THE PHYSIOLOGICAL VALUE OF THE VITAMINES.*

BY A. D. EMMETT, PH.D.

Pertinent questions as to what vitamines are, and how they act in the physiological economy have been and are now occupying the serious attention of many investigators throughout the scientific world. At first, when Funk[1] announced his vitamine deficiency theory, there was a great deal of skepticism on the part of many and especially among medical men who have been schooled essentially along the line that disease is fundamentally due to infection. In the last three years, however, our medical, biological and chemical journals have issued an almost unceasing array of articles bearing on subjects relating to vitamines. As a result of these studies, I think I can show you that this theory is based upon sound scientific facts, and that the future possibilities in this field of study are very suggestive of fruitful and beneficial results in the treatment of certain general malnutritional ailments to which mankind is heir.

In the very beginning, it should be frankly admitted that biochemists and physiologists are, as yet, very much in the dark as to the exact chemical nature of the vitamines and as to the precise definiteness of their pharmacological action. Such progress has already been made, however, that it has had the effect of stimulating investigators to do further work.

By way of introduction, a brief résumé of the early history of vitamines and a general description as to what they are will aid in a better understanding of their physiological value. It was Eykmann,[2] Grjins,[3] Schumann,[4] Chamberlain and Vedder,[5] and others, who were first impressed with the idea that the diet had a definite relation to a specific disease, beri-beri. It was found that when the oriental peoples consumed an overabundance of white or milled rice, they gradually acquired the symptoms of this disease, and that when they substituted unmilled or natural rice, the pathological condition was corrected. Fraser and Stanton[6] were able to cure beri-beri by using an extract of rice polish, and Funk[7] succeeded, in 1912, through his series of researches, in isolating a curative fraction from extracts of rice and brewers' yeast to which he gave the name vitamine.

Now we consider that there is more than the one vitamine, and designate the "vitamines" as one of the essential nutritive groups which must absolutely be present—along with the right amount of protein and mineral salt, and a sufficient amount of carbohydrates and fats for energy—to produce normal growth and development.

Due to the studies of Hopkins,[8] Osborne and Mendel,[9] McCollum and associates,[10] and others, we recognize two general types of vitamines—the water-soluble and the fat-soluble. The water-soluble group includes: (*a*) the original antineuritic vitamine of Funk; (*b*) the growth-promoting vitamine; (*c*) the yeast-stimulating vitamine; and (*d*) the antiscorbutic vitamine. Besides these there are some suggestions being put forth which tend to show that there are other water-soluble vitamines.

As to the fat-soluble vitamines, we have considered that there is, so far, but the one form—designated by McCollum as fat-soluble A. Mellanby[11] has thrown

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out the suggestion, though, that there may be another one, which relates to dental caries. Then, too, Myers and Voegtlin[12] have just shown that the water-soluble antineuritic vitamine, when separated out, is in turn soluble in certain oils.

It is thus easily seen that the researches directed toward a study of the chemical nature of the vitamines has been tremendously complicated by various factors. Thus, the biological and physiological evidence indicates that there are probably more than one or two. On the other hand it is possible that there may be a primary, or alpha or nuclear vitamine base or group and that the other vitamines are derivatives of it. Another supposition might be that this primary vitamine is susceptible to variations in the acid or alkaline menstruum of the different body fluids; and also that certain metabolic end-products may act as inhibitors.

Funk[13] considers that the antineuritic vitamine is possibly related to nicotinic acid, or adenine or some of the pyrimidines or purins. Williams[14] is of the opinion that this vitamine is a pseudo-betain and that it has tautomeric properties, that is, existing in both active and inactive forms, or in uncombined and combined forms.

This antineuritic vitamine is precipitated by such reagents as phosphotungstic acid, mercuric chloride, silver nitrate and picric acid. It is adsorbed by fuller's earth and mastic. It is stable in dilute acids, even on boiling; and is less stable in alkaline media. One thing stands out prominently in this work, namely, that this vitamine is very easily altered, and in the concentration and purification of it, like the active principle of many of the glands, it becomes less potent.

As to the isolation and chemical nature of the other water-soluble vitamines referred to, practically nothing has been accomplished. They have certain thermostable properties and are more or less affected by alkali. The antiscorbutic water-soluble vitamine is not adsorbed by fuller's earth, and is very easily destroyed or altered by heat.

The fat-soluble A vitamine is not a lipoid, does not contain phosphorus or nitrogen, is fairly stable to heat, and is considered by Steenbock and Boutwell[15] to be possibly associated in its chemical structure with carotin, the yellow pigment of butter oil. Other studies, particularly those of Palmer and Stephenson, indicate that there is little if any relationship in this connection.

Now, as to the physiological value of the vitamines, it has been shown experimentally that, in the absence of the antineuritic vitamine, fowl will come down with avian beri-beri. From the extensive work of McCarrison[16] on pigeons, the symptom complex of this disease is a gradual loss of appetite, a decline in weight, a lowering of the body temperature, a decrease in the red blood cells, a general derangement in the endocrine glandular functioning causing atrophy in some glands and hypertrophy in the adrenals, a partial or complete paralysis, and finally, if no treatment is administered, death.

Histological examination of the nerve tissues by the Marchi method shows a certain amount of degeneration and on this basis it has been stated repeatedly that a lack of this vitamine causes nerve degeneration. McCarrison and others are of the opinion that instead there is an accumulation of toxic substance, which inhibits the normal passage of the nerve impulses. Otherwise, it is difficult to explain the rapid recovery following treatment.

According to Funk,[17] there is a relation between the amount of carbohydrate and the absence of the vitamine in respect to the rate of hastening the onset of

the disease. He found that the sugar in the blood was increased by a lack of vitamine and that an excess of starch in the diet aggravated the condition still more.

McCarrison,[16] using monkeys, found that a diet that was low in vitamines and protein but high in fat or starches or both, brought about a condition which was very prone to cause disease, particularly of the gastro-intestinal type, and that this condition tended to greatly favor bacterial invasion of the blood and tissues. He also concluded that an *absence* of the water-soluble vitamines in the diet was of less value from the practical standpoint of disease than was a *subminimal* supply of the vitamine. For, in the first case, there results a rapid dissolution and death, especially if the other nutritive requirements are not met, while in the second case, there is a tardy dissolution with the concurrent appearance of disease in one form or another.

McCarrison says "The results recorded in this paper may afford some explanation of the genesis of that great mass of ill-defined gastro-intestinal disorders and vague ill health which forms so high a proportion of human ailments at the present day."

As to the water-soluble growth-promoting vitamine, Hopkins, in 1913, was the first to note that when all the supposed known dietary factors were present for normal growth, young rats grew for a time then ceased. Upon adding a minute quantity of raw milk—too small to contribute anything to the number of calories, or to the protein or mineral salts—the animal began to grow markedly. He associated this finding with Funk's vitamine deficiency theory and considered that here was another vitamine than the antineuritic—the growth-promoting factor. Osborne and Mendel,[9] as well as McCollum and associates,[10] and others have confirmed this. McCollum, however, takes the position that this vitamine is the same as the antineuritic while in our laboratories we[18] have introduced evidence which suggests that such a conclusion may be questioned, for the present at least.

In the absence of this second vitamine, the rat soon ceases to grow (measured by weight) and remains stationary. If there is an apparent *total* lack of this vitamine, then the animal declines in weight and later reaches a stage when it cannot control its movements and shows a positive loss of the power of locomotion due to partial paralysis of the posterior extremities. In our laboratories we have also made histological examination of a number of the tissues, including glands, of several rats that were suffering from a lack of this vitamine. There was found to be a general derangement in the digestive system as well as in the endocrine glands. The thymus, for example, was almost completely atrophied while the adrenals were enlarged. The livers also showed definite indications of fatty infiltration and in some cases even degeneration. This is suggestive of an altered carbohydrate metabolism.

If an extract containing this vitamine is introduced in the ration, or given to the rat subcutaneously or *per os*, there will result a rapid and quick response, and in due time a complete recovery in loss of weight with subsequent normal growth. Eddy and Roper[19] treated marasmus babies and were able to stimulate growth and normal recuperation. Daniels and Byfield[20] found that this water-soluble vitamine accelerated the metabolism in convalescent children.

Again, Uhlmann[21] in a series of tests with extracts containing the water-soluble vitamine found that the presence of vitamines in the extract bore a close relation

to the salivary, gastric, bilary and pancreatic secretion; that nerve action was stimulated and body tone regulated; that they caused vascular dilation, lowering of the blood pressure, and stimulated tonus and pendulum motion of the intestine.

Voegtlin and Myers[22] consider that the antineuritic vitamine acts in the same manner as secretin which stimulates the secretions of the pancreas and liver. There have been data put forth to refute this idea but it is still tenable. Dutcher[23] has presented the theory that this vitamine may increase the production of catalase and in turn aid in the oxidative processes. He also throws out the suggestion, which has been made previously, that vitamines are closely allied with the active principles of the endocrine glands. In this connection, it might be added that we[24] have shown the development of the tadpole, other essential factors being supplied, depends in part on the presence of the water-soluble vitamine.

The third water-soluble vitamine, which is designated by some as water-soluble C and by others as the antiscorbutic vitamine, has a definite and specific relation to scurvy. This vitamine, as has been mentioned, has two characteristic properties which distinguish it chemically or physically from the former two vitamines—namely, it is not adsorbed by fuller's earth and is extremely unstable to increasing temperature. Harden and Zilva[25] have shown that after neutralizing the acid and removing the salts formed, the filtrate contains the vitamine. This proves conclusively that the supposition that has been believed by many, namely, that the organic acids were the corrective agents in scurvy, is wrong. The lack of this vitamine is, therefore, the direct cause of scurvy. According to Zilva and Wells, and later Howe, the pulp of the tooth is very appreciably affected by a deficient antiscorbutic diet.

Hess[26] recognizes two types of scurvy, the subacute form which occurs during the first few months of infancy and the acute form which is more easily observed in children and adults by a looseness of the teeth, bleeding gum, etc. Pediatricians are agreed that much more care should be exercised in guarding against scurvy in the feeding of infants and that their food should be supplemented with some material as orange juice. Here, however, we are often met with cases where a baby's stomach is too sensitive to withstand the free acid, and special preparations should be employed.

The fourth water-soluble vitamine that we referred to relates to the stimulation of the growth of the yeast cell. Williams[27] and then Bachman[28] put forth evidence which showed clearly that extracts that contained the antineuritic and growth-promoting vitamines carried a stimulus, which, when the extract was added to a synthetic media (that would not support growth of the yeast), caused a marked accelerating effect on the yeast, either in the increased number of cells or the degree of fermentation. Williams considers this vitamine to be the same as the antineuritic. We have recently been able to demonstrate that the yeast-stimulating vitamine is apparently biologically independent of the antineuritic and the rat growth-promoting vitamines.

Besides these water-soluble vitamines, there appear to be others, or at any rate, other organisms seem to require vitamines for normal functioning. For example, certain fungi and certain bacteria need vitamines. Davis and I have done some preliminary work on the specific vitamine requirements of bacteria and the tentative data suggest that in this case the extracts that stimulated yeast growth had no effect while special ones were efficacious with the bacteria in question.

Having considered the water-soluble vitamines, let us turn our attention to the fat-soluble form—that is, "vitamines" that are soluble in fat. Osborne and Mendel,[9] and at about the same time, McCollum and associates,[10] working independently, discovered that young rats required for complete normal growth and normal functioning another member of this vitamine group and McCollum proposed the name fat-soluble A. Without this vitamine, all the other dietary essentials being present, rats grow for a while then growth ceases and there is a subsequent loss in weight. At the same time, an eye condition, xerophthalmia, develops, and complete blindness will eventually result unless the missing factor is supplied. The general condition of the animal is a syndrome of malnutrition. We have found that on this dietary plane, rats are very susceptible to lung infections—as pneumonia and bronchisepticus. This is in marked contrast to the animals fed a ration with the other vitamines missing and the fat-soluble A present, although an exception should be made in the case of the antiscorbutic vitamine. Guinea pigs are very prone to infections of the pneumonia type in this case.

Mellanby[11] and others associate the etiology of rickets with a deficiency of the fat-soluble vitamine. Along with this faulty diet one should also consider a possible depletion of the calcium and phosphorus salts. Mellanby also suggests the possible relation of a subminimal supply of fat-soluble vitamines in infancy to the later development of dental caries. Beside these, many are inclined to attribute pellagra as being due to a low supply of this vitamine along with a supply of a poor quality of protein and a deficiency of calcium salts.

In general, it may be said that *all* the known vitamines are needed for normal development and functioning. When one form is missing the clinical picture shows a pathological resultant effect of some sort. Thus, Stark[29] in clinical tests, and later McCollum and Simmonds in careful experimental studies on rats, showed very clearly that the vitamines have a definite relation to the development of the young during pregnancy and also during the period of lactation. McCollum concluded that if the food given the mother was lacking or deficient in one of the vitamines, the milk would be correspondingly affected. As a result the young would not develop normally. This is a very fundamental fact for if the infant is started on a faulty vitamine plane, especially if some of the other nutritional essentials are not properly balanced, the ultimate results will be serious, unless the diet is modified or there is added some supplemental agent containing the vitamines.

From what I have cited in this brief résumé and from the illustrations and charts, the evidence points with certainty to the undeniable fact that the so-called vitamines have a definite place in the physiologic economy. It appears that they act as powerful stimuli, regulating the metabolism so that a sluggish or retarded systemic cycle of physiologic reactions shall be brought more nearly into its proper rate of speed or motion. Whether this is a case of proenzymic action or not is only conjecture. Whether we have a direct quickening or accelerating action, or whether it is first a case of stimulating the removal of certain toxic metabolic end-products that have accumulated due to subminimal amount of vitamine, is not known.

While the vitamines are very important indeed, at the same time it should be borne thoroughly in mind that the proper balancing of the other nutritive essentials—proteins, carbohydrates, fats and mineral salts—are equally important.

There is a definite interdependence of these nutritive groups, which calls for the judgment of the trained medical man to determine how it should be varied.

Again, it should be borne in mind that in the large majority of cases, the studies that have been made in this field have been based upon the source of vitamines as they exist in extracts and not as isolated chemical bodies. Therefore, it may and probably will in some cases be shown in the future that some of these deductions will be found to be not as exact as they have been stated. Bear in mind, however, that these studies are pioneer, as it were, and that the majority of the investigators have gone and are going forward with open minds, thoroughly convinced that ultimately (perhaps in the near future) the vista will become clear and then we can talk with more exactness and more precision.

In the meantime, it seems only rational to advocate the therapeutic usage of the different vitamines in concentrated form—namely, the fat-soluble A (present in egg-yolk, fat, green leaves, etc.); the water-soluble B (present in yeast, rice polish, etc.); and the water-soluble C (present in orange juice, fresh tubers, etc.). I see no fundamental objection to this provided it is thoroughly understood that such concentrated vitamine products are intended to be used solely at the discretion of the physician who is in a position to diagnose the case and decide whether he is dealing with a type of malnutrition, the supplemental treatment of which will be assisted with vitamines.

Now I have intentionally avoided referring to the various sources of vitamines in raw materials, food and food products. This phase of the subject hardly comes within the scope of the topic before us. The two ideas should be kept distinctly separate for many reasons, but chiefly to us on the grounds that we are dealing with the treatment of a diseased condition and not preventative medicine. Also I have avoided discussing methods of extracting and isolating vitamines. Either of these phases of the subject would have occupied the entire time that has been placed at my disposal. Then, too, I have taken it for granted that at the present unsettled stage of vitamines you as pharmacists would be primarily interested in the physiologic value of vitamines so that thereby you would gain a general idea, at least, as to their complexity and be in a better position to coöperate with the physician in the rational usage of them.

MEDICAL RESEARCH LABORATORY,
PARKE, DAVIS & CO.,
DETROIT, MICH.

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CLINICAL AND PATHOLOGICAL LABORATORIES.*

THEIR MAINTENANCE, SERVICE CHARGES AND SCOPE OF WORK.

BY HENRY J. GOECKEL.[1]

As clinical laboratory examinations, that is, the chemical, microscopical and biological examinations, are becoming of more value in the diagnosis and control of disease, the employment of such methods is increasing. With this increase many more laboratories are being established in hospitals, and as state, municipal and private institutions.

From an apology for a laboratory, such departments are becoming of paramount importance in hospital equipment. Many of the more recent institutions have set aside an entire building or unit of structure for this purpose. From an equipment for making a routine urine examination, and examining a few specimens and tissues microscopically, they are now equipped for far more extensive work. The time has come when no hospital can lay claim to recognition unless it has a well-equipped laboratory.

This being the case, it will be timely to discuss the various factors involved in, and their bearing on, the cost of such service. This is especially opportune, because many electing this branch as a vocation are not trained in industrial or business methods. Many who are concerned with the establishment of such laboratories, especially in connection with hospitals, pharmacies, and with medical groups, are not conversant with the subject.

The factors to be considered will vary with the given laboratory. The following will probably include all to be considered:

The first comprehensive item to be considered is the proportionate charge for what may be called the overhead or fixed expense. This is the cost of main-

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[1] Pathologist and biological chemist of Muhlenberg Hospital, Plainfield, N. J.