equilibrium point of the reaction more in one direction or the other, thus increasing the velocity.

It is our intention to continue this study on the destruction of vitamin under the influence of experimentally measured oxidation and reduction potentials as soon as conditions permit.

The Antiberiberi Vitamine

By Casimir Funk

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The antiberiberi or B vitamin is the most important and the most frequently studied of this class of substances. Its discovery was contingent, as is well known, on the elucidation of the etiology of beriberi, a far-eastern disease, due to continuous consumption of polished rice. This vitamin is more or less distributed throughout the whole animal and vegetable kingdom, but in the cereal grains it is located chiefly in the germ and in the peripheral layers and therefore easily lost during the cleaning process applied to the grain.

As regards the function of this substance in the body, we know only that in its absence from the food general cachexia develops, similar to a severe inanition, the whole picture originating, as it seems, in the involvement of the central nervous system. Among the more important pathological changes is the increase in size of the suprarenals, going hand in hand with an increased adrenaline output. This observation put forward by McCarrison applies for the time being only to avian beriberi and may be brought into relationship with the present writer's observation that an increased carbohydrate consumption hastens the onset of the disease, showing perhaps that B vitamin plays a role in the carbohydrate metabolism. Contrary to this, a higher protein supply diminishes the needs for this vitamin and acts as a vitamin saver.

The present paper will deal briefly with the chemistry of B vitamin. The chemical studies deal almost exclusively with rice-polishings and, particularly, yeast, and were started about ten years ago. In dealing with this phase of the subject, very serious experimental difficulties were encountered, owing to the fact that no precipitation or color reaction specific for B vitamin is known. This substance is present in the starting material in minute quantities (as far as our present evidence goes, there is no more than a fraction of a milligram in a pound of dried yeast) and has to be separated from a large amount of inactive material, which is only partly becoming chemically known to us. The first endeavors were therefore directed toward a separation of the active material by the simplest possible procedure. Limiting the steps to a minimum is very important for the final success, as the time taken for the operations is an important factor in obtaining the final fraction in an active state. This separation was effected first by an extraction with alcohol, then by precipitation with phosphotungstic acid, tannic acid being used for the same purpose by Suzuki. Later came the adsorption with fuller's earth, introduced by Seidell, then the alcohol precipitation method of Osborne and Wakeman, the precipitation with bismuth potassium iodide by Hofmeister, adsorption with norit by Eddy, and so on. Unfortunately none of these methods shows any specificity for B vitamin, and the fractions obtained are contaminated with a large quantity of inactive material. More promising was the method of fractionating the phosphotungstates obtained from yeast by means of acetone, the active fraction being insoluble in this solvent and representing roughly only 10 per cent of the total precipitate.

ISOLATION OF B VITAMINE

The isolation of B vitamin was attempted by a number of investigators. In 1911 to 1913, the writer was able, by

precipitation of the decomposed phosphotungstates with sublimate, silver nitrate in acid and finally in alkaline solution, to obtain a series of crystalline substances, one of which proved to be nicotinic acid, and another which after recrystallization to a constant melting point still exhibited marked curative properties for beriberi pigeons. These substances were analyzed at that time and their formulas tentatively established, but it is still uncertain whether we were dealing with B vitamin or whether the substance was yet contaminated with adsorbed active material—this in spite of the repeated crystallization. Whatever may be the final outcome, we had in hand a new substance which is worthy of further study. The vitamin research has brought to light a series of new substances, present in the starting materials in very small amounts, among them nicotinic acid, aschamine, and a recently isolated reducing substance, glucathion, obtained by Hopkins. It is clear, therefore, that the above-described attempts did contribute materially, and will do so more in the future, toward increasing our knowledge of chemistry of the living cell. From all the known reactions for B vitamin the most promising for the present is the precipitation with silver in alkaline solution. By using this method we obtain this vitamin in concentrated form, together with other substances which are largely known to us chemically. Among the substances which are carried down in this fraction, we know already the pyrimidines, histidine, histamine, glucathion (peptide composed of glutaminic acid and cysteine), guanidine, carnosine, and nicotinic acid, and we possess therefore ways and means to effect a successful separation of some of the impurities.

By using the combination of his fuller's earth method with the writer's silver method, Seidell was able to obtain a silver fraction which represents a small part of the initial yeast material. As we were able to ascertain in collaboration with Mr. Levy, this fraction contains a large proportion of inactive impurities.

NOMENCLATURE

In concluding the part of this paper relating to attempts at isolation of the B vitamin, the author wishes to dwell a moment on the question of nomenclature. A former assistant, Dr. Drummond, recently suggested dropping the final "e" in the term vitamin. This suggestion did not come as the result of experimental evidence, but merely on theoretical grounds. This may seem a trifling matter, but the suggestion has found already a number of followers. The name vitamin was given in the first place not so much to indicate the amine nature of these substances as for euphonic reasons. We were anxious to choose a name which would "take" and would stimulate workers in this new direction—and it did take. The final "e" was given to indicate the nitrogenous nature of these substances, in which the writer thoroughly believes. After all, the present evidence tends to show that the vitamins act as bases as well as acids and such substances have the ending "e" in the English language. By dropping the "e" very little is changed, the amine still being pronounced. In the absence of experimental evidence against these views, there is little necessity to effect a change of nomenclature at this time. In connection with a possibility of an acidic nature of this substance, we have sometimes succeeded and sometimes failed to distil in vacuum the free ester of the substance which acts on yeast. The experimental difficulty here is in the effective separation of the nondistilling material, which accumulates and causes decomposition at higher temperatures, showing at the same time the complexity of our present vitamin fractions.

IS B VITAMINE A MIXTURE?

Another problem of great interest is whether the substance with which we hitherto have dealt as B vitamin is one or

a mixture of substances. For some time, indications have been present in the literature showing that we are dealing here with a mixture. In particular, Abderhalden and Schauermann have contended that there is a substance which acts on the nervous system, another one which acts in maintaining weight. In this country Emmett advocated the view that the substance for rats is not the same as for pigeons and is still different from that which acts on yeast. These views are undoubtedly correct, to a great extent. Recently, in collaboration with Dr. Dubin we succeeded, by fractional adsorption with either fuller's earth or norit, in separating at least two substances in a fairly complete manner. This separation will enable us to study these two substances more closely and also to determine their respective value in animal nutrition. Another field which will be influenced by the above separation is the nature of the substance which is important in the metabolism of lower organisms. Recently, the so-called yeast method was introduced for the quantitative determination of B vitamin. The above separation shows clearly that we are dealing here with two substances at least, one which cures beriberi, and the other which acts on lower forms of life. This second substance we propose to call D vitamin. The yeast method, showing the relative content of extracts in vitamins of the B-type, can no longer be regarded as specific for antiberiberi vitamin. Yeast is favorably influenced by D vitamin and we have no doubt that we are dealing here not with an improvement of the medium, as some workers have contended, but with a specific substance; this presents a new field of investigation for the future. As regards bacteria, the problem is more complicated, as these microorganisms require at least two substances, one present in the blood, possibly connected with the blood pigment, and another substance of the nature of the above-described D vitamin. Here also our adsorption procedure offers a distinct advantage over the methods hitherto employed, as it eliminates a large proportion of the impurities from the start.

LACK OF CHEMICAL KNOWLEDGE

In the chemical work on B vitamin we are still struggling with a difficulty which lies in the way of progress and success. The present methods seem to injure the activity of the substance. This is undoubtedly due to our lack of knowledge of the chemical characteristics of these substances. It seems that in the case of B vitamin we are not dealing, as in the case of other vitamins, with the injurious effect of oxidation. There is something else to be looked for and it seems to the writer that so long as we do not possess a specific precipitation method or know how to stabilize this substance so that one would have the time to study its characteristics, no definite progress can be made. Unfortunately the vitamins, like the rare gases of the air, have so far shown exceedingly little affinity for the known reagents, most of which no doubt have been tried. For the present, therefore, we have to contend with the elimination of impurities; this necessitates slower procedure and we are handicapped by the imperfect chemical knowledge of the latter.

Views are often expressed as to the exaggerated importance assigned to the vitamins. There is no doubt that vitamins do not mean everything in nutrition. One cannot live without vitamins, but one cannot live with vitamins only. There are some who hold that the vitamins are only a fad in science, which will pass away as many other fashions have passed. This is not so; the vitamins are here to stay. Their chemical nature may be difficult to establish, but the price of the efforts will be well worth while. It will open up new viewpoints and bring new ideas. It is therefore a pity that only few organic chemists venture into this field, offering possibilities of new and unexpected developments. For the

chemical study of the vitamins, vitamin B, or perhaps better still the above-mentioned vitamin D, offers the best opportunity on account of greater stability. We have to start with the study of the most stable vitamin. Knowing the chemical nature of one such substance, it may prove easier to arrive at the others.

Experiments on the Isolation of the Antineuritic Vitamin

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It is generally acknowledged in recent contributions to the subject that no trustworthy information concerning the chemical nature of any vitamin has, as yet, been obtained. On the other hand, the study of the functions of the several vitamins is progressing rapidly and is closely approaching quantitative exactness in some cases.

The failure to make substantial progress in the chemical isolation and identification of vitamins is due to the length of time required for, and the uncertainties of, the physiological tests available for controlling the fractionations, and more particularly to the readiness with which the characteristic vitamin activity disappears during the laboratory manipulations. In this respect it is a common experience to find that the product finally isolated is devoid of the characteristic physiological action of the original crude material. Some investigators consider it an axiom that the better the final product appears from the chemical standpoint, the worse it will be found when tested for its physiological activity.

It is probable that by far the greater number of the experiments which have been made on the isolation of vitamin are useful principally in showing how the problem cannot be solved and have, therefore, been withheld from publication. A considerable number of the present series are of this type and are omitted. Although those which are given do not furnish a solution to the problem of the true chemical nature of vitamin, they appear to supply another stepping stone toward this goal. A method is described by which a relatively stable silver precipitate, which undoubtedly possesses considerable vitamin activity, can be obtained. It is hoped either that the identity of the active constituent of this product can be established or that the pure vitamin itself may eventually be isolated from it.

The discovery of this new vitamin complex was briefly described in a preliminary paper¹ published several months ago. The reaction involved has since been studied in greater detail and much additional information obtained in regard to it. Briefly, it consists in subjecting a concentrated vitamin extract to successive precipitation with aqueous silver nitrate and ammoniacal silver nitrate. The latter precipitate has been found to contain by far the greater part of the vitamin originally present. Since it is probable that the method of preparing the aqueous vitamin extract used for the precipitation contributes largely to the success of the procedure, this will first be described.

PREPARATION OF CONCENTRATED VITAMIN EXTRACT FROM BREWER'S YEAST

The fresh yeast, as obtained from the brewery, is allowed to autolyze and the thick liquid thus obtained is filtered. English fuller's earth² is added to the clear autolyzed filtrate in the proportion of 50 g. per liter and, after thorough shaking,

¹ Atherton Seidell, "Preliminary Note on a Stable Silver Vitamin Compound Obtained from Brewer's Yeast," *Public Health Reports*, April 1, 1921, p. 665.

² The variety occurring at Surrey, England, and imported by Eimer and Amend.