THE PRESENT STATUS OF OUR KNOWLEDGE OF THE VITAMINS*

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INTRODUCTION

It is important that brief consideration be given to the background from which the vitamin hypothesis has evolved, not only for the information thus conveyed but also because such an account will clearly illustrate the evolution of one phase of the science of nutrition and will indicate the current tendency of thought in this field. It is, of course, difficult to determine the precise beginning of the conception of the accessory food factors. Throughout the earlier history of nutritional physiology one finds frequent references to experimental observations which, from the vantage point of our present state of knowledge, could be interpreted to be the beginnings of the development of the vitamin hypothesis. Significant and suggestive as were these occasional observations, it must be admitted that not until the early part of the present century were investigations systematically planned for the purpose of demonstrating the existence of accessory food substances and of elucidating their various properties. In this connection one should not lose sight of the fact that the physical and financial equipment and the progressive point of view of the agricultural experiment stations and the scientific foundations, as well as the large universities, were factors of great importance in furthering the extremely rapid expansion of our knowledge of nutrition in general and, with it, of the vitamins.

The discovery of the accessory food factors was a natural outgrowth of a gradual change in the conception of the constitution of a perfect food. From the time of Hippocrates until as late as 1830 it was generally believed that all of the various animal and vegetable products which serve as food contained one thing in common, namely, the so-called universal aliment. The organs and tissues

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of the body abstracted that which maintained their composition constant and permitted their normal functions, irrespective of the wide variety of forms in which food was offered; this was proof of the existence of a substance common to all foods, the universal aliment.

William Beaumont, the pioneer American physiologist, in his book published in 1833, subscribed to this idea. But the next year it was suggested that a perfect food must contain some fat, carbohydrate and protein. From this time until the end of the century the conception of food changed little, save that under the influence of Liebig and Voit and their pupils, the inorganic salts were raised to an important place in nutrition. Thus it came to be accepted among dietitians and animal husbandrymen that the chemical analysis of a food was a practical and reliable index of its nutritive value. Although almost throughout the nineteenth century there were occasional isolated statements to the effect that experience indicated that the formula of a food could scarcely be simplified to fat, carbohydrate, protein, salts and water, it was not until the first decade of the present century that Pekelharing[6] and, independently, Hopkins[4] suggested that unrecognized substances in plant and animal tissues were essential constituents of the diet. Within six years, experiments of far-reaching importance had already demonstrated that nutritive success required the presence of mere traces of hitherto unidentified factors in the food. It was into a philosophical atmosphere motivated by this point of view, that the vitamin hypothesis was born.

From the foregoing discussion it is apparent that, in according the vitamins an indispensable place in the diet, we do not consider them valuable because they furnish energy; they are not in the class of fats, carbohydrates and proteins in this respect. On the contrary, we might roughly consider them along with the hormones and those inorganic elements like iodine, copper and manganese which are required only in fractions of a milligram. These might all be looked upon as stimulators or conditioners of cell metabolism, perhaps a type of biochemical catalyst.

The mass of pertinent experimental evidence at present available forces the conclusion that the conception of the vitamins is not a fad; indeed, it is rapidly ceasing to be looked upon even as a biochemical novelty but is rather accepted as one of the fundamental principles of nutrition. As will be brought out in the subsequent discussion, progress has reached the point where the chemical

formulas of some of the accessory food factors are known with reasonable certainty.

A further word about systematic nomenclature. In the early days of their discovery, the vitamins were named according to their solubilities together with a designating letter, viz., fat-soluble A or water-soluble B. There subsequently developed a system of naming which employed what appeared then to be the outstanding pathological symptom occurring in the absence of the appropriate vitamin. Further work has demonstrated that this system is not desirable, although one frequently encounters the double names such as vitamin C, the antiscorbutic vitamin; or vitamin A, the anti-ophthalmic vitamin. The accepted system of naming the accessory food factors at present employs only letters. The meanings of these will be made clear in the discussions to follow.

VITAMIN C

DISCOVERY

Scurvy was one of the earliest known of what we now recognize as deficiency diseases. Until the beginning of the nineteenth century scurvy inevitably appeared when, by force of circumstances, groups of people were subjected to a rather strictly limited food supply. Thus, military history repeatedly records outbreaks of this disease among troops on long expeditions; scarcely a sea voyage of discovery or of commerce was entirely free from it. Scurvy frequently appeared after crop failures, notably in countries that depended upon the potato as a staple food, and inmates of institutions were frequently victims of the disease when, by necessity or by design, the food supply was restricted. After 1795, when a ration of lemon juice was allowed to all sailors in the British navy, the incidence of scurvy showed a marked decrease, and the same can be said of the disease among the sailors of the merchant marine after the tardy adoption by the British Board of Trade of a similar rule in 1865. The essential identity of infantile scurvy with that occurring in adults was established in 1883. The disease in infants is largely a disorder of modern times, and appears to have increased with the introduction of artificial feeding methods and the heat treatment of the milk which usually forms a part of the formula. It should be stated here that during the past twenty-five years, with the excep-

tion of the period of the World War, infantile scurvy has steadily decreased as the discoveries from animal experiments have been applied to the nutrition of babies.

It was early realized that the cause of scurvy had some connection with the absence of certain constituents from the diet, and fresh vegetables, fruit and fresh meat have been traditionally looked upon as protective foods. History abounds in fascinating stories of the striking cures brought about by the administration of various fresh plant tissues to sufferers from scurvy. However, though this long experience with scurvy provided a background on which to build, the crystallization of the conception of a responsible accessory food factor in connection with scurvy dates from the experimental production of this disease in the guinea pig by Holst and Froelich[2] in 1907.* Before this time scurvy had been attributed to acidosis, to a lack of potassium, to a deficiency of citric acid, and to infection. In spite of the accumulated evidence the conception of an indispensable food factor of hitherto unknown nature had apparently not received general credence. The studies of Eijkman in the 1890's set the stage, as it were, for the proposal of the vitamin hypothesis. Holst and Froelich[3] determined the conditions necessary for the production of experimental scurvy, investigated the curative properties of certain green foods, and concluded that the disease was caused by the absence of an unidentified substance occurring in these foods.

PATHOLOGY

There appear to be two fundamental lesions in scurvy, namely, localized hemorrhage and fragility of the bones. The cementing substance between the endothelial cells of the capillaries is deficient so that the blood vessels are weakened. The result is that blood escapes into various tissues and cavities. Hemorrhage into the joints of the knees and wrists of guinea pigs occurs early, and tenderness and swelling are among the first symptoms of scurvy in these animals. Blood escapes into the connective tissue sheaths of the muscles and under the skin so that there are discolored areas on the extremities of human patients with scurvy, and these seem more pronounced where there have been bruises, as on the shins. Hemorrhage under the periosteum is commonly seen, the hematomata later

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* Theobald Smith[9] in 1895 recorded the production of scurvy in guinea pigs, but there is little to indicate that he realized the significance of this observation.

organizing and becoming more or less ossified. The gums become swollen, hemorrhagic and ulcerated, the teeth loosen in their sockets and, in the guinea pig, the jaw bone is markedly eroded.

Enlargement of the adrenal glands and early hemorrhage, infiltration, and degeneration of the cells of the cortex have long been noted in scurvy. Doubtless these facts will receive renewed attention in view of the very recent work on the chemical nature of vitamin C.

Part of the swelling of the wrists and knee-joints and at the costochondral junctions in the ribs in scurvy is due to changes in the bone itself. There is apparently a deficient production of organic matrix so that mineralization cannot occur. Instead of the regular columns of cells in the ossifying cartilage and in place of full well-defined trabeculae, there is marked disintegration and disorder in the cartilage with small misshapen trabeculae and fractures of the rarified bone so that the epiphyses of the long bones often separate and may be forced down into the debris; frequently an organized hematoma under the surrounding periosteum acts as a natural splint.

Structural changes in the teeth also occur very early in the disease and the effort has recently been made not only to use this dental response as a criterion in the assay for vitamin C, but also to relate on this basis a lack of vitamin C to dental caries. From the available evidence it appears that in the absence of vitamin C, the normal formation of intercellular cementing substance, perhaps, mucoid, is prevented, with the resulting structural changes in the capillary walls and in the bones.

In the guinea pig the symptoms of scurvy are usually a loss in body weight, lethargy, a tendency to remove the weight from the feet either by holding up one leg or, in advanced stages, by lying on the belly and extending the legs. The joints are tender and often hemorrhages can be seen through the skin on the inside of the thighs. In human patients the skin is sallow, there are pains in the joints, disinclination to move, general malaise, and hemorrhages in spots on the extremities, especially where bruises have occurred. The gums are also affected showing puffiness, hemorrhage and ulceration.

It has been recognized that in infants there may exist a subacute form of scurvy during which growth is retarded and there is irritability, restlessness and even physical discomfort. In the more advanced stages the symptoms are more or less typical of those in adults.

Since the establishment of the vitamin-deficiency theory of the etiology of scurvy, the incidence has dropped steadily. During the World War there was an increase both among infants and adults but since then the disease has decreased until, with the exception of infrequent epidemics, scurvy is more or less of a rarity. However, under the present conditions of economic stress, it would not be surprising if this disease manifested itself again among those groups whose food is severely restricted and who are without guidance in applying available funds to the selection of an adequate diet.

Quantitative Determination and Distribution of Vitamin C

The only method thus far found satisfactory for the quantitative estimation of vitamin C is that of the bio-assay. It has been found that not all animals are susceptible to scurvy; man, monkeys, and guinea pigs will develop it whereas the rat, dog, chicken, and calf apparently are immune to the disease. The guinea pig is universally employed in the determination of vitamin C because of its small size, of its susceptibility, and because scurvy in this species closely resembles the disease as it occurs in man. Guinea pigs of definite weight and age are given an experimental ration free from vitamin C. One such ration in wide use consists of rolled oats, bran, heated skim-milk powder, butter fat and sodium chloride. On such a ration alone the signs of scurvy appear in less than two weeks. The material to be assayed is added to the ration in different amounts for different guinea pigs at the beginning of the experiment, and the least quantity of material sufficient to protect the experimental animals from scurvy over a period of 70 to 90 days is said to contain one unit of vitamin C. The criteria for the presence of scurvy are changes in body weight, changes in the jaw, teeth, ribs and joints, and hemorrhages in the ribs, intestine, joints and muscles.

On the basis of this method of testing it has been found that 3 cc. of tomato juice, 1.5 cc. of orange or lemon juice, and 5 cc. of lime juice each contain one unit of vitamin C. The remarkably high potency of orange and lemon juice is worthy of comment; indeed the international unit of vitamin C, recommended to the Permanent Commission on Biological Standardization of Health Organization of the League of Nations is the antiscorbutic value of 0.1 cc. of fresh lemon juice.

Data on the distribution of vitamin C emphasize at the outset

the wide distribution of this factor in plant tissue and the paucity of it in animal foods. The citrus fruits, the tomato, and certain of the leafy vegetables are the richest sources of vitamin C. In general, it has been found that the leaves of vegetables contain more of the antiscorbutic factor than do the stems, and these, in turn, contain more than the roots and tubers. A recent study has brought out the striking fact that the vitamin C content of apples differs with the variety, one variety being approximately as potent as orange juice whereas apples usually are regarded as only one-sixth as rich. Furthermore, the pulp immediately under the skin has more vitamin C than that nearer the core. From the Table it is also apparent that certain seeds before drying, like corn and peas, as well as seed-pods like string-beans are good sources of the antiscorbutic factor. Dried legumes and grains are poor as antiscorbutic foods until they are germinated, when the vitamin C content is greatly increased.

SOURCES OF VITAMIN C IN NATURE

Most Potent	Excellent	Good
Lemon	Citron	Apple*
Orange	Lime	Grapefruit
	Strawberry	Banana
Tomato	Pineapple	Pear
Cabbage	Peach	Grape
Spinach	Raspberry	Watermelon
Lettuce	Tangerine	
Onion		
	Rhubarb	Beet
	Celery	Carrot
	Turnip	Cauliflower
		Cucumber
		Potato
		Sweet corn
		String-beans (cooked)
		Peas (cooked)
		Spinach (cooked)
		Pepper (green)

As a class animal foods are practically devoid of vitamin C. Milk usually contains vitamin C but the amount is variable.

A word should be added on the value of milk, as a source of vitamin C, inasmuch as this food is such an important part of almost every dietary. The antiscorbutic value of milk appears to vary with the amount of vitamin C in the feed of the animal producing the milk. In general, milk from cows which are entirely stall-fed can

* One variety of apple has been found to equal the lemon as a source of vitamin C.

be expected to be less rich in vitamin C than milk from cows on pasture. One is forced to conclude, therefore, that milk cannot be seriously considered as a reliable source of the antiscorbutic factor.

CHEMICAL NATURE OF VITAMIN C

The newer contributions to our knowledge of vitamin C have been concerned largely with the chemical behavior of this factor, the possibility of obtaining concentrates of it and, very recently, with the efforts to identify it and to isolate it in crystalline form. Experience has shown that, as it occurs in nature, vitamin C is extremely labile; indeed, Holst and Froelich commented on its ready destruction by heat. Subsequent studies have indicated, however, that the heat lability is conditioned by the hydrogen ion concentration of the system as well as by the presence of oxygen. Other conditions remaining the same, vitamin C is much more rapidly destroyed by heating at a neutral reaction than when the system is acid; the natural acidity of tomato juice is an important factor in conserving the antiscorbutic potency of ordinary canned tomatoes. Alkalinity favors rapid destruction of vitamin C even at low temperatures. That oxidation is the important factor in the destruction of vitamin C at 100° C. is shown by the complete loss at either acid or alkaline reaction when oxygen is bubbled through the liquid, whereas decitrated lemon juice showed no loss of potency after boiling for two hours in an atmosphere of carbon dioxide. Even alkaline decitrated lemon juice shows little loss of vitamin C at room temperature if oxygen is excluded. The antiscorbutic factor has retained its potency for as long as five months in the presence of acid and when oxygen was excluded.

The efforts to concentrate vitamin C have been applied largely to decitrated lemon juice. In one investigation[11], after removing the sugar by fermentation, precipitating inactive material with alcohol and concentrating, the active principle was precipitated with basic lead acetate. Removal of the lead left a concentrate very low in total solids and with little loss of antiscorbutic activity. This material exhibited reducing properties, though whether this was due to the vitamin C or to some contaminating compound is not certain. The removal of this reducing substance resulted in a decreased activity of the vitamin preparation and it has been looked upon as a protector of the vitamin. The active concentrate contained a very small amount of nitrogen. Dialysis experiments indicated that the

antiscorbutic factor had molecular dimensions not widely different from those of the hexose sugars.

Late in December of 1931 it was reported[7] from Norway that vitamin C was a derivative of narcotine, one of the alkaloids of opium. It was alleged that the compound was produced from narcotine in the ripening process in apples and citrus fruits. Scurvy was prevented by doses of 20 to 30 gamma per diem, but life was not prolonged. However, other investigators in the same field have not been able to duplicate these results.

In this country another series[8] of studies on decitrated lemon juice has yielded a concentrate of vitamin C 10,000 times more potent than the original juice, and with only 0.09 milligram total solids per cubic centimeter of the original lemon juice. This concentrate was made by decitrating lemon juice with an excess of neutral lead acetate and precipitating the lead complex containing practically all of the vitamin C with ammonium hydroxide at pH 7.4 to 7.6. There was no indication of more than one active factor involved and this appeared to be acidic with a strong reducing activity.

Experiments[5] reported this year describe the further fractionation of this concentrate by precipitation from an acetone solution with petroleum ether and subsequent solution in anhydrous ethyl acetate. Crystals were obtained from the ethyl acetate solution which were active in curing experimental scurvy in daily doses of 0.5 milligrams. Recrystallization did not change the antiscorbutic potency of this preparation. The crystals had an optical rotation of $\left[\alpha\right]_{20}^{D} = +25°$ and a melting-point of 183-185° C. They appear to be identical with the hexuronic acid ($C_6H_8O_6$) isolated from bovine adrenal glands, which had previously been shown to protect a guinea pig from scurvy for 90 days in daily doses of 1.0 mgm.[10] This hexuronic acid[1] is probably a 6-carboxylic acid of a ketohexose not related to d-fructose or to the ketose corresponding to d-galactose. It is readily oxidized even with atmospheric oxygen in the presence of alkali and a trace of copper, and forms a p-bromphenyl osazone. The following formula shows the relative simplicity of the compound and its possible structure:

$$\overset{\displaystyle \lceil \text{———O———} \rceil}{\text{CO·CHOH·CHOH·CH·CO·CH}_2\text{OH}}$$
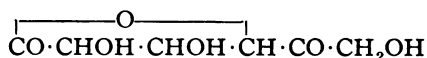
Additional corroborative experiments have not yet been reported from other laboratories.

Little is known of the physiological action of vitamin C. In the light of the foregoing discussion of hexuronic acid it is of interest to point out that the characteristic lesions in scurvy have been attributed in the final analysis to a defect in the intercellular cement substance in the walls of the blood vessels and in the bones. Despite a plentiful supply of calcium and phosphorus little mineralization takes place in susceptible species in the absence of vitamin C; apparently the organic pattern for the bone is defective. The organic matrix of the connective tissues includes mucoid which yields on hydrolysis, glucuronic acid among other compounds. Whether or not the fraction of a milligram of hexuronic acid said to satisfy the daily requirement of the guinea pig for vitamin C contributes directly to the building up of mucoid in the growing body is difficult to say, although this seems somewhat doubtful. The common enlargement of the adrenal glands in scorbutic animals and the recent discovery of hexuronic acid in the normal glands, might indicate that vitamin C acts as a stimulant to endocrine function, an argument somewhat more in line with the current conception of vitamin function.

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VITAMIN B

DISCOVERY

The discovery of vitamin B came about chiefly through the efforts of numerous workers to find the cause of the disease beriberi. This condition is of wide occurrence, especially among the people of the Far East whose staple food is white or polished rice. Beriberi has frequently appeared in epidemic proportions in China, Japan, the Philippine Islands, the Federated Malay States, and in South America. Between 1878 and 1883 the average number of cases treated in the Japanese navy was 323 per 1,000 per year. In this disease there is a degeneration of the peripheral nerves, which soon results in a paralysis particularly of the lower limbs. This paralysis may be associated with marked atrophy of the muscles and definite evidences of inanition, a condition frequently spoken of as the "dry" form of beriberi. In the "wet" form of this disease, edema is a prominent feature of the symptom complex. Other symptoms of interest are a characteristic cardiac hypertrophy and gastro-intestinal disturbances.

During the last decade of the nineteenth century the Dutch physician, Eijkman[4], living in Java, noticed a beriberi-like condition in his barnyard fowls. He made numerous experiments in an endeavor to determine the cause of this condition, and for many years believed it to be due to the presence of a toxin in the food. The work of Chamberlain, Vedder and others[11], between 1909 and 1912, led to the view that beriberi is not due to a toxin in the rice but rather to the absence from the diet of some substance of vital importance. In 1911 Funk[5] reported the preparation from rice-bran and yeast of chemical fractions possessing great potency in the cure of the experimental beriberi which is so easily produced in pigeons. He believed that this hypothetical substance was an amine of vital importance to the body, and therefore proposed as a name for it the term *vitamine*. It should be emphasized here, that the proof of this idea, that a disease results from absence of some dietary essential, such as Funk's vitamine, involves the demonstration that other animals receiving precisely the same experimental ration together with a small but adequate amount of the active chemical fraction, fail over a most extended period to develop the disease.

When it was found that other substances are essential dietary

factors, fat-soluble vitamin A, for example, the term vitamine came
to be used in a generic sense. McCollum[7] introduced the use of
letters to indicate different members of the group. Funk's term
vitamine suggests to an organic chemist that the compound contains
nitrogen. There was evidence that not all of these dietary essentials
contain this element. Drummond[3], therefore, suggested that the
final letter "e" of this term be dropped, thus giving the word *vitamin*
as the group name, a term which carries no connotations with
respect to the elements present in the dietary essential in question.
When the chemical structures of the various vitamins are elucidated,
new names may be devised based upon the molecular structures of
the compounds.

PHYSIOLOGY

Following the observations of Funk it has been shown by many
workers that the antineuritic or antiberiberi vitamin B not only
prevents or cures the symptoms of human and experimental beriberi,
but corrects the loss of appetite which develops in man and animals
subsisting on a diet lacking this factor. Certain gastro-intestinal
symptoms which frequently characterize the picture of beriberi are
also relieved by administration of this vitamin. The "hyper-
trophied" heart rapidly returns to normal size when the antiberi-
beri factor is given[1]. Numerous students of nutrition have also
shown that this vitamin is required for growth.

The loss of appetite characteristic of lack of vitamin B is worthy
of special comment, because its occurrence is too often unrealized
and because it has been shown to occur in man as a hidden complica-
tion in different diseases. Perhaps the best way to present this
anorexia for consideration is to describe a typical experiment on
the dog.

The animal is fed daily an appropriate amount of the experi-
mental ration which is complete so far as known except for the anti-
neuritic vitamin. For many days, usually over a period of about
three weeks, the dog eats promptly all the food offered. On the
day when the anorexia first appears only a small amount of the
food is eaten over a period of several hours. This failure to eat
all the food offered is exhibited for several days. As a result of
this failure to eat enough food the animal loses weight. The dog
is then given a substance such as beef extract, which does not contain
vitamin B, but which is believed by many physicians to be valuable in

combatting anorexia. The material is administered by stomach tube in order to avoid changing the taste of the standard ration. On the following day the animal again exhibits anorexia. Then the dog is given a liberal quantity of some substance believed, because of rat tests, to contain large amounts of the antineuritic vitamin. The following morning all of the food offered to the animal is eaten voluntarily within a few minutes, and this behavior is observed over several days. It has been shown that this effect is quantitative, that is to say, the number of days over which the appetite is maintained is related to the size of the animal and the amount of the vitamin-containing substance given. This relationship has been observed in the mouse, rat, pigeon and dog, and because of its quantitative nature, when observed under certain appropriate conditions, can be made the basis for estimating the amount of vitamin present in foods.

The antiberiberi vitamin is also required for growth. Upon this fact have been based many of the tests of foods for their content of this dietary factor. The method used here consists in feeding the young rat on a diet adequate except with respect to this vitamin. Instead of growing normally and showing the usual gain in body weight over different periods of days, the rat gains in weight for only a short period, and then shows a definite decline. When the animal receives material containing the missing vitamin, the food under examination, for example, there is prompt recovery and resumption of growth. By comparison of the effects obtained with different amounts of different foods, it becomes possible by this method to list foods in the order of their content of this vitamin.

Numerous tables[8, 12] have been published presenting the vitamin B ratings of different foods. In general, it appears that yeasts are excellent sources of this vitamin, but that yeasts also may vary considerably in this respect. Wheat germ is also very rich. The whole grains and the legumes are good sources of this factor. Contrary to popular opinion, the fruits and vegetables as a group are rather low in content of vitamin B. The reason for this doubtless lies in the fact that in many of the tests of these foods, the dried product was used; when the water normally present in the fresh fruit and vegetable is taken into account, the rating of the natural food becomes quite low.

The relatively high value of liver, heart, and pig muscle (pork) is in marked contrast to the low value of beef muscle. Oysters are a good source as compared with clams which are very poor indeed. Milk has a rather low rating; when the solids of milk and eggs are compared, these foods are found to be about equal.

It is not possible to review the numerous suggestions that have been made concerning the function of vitamin B. Let it suffice to point out that some have been made on the assumption that this substance is required by every cell of the body; others have attributed to this vitamin some rôle in the functioning of special tissues. Vitamin B has been suggested as required in (1) the metabolism of foodstuffs, particularly carbohydrates; (2) oxidation-reduction mechanisms; (3) immunity reactions; (4) metabolism of cell nuclei; (5) the body's supply of special hormones; and (6) enzymes. There is considerable evidence to support the view that the antineuritic vitamin is an essential constituent of cell nuclei. In spite of a tremendous amount of research in this general field it must be admitted that the question as to the exact function of this vitamin remains unsolved.

Vitamin B is of interest to the medical clinic in several ways[2]. Beriberi, which represents an extreme deficiency of this factor, has already been touched upon. The newer possibilities of interest center around the anorexia which occurs when there is a moderate shortage of this dietary factor. Eddy and Roper, Hoobler, and Dennett have shown that certain undernourished infants owe their loss of interest in food to lack of this dietary essential. Newburgh, at the University of Michigan, had occasion to treat a young woman whose voluntary food intake had reached a very low level, represented by about 500 calories per day; upon receiving liberal amounts of a good source of vitamin B, this girl voluntarily increased her daily food intake to reach a level of about 2,000 calories. An extreme case of tropical sprue with the patient in coma was injected with liberal amounts of a concentrate of antineuritic vitamin B, prepared by Block of our laboratory, with a result which may well be termed miraculous. A case of pellagra in the New Haven Hospital was also treated with Block's concentrate with promising results. These findings, in cases in which refusal to eat constitutes the outstanding problem, suggest numerous other clinical conditions in which trials of vitamin B therapy appear promising.

Vitamin B Complex

What was formerly called vitamin B has recently been shown to consist of at least two physiologically active substances, one the antiberiberi factor, and the other the antidermatitis or "antipellagra" (?) substance. Both of these factors are water-soluble and appear to be closely associated in nature. The English investigators have adopted the custom of naming these factors B_1, the antineuritic substance, and B_2, the antidermatitis agent; many of the American workers use the term vitamins B and G, respectively, for these dietary essentials. In view of these developments many of the earlier experiments on so-called vitamin B must be repeated in order to ascertain the part played by each member of what has come to be called the vitamin B complex.

A deficiency of vitamin B_2 (or G) results in cessation of growth and the development of a dermatitis similar to that observed in human pellagra. One of the most recent contributions to this subject is the observation that eye cataract develops in young rats allowed to subsist for long periods on a diet lacking this factor. It is pertinent to the present discussion to point out that much more work needs to be done in relation to the problem of the production of dermatitis by means of faulty diet. In all probability human pellagra is a complicated condition[9]. It is not to be presumed that many people live on exactly the same diet as do a number of rats in a nutritional investigation.

Examination of foods for the presence of vitamin B_2 (or G) indicates that yeast, muscle, milk and eggs are good sources of this factor. All of these foods have been shown to be of value in the treatment of human pellagra, as well as in allowing growth and in preventing the symptoms of so-called experimental pellagra. In contrast to this we find the whole-grain cereals listed as poor, and such foods as honey, lard, and numerous milled products that might be mentioned, as practically devoid of this vitamin. Whereas the whole-grained cereals are poor in content of B_2 (G), they are good sources of B_1. In milk the condition is the reverse; this food, although rich in B_2, is only a fair source of B_1. Therefore, these foods supplement each other. The cereal-milk combination is thus a good one to use from the standpoint of obtaining a good supply of the vitamin B complex.

Within the past few years many workers have reported results

suggesting the existence of still other water-soluble factors present in the vitamin B complex. For example, Williams and Waterman, and later Eddy and associates, claimed the existence of a vitamin B_3, required to secure maintenance of body weight in the bird. We have always regarded this claim as unconvincing, believing that the phenomena upon which the claim was based could be more easily explained as due to an insufficient amount of the already known antineuritic vitamin, the B_1 factor. It is with great interest, therefore, that we notice Williams and Eddy, in their last report to the Carnegie Institution, adopting the view that this is not unlikely.

Likewise, Reader claims the discovery of a B_4 factor required to secure growth in the rat. It is pertinent to observe that the phenomena cited by Reader suggest a lack of antineuritic vitamin. The fact that a sample of the Jansen-Donath crystalline preparation of antineuritic vitamin B_1 can serve as Reader's B_4 when given in daily doses as small as 0.002 mg., supports this view.

Still other factors have been described as members of the vitamin B complex, but further research must be undertaken before any definite pronouncements concerning them can be made. This represents a valuable field of study.

CHEMISTRY

What is known about the chemical nature of the B vitamins? They have at least one property in common, namely, that they are soluble in water. The antineuritic B_1 factor is sensitive to heat, particularly in the presence of alkali; vitamin B_2 is much more heat-stable. So far as is known, the B_2 factor is a neutral substance; it has not been concentrated sufficiently to allow any very definite statements as to its nature. It is adsorbed on fuller's earth, particularly in a strongly acid medium, but is extremely difficult to remove from the adsorbate.

Considerable research has been done in an attempt to isolate the antineuritic factor. Beginning with the experiments of Funk this problem has been attacked vigorously. In 1927, Jansen and Donath[6], working in Java, announced the isolation of a crystalline product having the formula $C_6H_{10}ON_2$, with a melting-point of 250° C. This material was basic in nature, forming characteristic salts such as a picrate and picrolonate, and double salts with gold and with platinum, etc. It was neither a primary nor a secondary amine. Jansen and Donath suggested that the nitrogen might be

part of a ring, either glyoxaline or pyrimidine in nature. Early in 1932, Windaus and associates[13] reported the isolation of this vitamin in crystalline form with the empirical formula $C_{12}H_{17}ON_3S$. It will be noticed that this formula is roughly twice that offered by Jansen and Donath, and in addition contains the element sulphur. The melting-point of this material is the same as that of the Jansen-Donath product. The sulphur is not present as simple sulphydryl, because the material yields a negative nitroprusside reaction; the sulphur may be removed by treatment with alkali and detected by the formation of lead sulphide. The latest communication on this subject is that of Van Veen[10], who has also prepared a crystalline material, the empirical formula of which is more nearly double that of Jansen and Donath, and includes sulphur, thus confirming Windaus and his associates. Van Veen's formula is $C_{12}H_{20}O_2N_4S$, with the same melting-point, 250° C. Like the other products cited, this material cures pigeons with daily doses of only 3 to 4 gammas (0.003-0.004 mg.). It is an interesting fact that mixtures of these three products yield the same melting-point, suggesting that these substances are identical. Doubtless the differences in the empirical formulae offered by the different groups of investigators are to be attributed to difficulties of analysis, particularly of the extremely small quantities with which the analysts have had to deal. As larger amounts of the crystalline products are prepared, additional work designed to disclose the molecular structure of this vitamin can be performed.

It is obvious that as the known vitamins in the vitamin B complex are isolated, it will be possible to repeat experiments supplying the purified product and thus, perhaps, discover still other factors, which were not appreciated previously, and which were supplied unwittingly in the earlier animal experiments.

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VITAMIN A

Discovery

The possibility that a suitable dietary measure would relieve vitamin A deficiency as expressed in disease of the eye may have been recognized long ago. Hippocrates is stated to have recommended liver to those afflicted with night-blindness. Inasmuch as night-blindness is now believed to be associated often with an inadequate supply of vitamin A, and liver is relatively rich in this factor, the ancient treatment seems rational. In more recent times, the explorations of Lewis and Clark in the Northwest Territory and of Livingstone in Africa provided the occasions for the observation and brief description of an eye affection which, like the first mentioned, can best be interpreted as resulting from a restricted dietary intake of vitamin A. The Lewis and Clark expedition journal of the date May 10-12, 1806 (vid. Browne[2]), relates that the Nez Percés Indians were accustomed to live chiefly on roots (as a rule possessing a low content of vitamin A) and that a number of the Indians came to be treated for scrofula, rheumatism and sore eyes. Then follows the interesting comment: "The scrofulous disorders we may readily conjecture to originate in the long confinement to vegetable diet; which may perhaps also increase the soreness of the eyes; but this strange disorder baffles at once our curiosity and our skill." Again, Livingstone (1857), chronicling the trials of his party (vid. McCollum and Simmonds[4]), wrote that the diet for a while was limited to sugarless coffee, manioc roots and meal, and that "the eyes became affected (as in the case of animals fed on experiment on pure gluten or starch)."

Toward the end of the nineteenth century, the frequent occurrence of either xerophthalmia or night-blindness in certain groups of poorly-nourished people was reported from several different parts of the world. Mori[5], in 1904, studied some 1400 cases of an eye lesion in young Japanese children, and showed that the condition was readily relieved by the administration of cod-liver oil. It is noteworthy that all of the foregoing accounts deal with some disturbance of the normal functioning of the eye. Other symptoms of vitamin A deficiency were discovered largely through the application of the experimental method, particularly in connection with the use of the albino rat.

Stepp[9] concluded in 1909, that some ether-soluble constituent of natural foods was essential for weight maintenance of mice and pigeons. However, the status of vitamin A as a distinct entity was first clearly elucidated by two groups of American investigators in 1913. McCollum and Davis[3] and Osborne and Mendel[6] demonstrated that certain fats, such as cod-liver oil, butter, and egg-yolk, contributed a dietary item indispensable to the growth of the white rat. Lard, olive-oil and almond-oil, though fats beyond question according to the ordinary chemical definition, were found to be practically devoid of the factor. Osborne and Mendel showed further that the particular deficiency was not only responsible for growth failure but, indeed, gave rise to the diseased eye condition and to the formation of concretions in the urinary tract. Thus the various symptoms were correlated and a definite syndrome established. The substance which alleviates this syndrome has been variously designated the anti-ophthalmic vitamin, fat-soluble A, etc., but latterly, simply vitamin A.

PATHOLOGY

The following description of the pathology of vitamin A avitaminosis is based, unless otherwise stated, upon observation of the young albino rat. In the course of three to four weeks of the animal's ingestion of the appropriately deficient diet,* the eyes exhibit unmistakable symptoms. The initial excessive lacrimation and photophobia are soon followed by the appearance in the inner canthi of a small amount of sanguineous secretion which later may become purulent. Retraction of the eyeball, inflammation of the conjunctiva and progressive swelling of the lids are also to be seen. Subsequently the cornea may become opaque and finally ulcerated

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* The technic now employed to secure vitamin A deficient animals for experiment under carefully controlled conditions is much the same as that initially used by McCollum and by Mendel. Albino rats weaned at the age of about three weeks are given a purified, or so-called synthetic, diet. The latter may consist of casein, starch, a balanced mixture of inorganic salts and lard. A supplement of irradiated brewer's yeast furnishes vitamins D, B and G. The object in arranging the diet, obviously, is to supply all of the known essential factors except the one under immediate consideration. Commercial preparations of starch and lard may be employed without further purification. Technical casein, however, is contaminated with butter fat and, of course, in turn with vitamin A. These must be removed by extraction with alcohol and ether, or the vitamin A must be destroyed by heating in contact with air.

(cf. Yudkin[11]).   Sterility develops in both sexes, the sterility in the female being due to a failure of cell implantation and not to cell resorption as in the instance of vitamin E deficiency.   Derangement of the œstrous cycle is an early sign of vitamin A deficiency and may be determined by periodic examination of vaginal smears.

Approximately simultaneous with the development of the eye lesion, growth ceases, and subsequently, various other indications characteristic of deficiency conditions in general become more and more marked.   These symptoms are, for example, priapism, a rough coat, angular body lines, muscular incoordination, and failure of the animal to preen itself and keep clean.   Occasionally, the nasal discharge as well as the urine may be bloody in character. If no vitamin A is administered, the condition of the animal becomes increasingly incompatible with life in consequence of drastic irreversible changes occurring in many parts of the body.   During the terminal stages of the deficiency, the animal is observed to be moribund. Little or no food is consumed.   Respiration and circulation are poor. The body temperature falls and finally death ensues.

Necropsy commonly reveals infection—often in the form of large abscesses—of the nasal sinuses, of the tongue, of the salivary glands, of the neck lymph glands with occasional perforation to the exterior, of the mucous lining of the trachea (resulting at least rarely, in the writer's opinion, in outright mechanical strangulation through the impediment of accumulated desquamation) of the lungs with the development of bronchiectatic cavities, of the kidneys and ureters, and of parts of the genital system.   Continued vitamin A deficiency may be attended by calcification of the kidney epithelium and by deposition of calcium phosphate concretions in the renal tubules and pelvis and in the bladder.   The lodging of debris and calculi in the ureter may cause blockage of the latter, with the result that the kidney and the ureter undergo extreme enlargement with subsequent necrosis.   Upon histological examination, the tissues of rats subjected to vitamin A deficiency exhibit a prevalent degeneration of the epithelium in the many widely scattered organs already enumerated (cf. Wolbach and Howe[10]).   This primary change, called keratinization, paves the way for bacterial invasion and the attendant secondary changes.   Marked alteration in the central nervous system and in the bone structure has also been reported.

Many species other than the rat, if not most of the higher animals, are strictly dependent on an exogenous supply of this sub-

stance which we call vitamin A.   Man and the monkey, guinea pig, calf, chicken, dog, rabbit and mouse require it, and the consequences of deprivation are quite similar among the various vertebrates listed. However, one would not suppose that human beings, even when poorly nourished, would commonly develop a complete picture of all the irreparable tissue ravages previously described.   Some part of the diet is likely to contain a mildly protective supply of the needed factor.   Even starvation will not ordinarily result in typical deficiency symptoms, because the body must go on growing or vigorously metabolizing until the reserve of the primary limiting factor is exhausted.   It is rather to be expected that the unplanned spontaneous human deficiency, though responsible for extremely grave consequences in many instances, would commonly be subtle or subacute in its manifestations.   Bloch[1] has recently reported an abnormally high premature death-rate among children who have suffered from the eye disease of dietary origin.   In other words, it is highly possible that, although the deficiency is apparently overcome, irreparable damage, temporarily hidden, may exact its toll in the course of a few years.   There is considerable experimental evidence with animals to support this view (cf. Sherman and Smith[7]).   The writer recalls an albino rat which, with the aid of a dietary supplement, recovered from its sickness arising from a brief state of vitamin A deficiency, and grew to be quite large and apparently healthy in every way.   However, necropsy revealed a stone partially encysted in the wall of the urinary bladder.

DISTRIBUTION

The determination of the quantity of vitamin A, like all bio-assays, necessitates the employment of some common denominator for the expression of potency.   The amount of food necessary daily to restore a body weight gain of 3 gm. a week to the albino rat previously definitely depleted of the factor in question is said to contain one unit of vitamin.   A new international unit has recently been defined.   It is based upon the physiological effect of one gamma (0.001 mg.) of crystalline carotene, a substance possessing several extraordinary characteristics.

In 1919, Steenbock[8] reported the important observation that vitamin A often occurred associated with the yellow color of plant structures, such as the carrot and the sweet potato.   It has since been demonstrated that one of the plant pigments responsible for

the yellow color, carotene, a hydrocarbon with the molecular formula, $C_{40}H_{56}$, cures A avitaminosis. Further investigation has shown rather conclusively, however, that this hydrocarbon is not the substance known as vitamin A found concentrated in the livers of animals, but that carotene is a precursor of vitamin A. The conversion of carotene to the vitamin occurs in the liver. In fact, experiments have been reported, indicating the presence in the liver of an enzyme capable of effecting this transformation. Carotene with related pigments is apparently synthesized in chlorophyll-containing plants. In the sea, these are represented by diatoms and algae which are ingested by small marine animals, the plankton, which are in turn devoured by a series of progressively larger animals until vitamin A or its precursor, carotene, finds its way into the codfish. The latter stores away a rich supply of the vitamin in its liver. Apparently, the plant materials owe their potency chiefly to carotene; those of animal origin largely to the substance which we choose to call vitamin A. Both of these, qualitatively at least, accomplish the same physiological purpose.

The following table summarizes our knowledge with respect to the vitamin A potency of various articles of the diet.

SOURCES OF VITAMIN A

Most Potent

Fish-liver oils

Excellent		*Good*		
Plant	*Animal*	*Plant*		*Animal*
Alfalfa	Butter	Vegetables:	Fruits:	Clam
Broccoli	Cheese	Artichoke	Apricot	Glandular organs:
Carrot	Cream	Asparagus	Avocado	Liver
Lettuce	Egg (yolk)	Cabbage	Banana	Kidney
Spinach	Milk (whole)	Celery	Orange	Oyster (raw)
Tomato		Chard	Peach	
Watercress		Clover	Pineapple	
		Corn (yellow)		
		Escarol		
	Minimal	Kale		
	Starch	Pea (green)		
	Sugar	Pepper (green)		
	Lard	Squash		
	Egg white	String-bean		
		Sweet potato (yellow)		

The reader's attention is directed to liver and liver oils, many green leaves and the dairy products which may be employed to make a substantial contribution of vitamin A to the diet. It is noteworthy that halibut-liver oil is a better source of the factor than even cod-liver oil. The physiologist's interest in vitamin A should be whetted by the observation that the retina, among the many tissues of the body, exhibits an unusual potency, it being intermediate between that of butter and that of cod-liver oil.

Perusal of the accompanying table might create the impression that Nature by promiscuous scattering of vitamin A or its precursor, carotene, among the natural foods has provided adequate insurance against any serious dietary deficiency of these substances. The fact is that in the world today millions of people exposed to the vicissitudes of poverty, war and social and political revolution, are subject to the constant menace not only of general food shortage, but also of improperly balanced dietaries. During the World War, there was an extensive outbreak of xerophthalmia among the children of Copenhagen, despite the fact that Denmark has long been a great dairying nation and therefore a large producer of vitamin A in relatively concentrated form. The explanation of this anomalous situation lay in the exaggerated export of the dairy products, with their included vitamin A, to other countries.

Many vitamin A concentrates are commercially available for therapeutic and prophylactic purposes. Generally, they consist of the non-saponifiable portion of cod-liver oil, although recourse is now also had to the very potent crystalline carotene. Thus it is possible to provide preparations richer in this vitamin than is cod-liver oil. Dehydrated preparations of appropriate green leaves have been used to fortify babies' food with this vitamin. Vitamin A has been successfully administered to experimental animals and to man by subcutaneous injection.
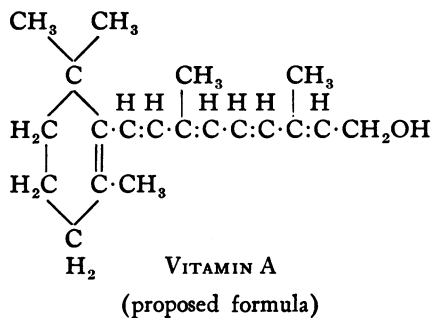
CHEMICAL NATURE OF CAROTENE AND VITAMIN A

The chemical distinction between carotene and vitamin A is based on the following grounds: (1) Carotene is intensely yellow: vitamin A concentrates possess very little color; (2) Carotene exhibits no characteristic 328 millimicron absorption band: vitamin A does; (3) With antimony trichloride, carotene develops a greenish-blue color with a characteristic absorption in the spectrum at 590 millimicrons: vitamin A gives an intense blue color with a

prominent band at 610–630 millimicrons. These properties, with the one in common of relieving a specific nutritional deficiency, are the landmarks which investigators have employed in many different types of physiological and chemical investigation of vitamin A. Colorimetric analysis has been used in a supplementary way in the commercial assay of cod-liver oils.

The chemical nature of vitamin A has naturally elicited the intense and persistent interest of the chemist. Five years ago comparatively little was known of the molecular constitution of either carotene or vitamin A. It is now possible, on the basis of many recent brilliant contributions by Karrer, Kuhn, and Heilbron and their associates, to present with considerable confidence the formulas for both substances.

$$
\begin{array}{c}
CH_3 \quad CH_3 \\
\diagdown\diagup \\
C \qquad\qquad CH_3 \quad\; CH_3 \qquad\; CH_3 \qquad\; CH_3 \qquad\qquad CH_3 \quad CH_3 \\
\diagup\diagdown \; HH \;|\; HHH \;|\; HHHH \;|\quad\; HH \;|\; HH \;\;\diagup\diagdown \\
H_2C \quad C\cdot C{:}C\cdot C{:}C\cdot C{:}C\cdot C{:}C\cdot C{:}C\cdot C{:}C\cdot CH{:}C\cdot C{:}C\cdot C{:}C\cdot C \quad CH_2 \\
\;|\quad\;\| \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \|\quad\; | \\
H_2C \quad C\cdot CH_3 \qquad\qquad\qquad\qquad\qquad\qquad H_3C\cdot C \quad CH_2 \\
\diagdown\diagup \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \diagdown\diagup \\
C \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad C \\
H_2 \qquad\qquad\quad \text{CAROTENE} \qquad\qquad\qquad\qquad H_2
\end{array}
$$

(probable formula of the β isomer)

$$
\begin{array}{c}
CH_3 \quad CH_3 \\
\diagdown\diagup \\
C \qquad\qquad CH_3 \quad\; CH_3 \\
\diagup\diagdown \; HH \;|\; HHH \;|\; H \\
H_2C \quad C\cdot C{:}C\cdot C{:}C\cdot C{:}C\cdot C{:}C\cdot CH_2OH \\
\;|\quad\;\| \\
H_2C \quad C\cdot CH_3 \\
\diagdown\diagup \\
C \\
H_2 \qquad \text{VITAMIN A}
\end{array}
$$

(proposed formula)
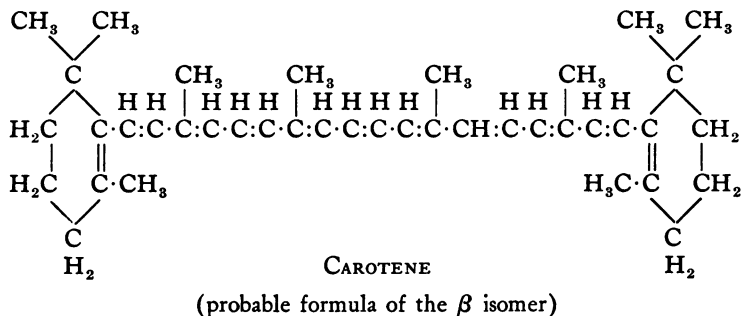
β carotene (natural carotene consists of α and β isomers, both of which are physiologically active) has a structure which is closely related to that of other carotenoid pigments, and the terpenes of

essential oils. These various substances have in common in their formulas, the isoprene residue or unit,

$$CH_2 = CH - \underset{\underset{CH_3}{|}}{C} = CH_2$$
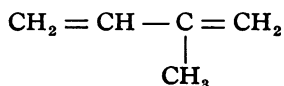
which can be obtained by the distillation of rubber. The formula for vitamin A, probably somewhat less certain than that for carotene, is equivalent to one-half of the symmetrical β carotene molecule with one molecule of water added at the point of cleavage. It is obvious that studies of the structure of vitamin A necessitated the production of the substance in a relatively pure state. Starting with the richest non-saponifiable fractions from fish-liver oils, chemists successfully accomplished this, essentially in two different ways; by adsorption on colloidal materials, and by distillation in vacuo at a pressure of 0.00001 mm. Sufficient progress in the study of the chemical structure of vitamin A has already been made to warrant the prediction that the synthesis of this important substance may soon be accomplished.

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VITAMIN D

DISCOVERY

In a historical discussion of rickets, Hess[7] refers to the "older" and the "newer rickets". The former belonged to the clinical and pathological era which embraces the long span between 1650 and 1918, whereas the "newer rickets" marks the present period still in fruition, which started with Mellanby, who produced the first evidence in 1918 of the dietary control of the disorder.

In his extensive feeding experiments Mellanby[12] found that "something associated with certain fats probably identical with the fat-soluble vitamine" (vitamin A) either prevented or cured rickets in puppies. The experience of clinicians did not permit acceptance of this suggested double rôle of vitamin A. Bloch, for example, observed that rickets did not develop in a very considerable number of xerophthalmic cases among Danish children fed diets lacking in the vitamin A factor. Presently McCollum and coworkers proceeded to clarify the situation by showing that cod-liver oil in which the vitamin A factor was destroyed by oxidation still retained its antirachitic potency; one group of rats consuming a vitamin A-deficient diet inevitably developed a xerophthalmia which was not relieved when the oxidized cod-liver oil was fed, whereas another group ingesting a rachitogenic diet was later cured of rickets when some of the oxidized oil was included in the ration. These investigators therefore interpreted their experimental data as showing that the antirachitic substance is distinct from vitamin A. Later this calcifying agent was called vitamin D, or the antirachitic vitamin. Thus, a new dietary factor was discovered, knowledge of which has aided in the conquest of the "largest" disease of infancy.

RELATION OF RICKETS IN ANIMALS TO HUMAN RICKETS

Concerning the relation of rickets produced experimentally in animals to human rickets, Park[14] cites the following facts:

The lesions in the skeleton, both gross and minute, are identical; the pathological conditions found to exist in the blood are identical; the rickets produced experimentally in animals may or may not be accompanied by symptoms of tetany, exactly as rickets in the human being may or may not be accompanied by tetany; the same remedial measures are effective in both. Not the slightest doubt can exist that rickets produced experimentally in animals and the rickets occurring in human beings is the same disease.

ULTRAVIOLET LIGHT AND RICKETS

The second propelling influence which contributed to the birth of the newer conception of rickets was the discovery that lack of ultraviolet energy plays a dominant rôle in the etiology of rickets. It was conclusively demonstrated that the disorder may be prevented or cured by suitable artificial irradiation when Huldschinsky, working in Germany where cases of rickets were prevalent at the close of the World War, showed that ultraviolet rays from a quartz mercury-vapor lamp prevented and healed clinical rickets. Later Hess and Unger of New York reported on the curative effects of sunshine in cases of infantile rickets.

Regarding the parts of ultraviolet and solar spectra which possess antirachitic potency, valuable information has been obtained. In the ultraviolet spectrum, antirachitic radiations are limited to wave lengths between 313 mμ and 265 mμ; in the solar rays, physiologically active wave lengths are confined to the narrower region of 313 mμ to 290 mμ. The latter value of 290 mμ represents the shortest wave lengths of sunshine which reach the earth's surface in the absence of atmospheric contaminations. Due to the effects of cloudiness, smoke and fog, rays shorter than 300 mμ seldom reach the earth. The law of the "vital importance of the minimum" applies to the indispensable solar rays which are supplied in the smallest amount and in least intensity.

ACTIVATION OF FOODS BY ULTRAVIOLET RAYS

In 1924 the striking observation was made almost simultaneously and independently by Hess and Steenbock and their respective coworkers, that many food products lacking in vitamin D potency could be richly endowed with antirachitic properties by irradiation with ultraviolet light. The foods which have been successfully irradiated include vegetable and animal fats, cereal and cereal products, fluid and dried milk, and green vegetables; on the other hand sugar, starch, gelatin, oleic acid, glycerol and mineral oil could not be so activated.

MEASUREMENT OF VITAMIN D VALUES

The so-called "line-test" devised by McCollum and collaborators[11] is one of the means employed to measure the vitamin D content of foods. This test is based upon the observations that the feeding of rachitogenic diets render the epiphyseal cartilages and

the metaphyses of the long bones entirely free from visible calcium deposits and that the "reappearance of the provisional zone of calcification after the addition of any substance to the rickets-producing diet constitutes a test for the calcium depositing power of the substance".

The rat unit of vitamin D which has been adopted by the Council of Pharmacy and Chemistry of the American Medical Association is defined "as that amount of vitamin D, which when uniformly distributed into the standard vitamin D-deficient diet—ration 2965 (Jour. Biol. Chem., 64: 263, 1925)—will produce a narrow and continuous line of calcium deposits in the metaphyses of the distal ends of the radii and ulnae of standard rachitic rats."[13]

The proposed international unit is defined as the vitamin D activity of 1 mg. of the international standard solution of irradiated ergosterol.

DISTRIBUTION OF VITAMIN D IN FOODS

In discussing the antirachitic values of foods, Sherman[15] notes that "naturally but unfortunately, the concentration of attention upon the enrichment of foods in vitamin D by means of artificial irradiation has led most people to overlook the fact that many natural foods contain significant amounts of vitamin D." Cod-liver oil still remains the most important natural source of the vitamin. Concerning its vitamin D content, interesting observations have been made by Hess, Bills and Honeywell. These investigators found that the potency of the antirachitic factor in samples of oil obtained from small, lean livers was 100 to 200 times greater than in samples yielded by large, fat livers. Apparently in the catabolism of the liver fat, the vitamin D factor becomes concentrated in the impoverished liver. In spite of the fact that the potency of oil obtained from individual cod livers may vary tremendously, commercial preparations of cod-liver oil represent a mixture of oils obtained from great numbers of livers with the result that the variation is much less than might be expected (vid. Blunt and Cowan[3]).

Other fish-liver oils including puffer-fish-liver oils and burbot-liver oils have been assayed and found to be more potent in the antirachitic factor than a good-grade commercial cod-liver oil. Fish-body oils including tuna fish, California sardines, and New-foundland herring have also been tested and found active. Very

recently, Dr. Martha Eliot[5], of the Yale School of Medicine, and associates have reported on the use of salmon oil as a cheap source of vitamin D. They found the oil to be "probably a more potent antirachitic agent than the average cod-liver oil".

Egg yolk is an excellent source of vitamin D. It is reported that physicians have been able to protect children against, and to cure children of severe rickets by feeding this food product. The concentration of vitamin D in egg yolk is not uniform, inasmuch as the content of the antirachitic factor is dependent upon the diet of the hens and the amount of ultraviolet irradiation furnished. Hart and coworkers increased tenfold the antirachitic potency of eggs by irradiating the hens.

Recently, attempts have been made to increase the vitamin D content of milk by various devices of feeding dairy cattle. There are indications that cows use the vitamin D in irradiated yeast more efficiently than in irradiated ergosterol. The question of the best method to employ in increasing the antirachitic factor of milk has been discussed by Krauss and coworkers[9] of the Ohio Agricultural Experimental Station. They remark:

> It would seem that the natural method for increasing the vitamin D content of milk would be through feeding the cow. This would eliminate special handling of the milk after it was produced, as is necessary when milk is irradiated. However, it would be very difficult to follow closely the potency of a milk supply, whose vitamin D content was dependent upon feeding operations carried on over scattered areas. . . . Such control could not be expected, and it is perhaps only for special, carefully supervised, and scientifically handled herds, producing special kinds of milk, that the practice may be at all warranted.

According to Hess and Lewis[8], milk activated by means of carbon-arc rays is a highly effective and reliable antirachitic agent in protection against infantile rickets. There are also movements in this country in the direction of the supplementation of milk with vitamin D concentrates.

Some investigations have shown that plants contain small amounts of vitamin D, but it may be stated that plants as a class are practically devoid of this accessory factor.

STABILITY OF VITAMIN D

The antirachitic factor in cod-liver oil appears to be remarkably stable. After passing a stream of air for 4 hours through cod-liver

oil heated at 120° C., Mellanby[12] found the oil to exert a strong antirachitic effect. When stored under suitable conditions in a cold, dark place, cod-liver oil has been found to retain its vitamin D potency for more than 25 years (vid. Clouse[4]). Irradiated food products maintain their acquired potency with little diminution for a varying period of time. Eggs boiled for 20 minutes do not lose an appreciable amount of their antirachitic factor.

ERGOSTEROL AND CRYSTALLINE VITAMIN D PREPARATIONS

Shortly after discovery was made that inert food substances could be successfully activated, it was concluded from a series of investigations that cholesterol and phytosterol were the particular components of the food which were rendered antirachitic by exposure to ultraviolet light. Later Rosenheim and Webster proved conclusively that purified cholesterol could not be activated, and no longer possessed the absorption spectrum in the ultraviolet region considered characteristic for cholesterol. As a result of a subsequent study these same collaborators stated that the naturally-occurring parent substance of vitamin D was ergosterol or a highly unsaturated sterol of similar constitution. Simultaneously Windaus and Hess announced that ergosterol, a contaminant of cholesterol, and not cholesterol itself, was the illusive "provitamin" of the antirachitic factor. Ergosterol, obtained by the French chemist Tanret from the fungus ergot, was previously thought to be limited in its distribution to plants, but has now been found to occur in both vegetable and animal tissues. It has the molecular formula $C_{27}H_{42}O$ and from its structural formula this sterol is observed to be a highly unsaturated product with a total of three double bonds; cholesterol, on the other hand, has only one such unsaturated linkage.

Regarding the nature of the substance termed "irradiated ergosterol" or viosterol, a tremendous mass of data, detailed and broad in extent, has been collected by the combined and cooperative efforts of the chemist, physicist, biologist and clinician (vid. Hess[6]). Research groups in this country and in Europe have worked unremittingly to produce the so-called "Vitamin D" substance. In 1928, in recognition of the merit of his studies in the chemistry of sterols and their relationship to vitamin D, Professor Adolph Windaus of Göttingen, Germany, received the Nobel prize[10].

During the last few years numerous accounts have appeared in rapid succession in the literature concerning the chemical and phys-

ical properties of substances which exhibit enormously high anti-rachitic potencies. The separation of crystalline preparations of vitamin D has been regarded as one of the most outstanding recent events in biochemistry. A crystalline antirachitic substance called calciferol was prepared by a group of English investigators[1]. This product, obtained from a purified and irradiated sample of ergosterol, was distilled and fractionally condensed in a high vacuum and exhibited an antirachitic potency of .05γ (daily dose). Some of the other properties possessed by this product were shown to be: melting point, 123-125° C.; high dextrorotation, $\left[\ \alpha\ \right]_{5461}^{20}\ +\ 260°$; and maximum absorption spectrum of 270mμ. Shortly after the preparation of calciferol, two products were made independently which exhibited greater antirachitic activity than calciferol. *Purified* calciferol prepared by Askew and others[2] displayed twice the antirachitic potency of the original calciferol preparation, as did the substance "D_2" which was reported almost simultaneously by Windaus and coworkers[16]. Askew and associates regard *purified* calciferol as representing "vitamin D" in a state of approximate purity.

A simple arithmetical calculation indicates that one part of the most potent vitamin D substance in two hundred million parts of food (assuming a food intake of 5 grams per day) suffices to heal rickets in the rat. These observations regarding the exceedingly minute amounts of dietary substances necessary for the health of the animal organism impress the individual afresh with the importance of the "little things" in nutrition.

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