneous			
Yeast, Baker's, dried	0	130–450	0
Brewer's dried	..	1,200–6,000	0

TABLE V

ASCORBIC ACID CONTENT OF FOODSTUFFS

	MG. PER 100 GRAMS
Cereals and cereal products	
Oats, sprouted, 1 day	2.5
" 9 days	25.0
dry	0; 11
sprouted, 4 days	20
" 5 days	42
Fruits	
Apple, variety unspecified	0.1 to 20
Apricots	0.8 to 16
Avocado pear	7.0; 13.0
Banana	1.0 to 15
Blueberries	5 to 75
Cherry	3.1 to 17
Currant, black	136 to 220.0
" " , juice	132 to 178 *
" red	50
" " , juice	44 *
Fig	2.0
" white heart	2.4
" inner	4.9
" outer	5.7
Cape gooseberry	26.5; 49
Gooseberry	27.6 to 47.0
" juice	27.3 *
Grape, white and purple	1.0 to 40
" juice	3.0 *
Grapefruit, juice	26 to 65 *
Guava	56 to 299
Huckleberry	44
Lemon	14.0 to 66
" juice	25.8 to 70.9
" whole peel	100.0
Lime, juice	16.8 * to 62.5 *
" " , sweet	31.2 and 58.8 *
" " , unripe	68.1 *
" " , ripe, fresh	39.6 *
" " , " old	23.8 *
Loganberry	20.4 to 48.4
Melon	1.6 to 20
" musk	59
" cantaloupe	15; 53
" water	1.0 to 7.0
Mulberry	6.6 and 21
Nectarine	24
Olive	15
Orange, pulp	16 to 47
" " , valencia	38 to 53
" " , navel	52 to 98.5
" juice	22 to 89 *
" " , Californian	63.2 to 70.6 *
" " , Sunkist, old	10 *
" " , " new	51 *
" " , "	32.5 to 64.7 *

* Per 100 ml.

TABLE V — *Continued*

	Mg. per 100 Grams
Fruits (Cont'd)	
Peach	8.0
" Clingstone	8.2 and 11.0
" Elberta	4.5 and 5.7
" Hardstone	8.5
Pear, Bartlett	3.6
Persimmon	6.1 to 20
Pineapple	10.4 to 62.9
" juice	5.9 to 75.9
Plum	<0.5 to 4.6
" dried prune	1.0
Pomegranate	15.6
Prickly pear, juice	16 and 20 *
Quince	10 and 16
Raspberry	30.5
" juice	20.8 to 32.6 *
Strawberry	46 to 77.5
Tangerine	10 to 36
" juice	10 to 78 *
Nuts	
Almond	0; 19.3
Chestnut, Spanish	32.3; 50
Cocoanut	0.4 to 13.4
Hazelnut	15
Walnut	30
Vegetables	
Artichoke, Jerusalem	5.8
Asparagus	12
Bean, variety unspecified, dry	1.25
" lima	23 to 61
Beetroot, fresh	2.7, 10
Broccoli	68
Cabbage	20 to 124.2
" , early summer	55
" , late autumn	30
" , juice	22 * to 93 *
" , Chinese	40
Carrot	1.0 to 31
" juice	4.0 *; 23 *
" tops	95
Cauliflower	19 to 101
Celery, stalks	1.0 to 5.7
Cucumber	1.0 to 17.8
Dandelion, leaves	8 and 42
Endive	19
Fenugreek	136.7 and 140.7
Grass, fresh	68; 75.3
Horseradish	52 to 160
Iris, leaves	110
Kale	34
Kohlrabi	16 to 100
Leek	4 to 33
" tops	50
Lentil, dry	3.0
" sprouted	15.0

* Per 100 ml.

TABLE V — *Continued*

	Mg. per 100 Grams
Vegetables (Cont'd)	
Lettuce	<0.5 to 22
Lucerne or alfalfa, fresh	73 to 380
" " " young plants	310 to 350
" " " flowering stage	225
" " " dried	5.7 to 160
Mint	63
Mushroom	1.9
Mustard leaves	81
" seeds	44
Onion, white	2.6 to 15
" tops	84
" spring	14
Parsley, green	140 to 280.8
" , root	14.0
Parsnip, greens	210 to 216
" , root	5 to 40
Pea, fresh, green	4.8 to 40
" , chick, sprouted	14.2
" , cow, leaves	70 to 190
" , " , seeds	6.7
Peppers, chillies or pimiento, green, fresh	11.7 to 330
Potato	11 to 36
Pumpkin or squash	1.1 to 22
" " " leaves	10 to 160
Radish	20
" pink	16.9
" white	15.0
" leaves	43 to 113.8
Rape, leaves	33; 48.3
" stem	17.8
Rhubarb, stalks	5.9 to 36.7
" juice	27.8 *
" leaves	30
Soy bean leaves	33.3
Spinach	6 to 124
Sprouts, Brussels	71.8 to 146
Sweet corn, young cobs	3.5 to 9
Sweet potato	16 to 91
Tomato	12.9 to 39
" juice	9.2 to 40
Turnip, root	17 to 43.4
" juice	19.1 *
" greens	39 to 120
Water chestnut	3.2
Watercress	24 to 76
Yam	6.1
Dairy products	
Milk, cow's, raw	<0.3 to 2.89 *
" " " pasture fed	1.64 to 2.18 *
" " " stall fed	1.59 to 2.27 *
" colostrum	1.62 to 3.20 *
" curd from cow's milk	0.7 to 1.0 *
" goat's	0.9; 8.5 *
" human	1.2 to 10.8 *

* Per 100 ml.

TABLE V — *Continued*

	Mg. per 100 Grams
Eggs	
Hen's egg, white	0
" " yolk	0
Duck's egg, white	0.3
" " yolk	1.3
Fish, Mollusks and Crustacea	
Clams, small white	17
" yellow	9.3
Fish, air, liver	16.0 to 114.4
" " muscle	6.0 to 27.7
Mussels, fresh-water	0.82 to 1.96
Roe, mackerel, soft	4.0
" perch, hard	10.0
" salmon, hard	14.0
Shrimp	2.5
Meat	
Blood, rabbit	2.3
Brain, ox	11.4 to 26.0
" rabbit	15 to 26
Gizzard, chicken	1.7
Kidney, goat	18
" , pig	14
" , rabbit	3.5 to 10
Liver, calf	33
" , chicken	22
" , duck	13
" , goat	26; 73.4
" , ox	24 to 68
" , pig	12 to 38
" , rabbit	8 to 40
" , sheep	25 to 46
Lung, rabbit	10 to 24
Muscle, goat	7
" , rabbit, red	1.1 to 3.4
" , " , white	0.7 to 1.6
" , " , red and white	0.42 to 1.5
" , ox, stomach	15.8 to 20.1
Heart, goat	8
" ox	4.6
" , rabbit	1.1 to 3.0
Pancreas, ox	12.2
Suprarenal, calf	99
" , ox	76 to 185
" , pig	115
" , rabbit	183 to 216
" , sheep	133
Miscellaneous	
Beer	0.2 to 0.5 *
Sauerkraut, canned	8.2; 21
" , juice	25 *
Tea, fresh leaves, Russian	113 to 187

* Per 100 ml.

TABLE VI

RIBOFLAVIN CONTENT OF FOODSTUFFS

	MG. PER 100 GRAMS
Cereal and cereal products	
Barley, unsprouted	0.01
" , sprouted, 1 to 8 days	0.10 to 0.22
Oats, unsprouted	0.02
" , sprouted 2 to 5 days	0.03 to 0.06
Maize	0.01
Wheat, whole	0.06
" , germ	0.033
Fruits	
Apricot, dried	0.057
Banana	0.0075
Lemon, juice	0.003 *
" , peel and residue	0.006
Orange, juice	0.0069 to 0.0089 *
Vegetables	
Cabbage, white	0.05
Carrot	0.02
Grass, fresh	0.05; 0.142
" , dried	0.6
Lucerne, dried meal	0.7
Pea, unsprouted	0.08
" , sprouted 8 days	0.28
Potato	0.0075; 0.01
Spinach, fresh	0.057
" , dried	0.57
Tomato	0.05; 0.071
Animal products	
Milk, cow's whole	0.1 * to 0.3 *
" , whey	0.045 *
Eggs	
Hen's, yolk	0.5 to 0.6
" , white	0.4 to 0.5
" , " dried	1.41
Fish	
Cod, liver	0.053
Eel, whole	0.18
" , " , young	0.37
" , " , full grown male	0.51
" , blood and muscle	< 0.1
" , liver	0.75 to 1.0
Meat	
Ox, blood	0.0025 *
" , brain	0.1 to 0.5
" , kidney	0.8 to 2.0
" , liver	0.1 to 2.4
" , ovary	0.1 to 0.5
" , spleen	0.05 to 0.1
" , suprarenal	0.5 to 1.0
" , heart	0.553
" , lung	0.89
" , muscle	0
Miscellaneous	
Beer, dark	0.029 *
Honey, pine	0.106
Wine, white	0.0081 to 0.0125 *
Yeast, dried, brewers'	1.8 to 3.0
" , " , bakers'	2.5; 3.6

* Per 100 ml.

Illustrations of the Effects of Specific Deficiencies

Fig. 1. Uterus of guinea pig deficient in vitamin A. The insert shows the columnar epithelium which lines the normal uterus. The contrast is striking, since the normal columnar epithelium has become transformed into keratinized cells. (Figure kindly furnished by Dr. S. B. Wolbach; insert by Dr. R. H. Follis, Jr.)

FIG. 2. Cross section of the kidney of a rat deficient in vitamin A. Note that the pelvis is filled with keratinized desquamated epithelium. (Courtesy of Dr. S. B. Wolbach.)

FIG. 3. Rib from an 8 month old child with scurvy. Note the increase of calcified trabeculae which are easily fractured and consequently do not project vertically as in the normal state (compare with insert—lower magnification). There are hemorrhages into the marrow spaces. (Courtesy of Dr. E. A. Park.)

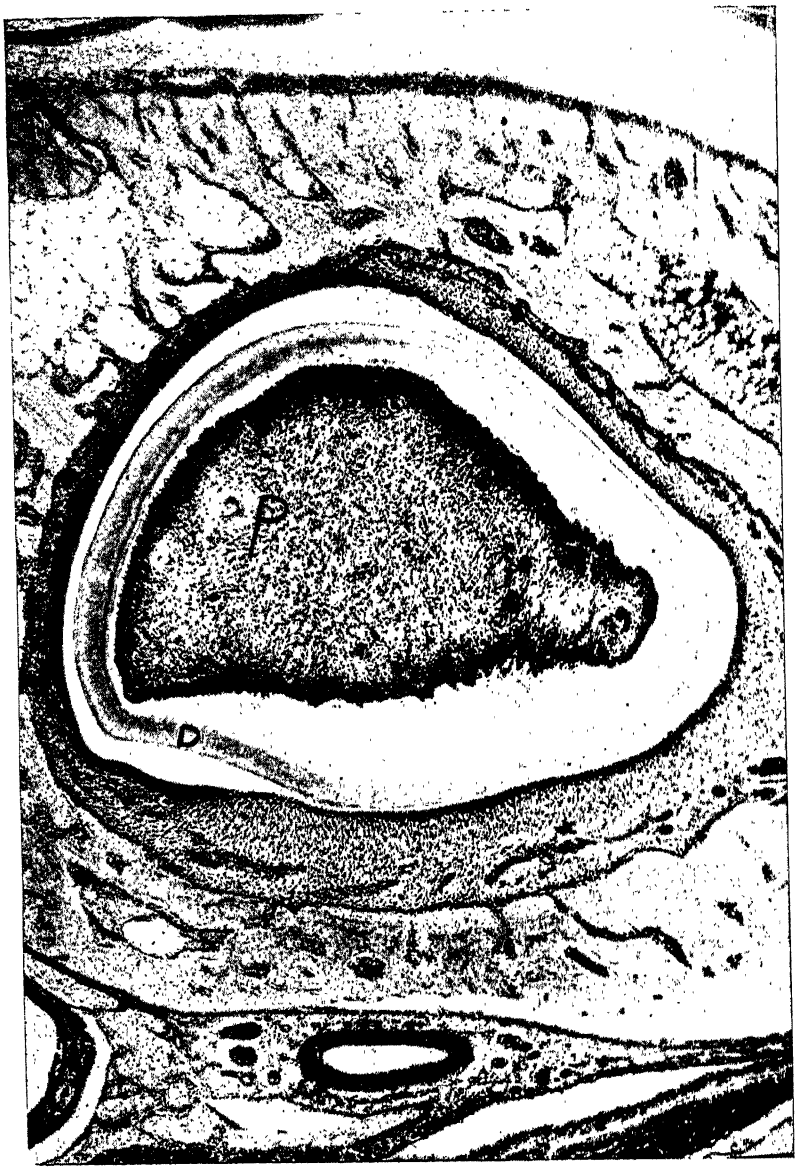

FIG. 4. Transverse section of incisor of guinea pig deprived of ascorbic acid for 12 days. Note the marked atrophy of the odontoblastic layer (O) and the retraction of pulp tissue (P) from the dentin (D). Part of the apparent pulp shrinkage is artefact. (Courtesy of Dr. S. B. Wolbach.)

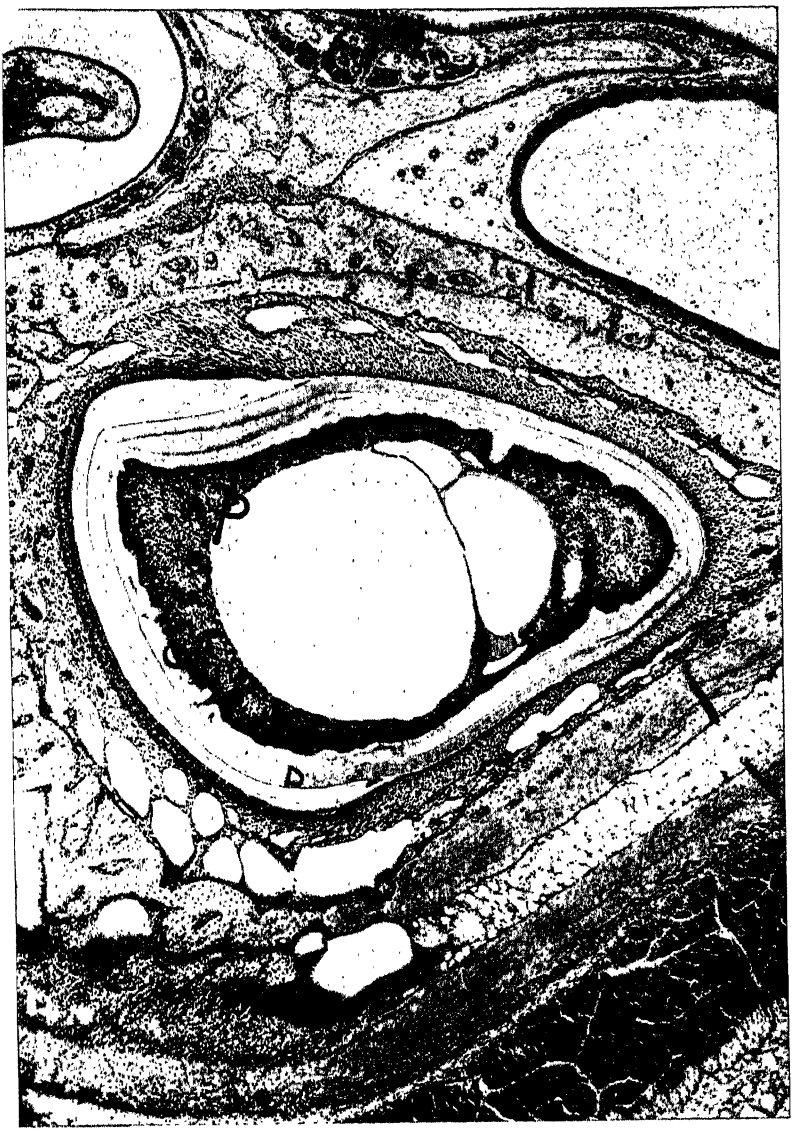

FIG. 5. Transverse section of incisor of scorbutic guinea pig 6 days after restoring ascorbic acid to the diet. Note that the odontoblastic layer (O) is contiguous with the dentin (D) and that newly formed dentin is present. The large cavity in the pulp (P) is artefact due to the accumulation of air during the washing process. (Courtesy of Dr. S. B. Wolbach.)

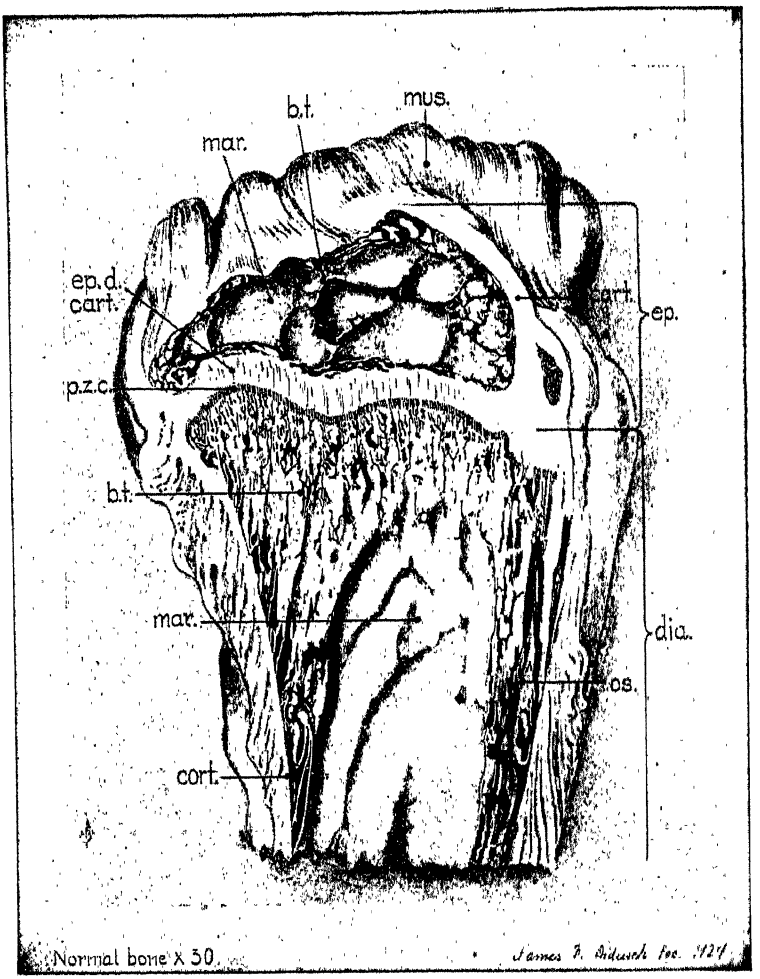

FIG. 6. Normal bone of a rat (proximal end of tibia). This is to be contrasted with Fig. 7, which is a ricketic bone, and also with Fig. 8, which shows advanced healing. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

Legend: b.t.—bone trabeculae; cart.—cartilage; cort.—cortex; dia.—diaphysis; ep.—epiphysis; ep.d. cart.—epiphyseal diaphyseal cartilage; mar.—marrow; mus.—muscle; p.z.c.—proliferative zone of cartilage; os.—osteoid; and met.—metaphysis.

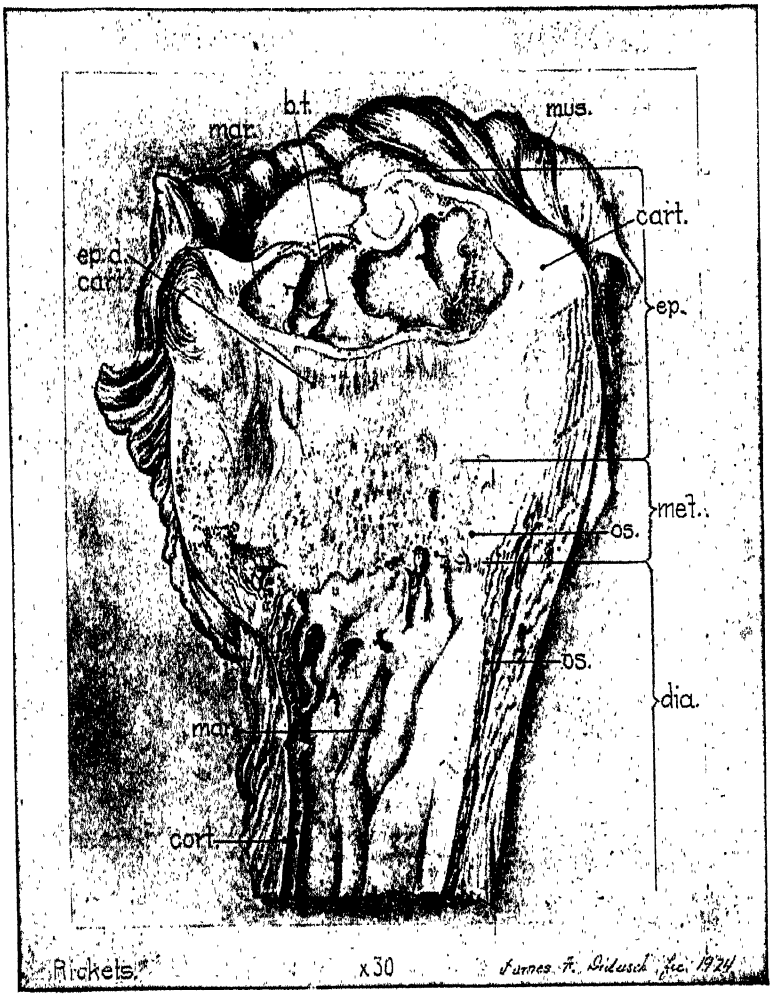

FIG. 7. Bone of a ricketic rat (proximal end of tibia) which shows the florid rickets produced by diet 3143 (high—Ca moderate—P). Note the enormous band of cartilage cells, the wide metaphysis which is absent from normal bone, and the excessive amount of osteoid tissue. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

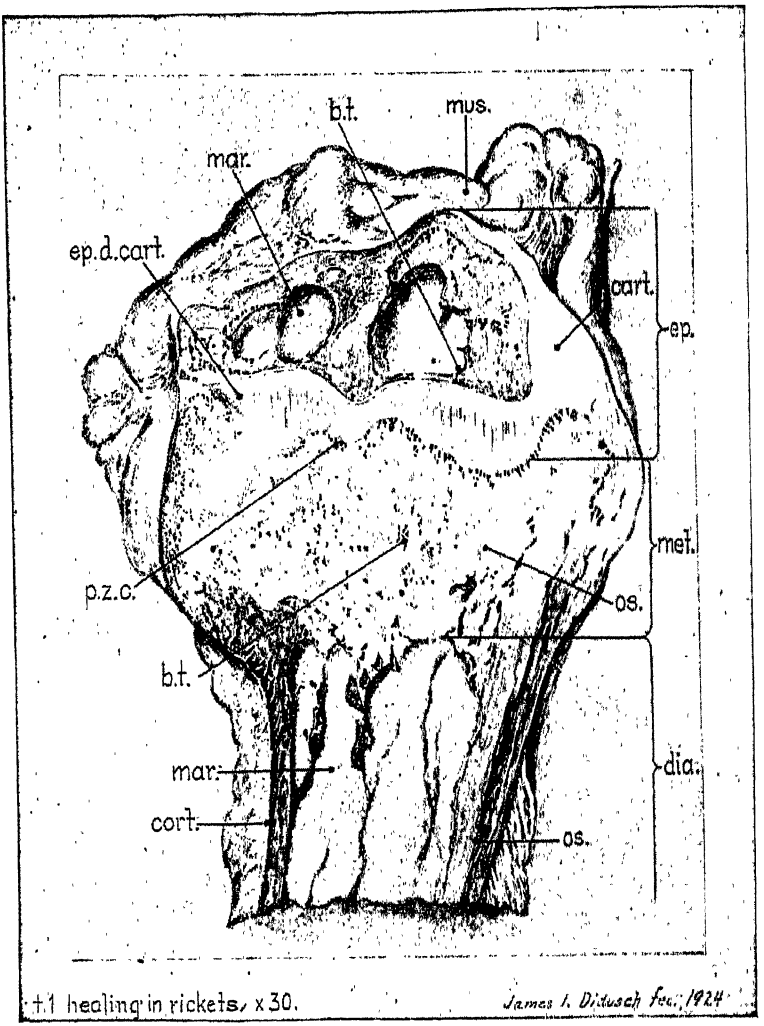

FIG. 8. First stage of healing of a ricketic bone. When vitamin D is administered to a rat whose bones are like that shown in Fig. 7 calcification occurs as shown at p.z.c. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

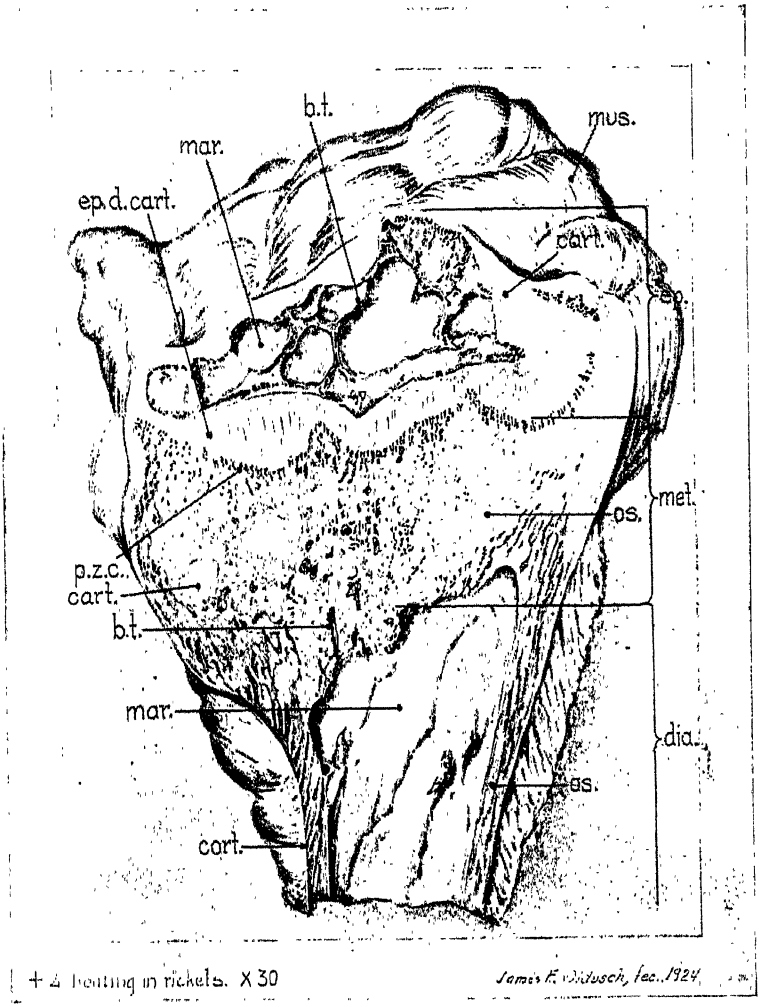

FIG. 9. Later stage of healing of a ricketic bone than that shown in Fig. 8. The calcification is shown in black at p.z.c. When the administration of vitamin D is continued the bone returns to the normal structure shown in Fig. 6. If the rickets is severe the bone calcifies with deformities. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

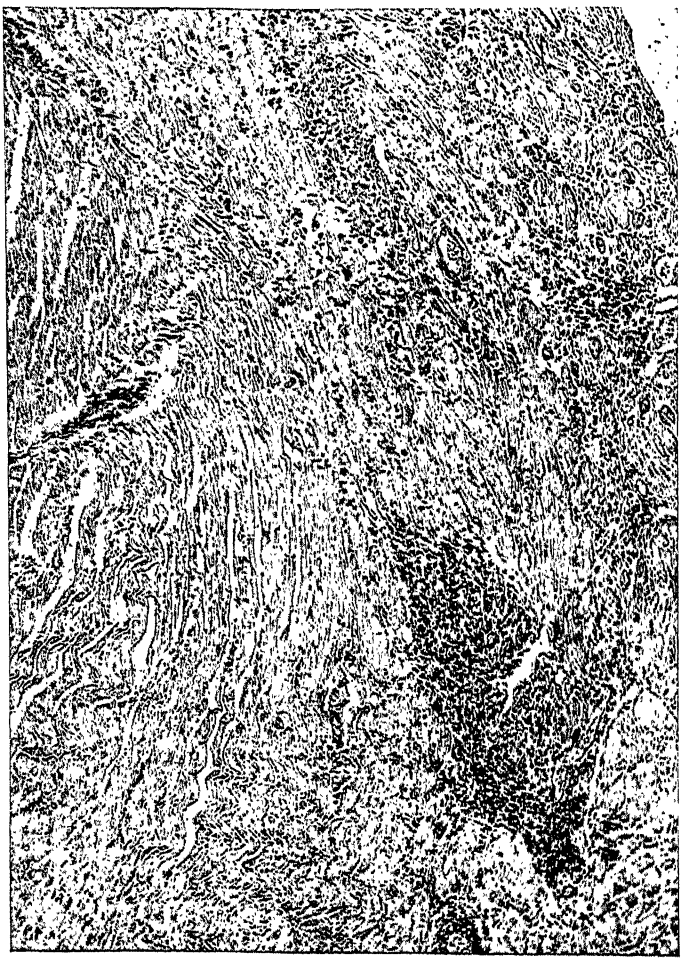

FIG. 10. Heart tissue of rat on a potassium-low diet. Note the foci of leucocytic infiltration where the muscle fibers have become necrotic. Subsequently these areas become replaced by scar tissue. (Orent-Keiles, Follis and McCollum.)

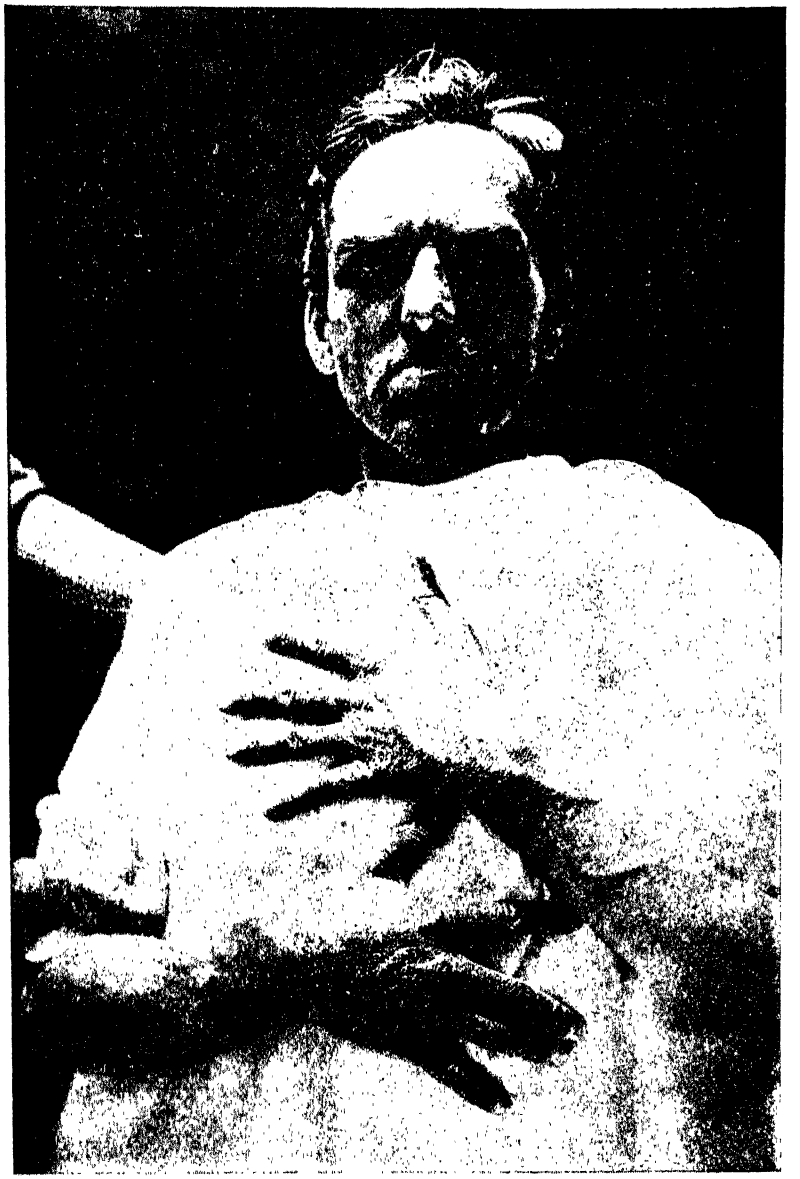

FIG. 11. Typical skin lesions on back of hands and fingers of pellagrin. It appears that a deficiency of nicotinic acid is related to the occurrence of pellagra. (Courtesy of Dr. MacNeal.)

FIG. 12. Paralysis in hind legs of female rat restricted to a vitamin E-deficient diet for approximately two years. (Courtesy of Drs. G. O. Burr, W. R. Brown and R. L. Moseley.)

FIG. 13. Gross effect of nicotinic acid deficiency in the pig. The basal diet was ground white maize 77·5, peameal 10·5, purified casein 6·5, cod liver oil 3, and salt mixture 2·5. (Biochem. J., 32, 10, 1938; courtesy of The Biochemical Journal and Dr. H. Chick.)

FIG. 14. Fig No. 18 (Fig. 13) three months after receiving 60 mg. of nicotinic acid daily with the basal ration. Note that all symptoms of deficiency have disappeared. (Biochem. J., 32, 10, 1938; courtesy of The Biochemical Journal and Dr. H. Chick.)

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