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THE CHEMISTRY AND BIOLOGICAL SIGNIFICANCE OF THIAMIN

By Dr. ROBERT R. WILLIAMS
BELL TELEPHONE LABORATORIES, NEW YORK, N. Y.

SOMETHING more than fifty years ago the Japanese Navy annually suffered an incapacitation of from 23 to 40 per cent. of its effectives. The disease which the Japanese called kakke was also prevalent among the civilian population, not only of Japan but of Asia generally. In China it was recognizably described more than 300 years ago. Its devastations among the Malay peoples of the peninsula and of the great islands of the East Indies is attested by the fact that the world at large has adopted the Malay term beriberi for the disorder. It is, however, no stranger in India, Siam and Burma.

It was the Japanese, however, who first afforded convincing evidence regarding its general nature. Takaki, in effect surgeon general of the Japanese Navy, observed in 1883 a disastrous outbreak during a six months' voyage of the training ship, *Riujo,* and being convinced of the nutritional nature of the disease ordered an experimental duplication of the cruise with the sole alteration of a change of ration. The comparative results of the two cruises were so striking that the changes in ration were presently made effective for the entire Navy with the result that the incidence of beriberi has never, since 1885, risen to as high a figure as one half per cent. of the force.

Takaki had little notion of the specific nature of the shortage in the diet. The beginnings of this disclosure were left for Eijkmann, medical officer in the Dutch colony in Java. In the course of experiments aimed at a study of the disease, he encountered its counterpart in chickens which for the sake of economy had been fed on waste rice from the hospital kitchens. He published his results in 1897. There followed many further studies from his laboratories, studies to which neither the world at large nor even the medical profession of the Orient paid prompt attention. A few discerning persons took them seriously. These were mostly Englishman in overseas service, Braddon, Fletcher and Fraser and Stanton. It was not till Funk in 1911, first working at Pasteur and later at Lister Institute, claimed the isolation of a specific substance to which he gave the name vitamine that the world at large took a hand in the study. The nutritional paralysis in pigeons with which Funk principally worked is perhaps even more striking than in chickens. A novice can readily reproduce the results and recognize the condition. All one has to do is to coop up the birds and feed them for a month on polished rice or white bread. In the throes of the disease the birds' movements are wholly incoordinated and consist in turning cart wheels or aimless floppings as if freshly decapitated.

While largely ignorant of beriberi, the rest of the world was becoming conscious in this interval of the necessity in foodstuffs of essential accessory substances. Many students contributed, but the names of Stepp, of Hopkins, of Osborne and Mendel and of McCollum are prominent. They worked largely witdh rats, noting the stunting of growth which ensues from a want of these substances. It was natural at first to suppose that all these phenomena were variable expressions of a single deficiency. Few had courage to believe that after all the extensive study of metabolism which had been carried on there should still remain a large number of unidentified essentials. It is indeed true that the beriberi preventing substance is a growth promoter of great importance for young rats, so much so that on certain diets at least the extent of growth depends on the amount of the substance in the food and generous supplies may lead to somewhat supernormal growth. The fact that this vitamin appears to play a role particularly with reference to nerve function has been allowed to obscure somewhat its broader role with reference to tissues in general. That its role is a general one is indicated by the wide-spread pathological changes which result from a deficiency of it, by its effects upon appetite, food consumption and skeleton size, and its therapeutic value in a somewhat diverse set of conditions.

Those who do not know beriberi will be curious as to how its affects human beings. For reasons which we do not know it takes two distinct forms, the wet and the dry. The contrast in appearance is conspicuous because of the accumulation of water in the tissues in the former case, while in the latter atrophy of the musculature becomes prominent. In most essential respects the two forms are alike. In both, there is numbness of the extremities amounting to anesthesia yet exquisite tenderness of the muscles to pressure. In both, the patellar and other reflexes are either exaggerated or lost altogether. In both, the heart is affected by an accumulation of fluid in the pericardium. Fluid is also usually present in the lungs. The right heart is most affected. Death, which often comes suddenly after some exertion, is due to heart failure. Response to treatment is often dramatic in the acute fulminating type of the disease, though complete recovery from the secondary effects of the disease, if it has existed for a long time, is usually discouragingly slow.

As we now know, the beriberi vitamin is only one of several substances which are of crucial importance in connection with various deficiency diseases. It was due to the presence in yeast, rice polish, etc., of these numerous substances of physiological importance that so much confusion arose about the B vitamins. This long delayed a satisfactory isolation of the substance in a pure state. For, if one has to depend on a biological test as a guide in the chemical procedures, he can be grossly misled if he does not choose the right test. There are several B vitamins, each of which contributes to the growth of rats so that the growth test in its cruder forms led us up many a blind alley. Tests which depend solely on the cure or prevention of polyneuritis have proved much more serviceable for our purposes. Happily, however, our mistakes and disappointments have helped to forward the progress of other workers toward other important goals concerned with dietary deficiencies.

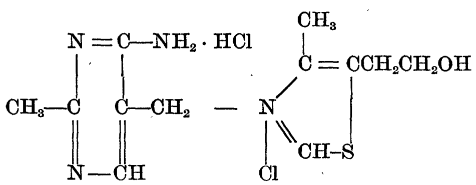
It was not till nearly thirty years after Eijkmann described avian beriberi that others, working in the same laboratory in Java, succeeded in obtaining the substance in pure form. To Jansen and Donath must go the honor of first isolating the pure substance. This was a fitting culmination of a long series of important contributions from the Dutch. For six years no one was able to repeat their work elsewhere, but their descriptions of the substance are unmistakable. Yields of it were so small that chemical study was greatly handicapped for lack of material.

This was to a great extent remedied by improvements in process which were put into effect in America. The nature of the undertaking with which we were confronted is well illustrated by the range of scale on which we were compelled to work. In the initial step we used a 1,300 gallon tank for extracting the rice polish; in the final step, a test-tube. The product was dissolved in 0.5 cc of water and caused to recrystallize by adding 10 cc of absolute alcohol. There are only 40 to 50 parts per million of the vitamin in the original rice polish, of which about one fourth could be recovered as crystalline material.

The object of all this work was not primarily to obtain a concentrated form of the vitamin with which to treat human beriberi. Relatively crude products would serve that purpose at least moderately well. The object was rather to learn the architecture of the molecule in order to ascertain what it does in the body and how it works. At first we had little thought of a practical artificial synthesis, as no one could foretell whether such a synthesis would be feasible even if we once learned the structure.

The method which the organic chemist follows in determining the structure of an unknown molecule is to tear it by progressive steps into fragments. When the fragments become small enough the chemist can recognize them by comparing them with simple substances whose architecture is already known. Having recognized the ultimate fragments, he must fit them together bit by bit to form the primary fragments and if possible the original molecule. It is like the familiar jigsaw puzzle, except that one can never see the picture, let alone the pieces, except in the mind's eye. It is all a rather abstruse business which takes on the air of reality even for the chemist himself only when he has in his hands the substance itself and finds that, though it has been constructed to a pattern existing in his imagination only, it possesses the properties of nature's own product. In this case it cures beriberi.

In presenting to you the study of the structure of the molecule, it will only be possible to give you some notion of the procedure. Like standing an egg on end it will seem easier if we first give the result and then tell you how it was obtained. The structure now universally accepted is as follows:



Its most conspicuous features are its two nuclei. By treating the vitamin with sulfite we were able to obtain one of these as a sulfonic acid, the other as a weak free base. By reducing the sulfonic acid with sodium in liquid ammonia, we were able to obtain a 2,5-dimethyl-6-amino pyrimidine which we eventually duplicated by synthesis after trying one after another the various compounds of the same composition in which the methyl groups are in other positions. We also were able to reproduce synthetically the oxy-sulfonic acid which we derived from the vitamin by hydrolysis of the primary product of sulfite cleavage.

As for the other product of sulfite cleavage, the weak base yielded on oxidation with nitric acid 4-methyl thiazole-5-carboxy acid, prepared synthetically by Wohmann nearly fifty years ago. Wohmann had never heard of vitamins and of course had no idea how or whether his information would ultimately be used. But he set it forth in publication and at long last it fulfilled its destiny. So shall it always be with hundreds and thousands of the humbler records of science if only they are correct. In this instance an erroneous analysis or melting point by Wohmann might have led us far astray, perhaps for years.

While the isolation of the vitamin required more than twenty years of intensive work in many countries, the development of its structure was fully accomplished in less than three years. Once the structure was known with certainty its synthesis followed in a few months. The synthesis of the thiazole portion came first by the condensation of thioformamide with bromacetopropyl alcohol. That of the pyrimidine is more roundabout and difficult but follows along lines which had already become familiar in the course of producing the various pyrimidines needed for comparison purposes during the establishment of structure. Finally the two portions were fitted together and the properties of the natural product were duplicated. Already hundreds of kilos of the compound have been produced commercially and it is finding an extensive use in medicine even in well-fed America.

This would have been a matter of great surprise to us who worked in the Philippines twenty-five years ago with what we called the "beriberi preventing substance." We then supposed that a deficiency of it could develop only when human dietaries are very restricted. We even supposed that its occurrence in foods was a matter of chance. We also supposed that this vitamin had peculiarly to do with the nutrition or function of nerves. Hence arose the name antineuritic vitamin and later "aneurin" was proposed by Professor Jansen, who was instrumental in first isolating it. Both these views have proven far too narrow as we shall see. In view of its extensive use in medicine, it has seemed necessary and proper to conform to the views of the American Medical Association regarding a name based upon chemical facts rather than conceptions of therapeutic value. Upon invitation, I suggested the name thiamin to imply that it is the sulfur-containing vitamin. This name has been tentatively adopted by the American Society of Biological Chemists and the American Institute of Nutrition and has been recommended to the International Conference for consideration.

During the period when the neuritic character of beriberi was still very prominent in all minds, studies were begun at Oxford upon the nature of the biochemical defect in the nervous tissue. Peters found first an excess of lactic and later of pyruvic acid in the brains of polyneuritic pigeons. The excess was not equal in all parts of the brain but was greater in the lower part and in the optic lobes. When the brain tissue of freshly killed polyneuritic pigeons was placed in Ringer phosphate solution in the presence of glucose, lactic acid or pyruvic acid, it was found to be subnormal with respect to its capacity to take up oxygen from the air. This deficiency in oxygen uptake was greatest in the brains of birds which were fully polyneuritic. Birds fed on polished rice for lesser periods of time yielded brain tissue of less subnormal respiratory capacity. Moreover, the deficiency of oxygen uptake was localized in the same parts of the brain as showed the greatest excess of pyruvic acid. When birds were treated with preparations of the vitamin and thus cured of their acute symptoms, the respiration of their brain tissue was found high enough to justify the assumption that it had partially regained its lost function. Still more striking was the later finding that it is not necessary to give the thiamin to the living birds. It may be added to the medium in which the polyneuritic brain slices are respiring and there produces the same effect of increasing the oxygen uptake and promoting the metabolism of the lactic or pyruvic acid.

Now all this is extremely important, not only because it tells us that something is chemically wrong in the brain but also that it is the carbohydrate metabolism which is impaired. This is derived from the fact that lactic and pyruvic acid are normal intermediates in the metabolism of carbohydrates by all cells. When yeast ferments sugar, it converts the six carbon chain of the sugar into two moles of the 3 carbon atom pyruvic acid by splitting the chain in two in the middle. Likewise muscular action in animals uses up sugar and produces lactic acid in its own cells. Fatigue of the muscle is intimately related to the accumulation of lactic acid in it. What is true of yeast and muscle is also true of many other cells which have been studied: *viz.,* that their living processes involve the conversion of glucose into vital energy via pyruvic acid.

As a beautiful confirmation of Peters' ideas about the biochemical defect in polyneuritis, there was isolated only a year ago by Lohmann and Schuster a substance which turns out to be the pyrophosphate of thiamin. It is present in yeast in amounts sufficient to account for all the thiamin which the cells contain. It has the properties of the cocarboxylase postulated by Auhagen in 1932. Without it yeast can not ferment sugar. Yet thiamin pyrophosphate itself can not ferment sugar unless it is accompanied by a specific protein which may be extracted from yeast and which, unlike thiamin pyrophosphate, is rendered inactive by a few minutes heating. The two together constitute an enzyme capable of converting pyruvic acid to acetaldehyde and carbon dioxide. It has antineuritic properties like those of thiamin itself and is capable of promoting the oxygen uptake of polyneuritic tissue.

Now free thiamin can not function as the coenzyme for the carboxylase of yeast. The pyrophosphoric acid is essential to this function. This is the second instance of a phosphorylated vitamin in the B group, for riboflavin phosphate has been shown to be the coenzyme of a yellow respiratory pigment which functions as a dehydrogenase. Since it is also true that nicotinic amide, which now appears to be the essential pellagra vitamin, is a part of the prosthetic group of cozymase and of a ferment of red blood cells, one is strongly tempted to believe that the water-soluble vitamins as a class are essential components of the enzyme systems by which living matter carries on metabolism.

As far as thiamin is concerned it seems clear that it enters into several enzyme systems. With polyneuritic brain tissue, increased oxygen uptake is the most conspicuous feature, the pyruvic acid consumed being sufficient to account for the oxygen only on the basis of its nearly complete conversion to CO_2 and water; in yeast, as we have seen, there is in effect no oxidation, merely a pure decarboxylation. Between these two extremes are the lactic acid bacteria of Lipmann, where there is oxidation *and* decarboxylation and the behavior of staphylococcus which, according to Krebs and Hills, results in a dismutation—*i.e.,* simultaneous oxidation and reduction with decarboxylation also entering in. Krebs' recent finding of citric and α keto glutaric acids in the urine of thiamin deficient rats conforms to Krebs' theories regarding the normal course of carbohydrate metabolism. Perhaps it will turn out that some positive knowledge of what happens to a sugar molecule in the body will be one of the principal fruits of the whole thiamin research.

Thiamin is present in so many hundreds of living tissues, animal, vegetable and microorganic, and has been demonstrated to play an indispensable role in such a considerable number of them, that the burden of proof now lies upon him who would dispute the *universality of its function* in living things. Yet it is present in tissues in very small amounts, usually less than one part per million. In seeds and in certain special animal organs, such as the liver, it may rise to three to five parts per million; only in the germs of cereals and in the cells of cereal-grown yeasts does it rise to thirty to one hundred parts per million. The lower saprophytic plants lack the power to synthesize it adequately and always respond with evidences of more vigorous vital function when given a liberal supply from some external source. Animals are unable to synthesize it from the elements at all. Only the higher plants can make it. Evidently they make it in the tops, probably in the leaves, transporting it thence to the roots which are unable to produce it.

Many interesting experiments regarding its function with respect to root growth have been performed by Kögl, by Bonner and by Robbins. It is to the latter that we owe some entrancingly interesting pictures which illustrate the fact that the growth of excised tomato roots in sugar solution depends quantitatively on the amount of thiamin added. In some of the experiments the fact is brought out that a mixture of the intermediates from which thiamin is made artificially will serve as well as thiamin itself for many plants. This indicates that the power to effect the final step of synthesis is often present in organisms or parts of organisms which have never acquired or have lost the power to effect the entire synthesis. It also suggests strongly that nature's method of synthesis is identical as to the last step with that whereby we made the vitamin in the laboratory. Some plants show a more conspicuous need of the thiazole part, some of the pyrimidine part, presumably because they differ with respect to their synthetic capacities for each part. Some plants, like all animals, must have the fully formed vitamin and can not effect even the final step of synthesis.

What does it mean with respect to the realm of nature as a whole? Its presence in seeds in large amounts seems to me highly significant, especially in view of the fact that seeds are often also repositories of carbohydrate dedicated to the nourishment of the seedling during the process of germination until the young leaves turn their green surfaces to the sun. More and more it seems that man commits a crime against nature when he eats the starch from the seed and throws away the mechanism necessary for the metabolism of that starch by the plant. Since plants have synthetic powers which men lack, the latter have small prospect of successful utilization of the starch unaided by the plants' enzymic component.

In fact as one surveys the situation in philosophical mood it seems that nature's entire economy of thiamin is hand to mouth. Her synthetic powers are barely adequate to keep life as a whole moving forward. She has to resort to symbiosis, whereby the synthetic deficiencies of one organism are made good by the scant surplus of others. She utilizes the dead remains of some of her children that others may grow and propagate their kind. She transports the substance from favored parts to those which are deficient in their synthetic powers. Very widely is it true that a generous external supply of thiamin increases visibly the vigor of the life processes of plants and animals. Probably the chemical instability of thiamin contributes materially to the economy of want.

It would be misleading to suppose that thiamin is the only vitamin which possesses a universal or nearly universal function in living cells. Vitamin C, vitamin B_2 and nicotinamide all appear to play somewhat similar roles. Thiamin is merely the most conspicuous example. The lack of no other accessory substance leads to so early, so profound and so universal a disaster.

These considerations also serve to explain the hitherto baffling problem of thiamin economy in human beings. It is a striking fact that the physiological consequences of thiamin deficiency affect every tissue. Pathological changes in beriberi affect nearly all internal organs. Also in practical trials of thiamin in therapy, favorable responses have been reported in a wide diversity of disordered conditions. In vain have we endeavored to find *the* specific effect of a deficiency of the substance and yet it is credited with almost panacean properties.

The reason is, we believe, that carbohydrate metabolism can not go forward in any living cell without thiamin. One of the outstanding achievements of modern biochemistry is the production of evidence that all cells, no matter whether of tiny unicellular organism or those of highly specialized tissues, have common needs. The liver, pancreas, the brain and each other organ of higher animals performs a special function for the entire assembly of cells which make up the body. But just as each home in a human society subsists by exchange of goods and services with others yet maintains its own kitchen and its own hearth so every cell carries on metabolism and generates energy within itself.

Mankind often lacks an ample supply of thiamin because nature generally must ration it carefully and further because he refines his grains and cooks his food. So when he suffers from constitutional disease his weakest organ may often show a benefit from an artificial supply whether the weakness be due to heredity, to past damage or to present severe strain upon the body economy, as in maternity, hard physical labor or in infectious fever. The apparent want is probably rendered more wide-spread by the fact that in human society the physically unfit increasingly survive and propagate their kind through interruption of the process of natural selection by humanitarian effort.

THE FUTURE OF THE CRELLIN LABORATORY[1]

By Dr. LINUS PAULING

DIRECTOR OF THE GATES AND CRELLIN LABORATORIES OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY

ABOUT twenty years ago, to house the division of chemistry and chemical engineering, which was beginning its rapid expansion under the direction of Professor Arthur A. Noyes, there was constructed the Gates Chemical Laboratory, the gift of Mr. Charles W. Gates and his brother, Mr. Peter G. Gates. This

[1] Address at the dedication of the Crellin Laboratory of Chemistry at the California Institute of Technology, May 16, 1938.