Thiamine

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Introduction

Thiamine was synthesized for the first time only in 1938.[1] This important scientific event occurred 27 years after Funk described the anti beriberi factor. It was as early as 1897 when Eijkman noted that certain fowls developed beriberi-like symptoms when fed on white rice and that far more prisoners had beriberi when living on polished rice than those who received unpolished grain. Thus it took 39 years for an original clinical observation to bear fruit.

Naturally, there was great interest in this new discovery and the medical literature was filled with reports of clinical trials with thiamine between 1940 and 1950. A review was published in 1951[2] in which it was stated that 230 or more diseases had been tested for response. Clinical observations suggested that this cheap, readily available agent did in fact have some effect on many of these conditions, but that it was variable, unpredictable, and in many instances so slow that no one could tell whether the improvement was spontaneous. Any agent which appears to be a "cure-all" is automatically suspect, so thiamine fell into therapeutic disrepute. Since thiamine does have a place in therapy, a realistic look at its role is timely.

Biochemistry

Thiamine occurs in at least four different forms in mammalian systems, the free or inactive molecule and three phosphorylated derivatives. There is also some evidence that there may be a methylated form necessary for the release of acetyl choline into the synaptosome.[3] [4] Its phosphorylation to mono, di(pyro), and triphosphate is similar to phosphorylation of adenosine and is importantly related to regulation of energy metabolism.

Little information exists on the role of thiamine monophosphate (TMP) although it is undoubtedly present in many tissues.[5] The pyrophosphate (TPP) is the best known since it is cofactor for at least 24 enzyme systems. Two important ones are pyruvic dehydrogenase, in which TPP is a catalyst in the synthesis of acetyl CoA, and a similar enzyme which dehydrogenates the branched chain keto acids derived from leucine, isoleucine and valine. It is also required by transketolase, an enzyme which occurs twice in the hexose monophosphate shunt. Without discussing details of the

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biochemistry involved, it is well known that TPP is a vital link in the metabolism of glucose and its deficiency is a major disaster in energy metabolism.

Thiamine triphosphate (TTP) is a form which is less well known or understood. It has a special role in the nervous system and its synthesis is catalyzed by a phosphotransferase which transfers a phosphate from ATP to TPP.[5] The main concentration of TTP in the nervous system is in the brain stem and upper cord, and some cases of Leigh's disease[6] have been found to be associated with a naturally occurring inhibitory substance which appears to block the formation of TTP in brain tissue. The precise action of TTP is not completely understood, but it is found in excitable membranes and in an experimental situation it is known to leave the membrane and pass into the surrounding perfusate when an electrically stimulated impulse passes through the nerve.[5] This suggests that it has an important bearing on phosphate exchanges which regulate energy metabolism in nerve tissue. It is also found in liver and heart, and of the four forms of the vitamin found in the nervous system, about 80 percent is TPP, 5 to 10 percent TTP, and the rest is present as TMP and free thiamine.[5] Thiamine deficient animals may become more aggressive or irritable, [8] [9] so the vitamin appears to have a direct effect on regulation of normal central nervous system function.

Clinical Deficiency

Nutrition

Clinical deficiency of dietary thiamine, probably with other nutrient deficiency, causes beriberi, and a wealth of literature on the subject has recently been disregarded in well developed societies on the assumption that this is a pure starvation disease that has been eliminated. Even if the characteristic symptoms and signs should be recognized, it might be assumed that they would clear rapidly after a small dose of thiamine for a few days. If no rapid response occurred it would then be assumed that the cause of the symptoms was not due to such deficiency. Low dose thiamine replacement is not effective in a chronic deficiency state, for several reasons. First, the water soluble synthetic salts of thiamine are poorly absorbed and an active transport system is required. In long term dietary deficiency, with consequent loss of energy, there may be defective absorption so that the physiologic doses have to be increased drastically. Second, phosphorylation activates the vitamin, and this process would be expected to be inefficient or inactive due to the loss of energy from the original deficiency. This vicious cycle might be the explanation for the clinical observation that beriberi requires large doses of thiamine for months.[10]

Biologic effects are related to whether thiamine deficiency is "pure" or mixed. In practice it is more than likely to be mixed. The age of the patient and the rate of induction of the biochemical state are influencing factors. Infancy beriberi is a viciously lethal disease which occurs in several different forms, depending on its acuity. One form was studied in breast fed Chinese infants in Hong Kong.[11] These infants received milk from their Bl avitaminotic mothers and revealed clinical characteristics which were surprisingly similar to those of Sudden Infant Death Syndrome (SIDS) as seen today in many countries. Sudden death occurred usually between two and five months, was rare under one month, or over one year, had a predilection for the apparently "healthiest" male infants, was more common in late winter, early spring, occurred usually at night while the infant was asleep and was associated frequently with a minor infection such as a cold. Autopsy findings were trivial as they are in modern SIDS. Fehily made a point of emphasizing that the acuity of this event appeared to be dependent upon the amount of milk ingested, thus underscoring the important fact that the nutritional demand for thiamine is closely related to caloric intake.

If beriberi causes sudden infant death, then its epidemiology is identical to that of modern SIDS. Recently it was shown that some infants who die from SIDS have very high concentrations of thiamine in blood[12] and the investigators have suggested that this represents thiamine toxicity. Perhaps the mechanism is a biochemical lesion in the activation of the vitamin resulting in accumulation of thiamine in its free unphosphor-ylated form. This explanation is supported by finding an occasional increase in serum

folate and B12 in the same infants. Clinically, the effect would be the same as dietary deficiency. In an uncontrolled clinical study[13] a group of 12 infants were reported to be relieved of their recurrent episodes of nocturnal apnea after administration of large doses of thiamine. Inactivation of cocar-boxylase was reported in experiments with dogs,[14] induced by fractional bleeding. Hypotension occurred in thiamine deficient animals after losing less than 4 percent body weight, and copious intestinal hemorrhage occurred in 86 percent. In thiamine fortified dogs significant hypotension did not appear until after 5 percent loss of body weight, and there was no intestinal hemorrhage.

Chronic infantile beriberi, characterized by vomiting, diarrhea, abdominal distension, neck stiffness, convulsions, dyspnea, cyanosis and tachycardia, is similar to the acute form of the disease seen in adults and called Shoshin by the Japanese. The more chronic varieties of the disease in adults are the "wet" or edematous form and the "dry" or polyneuritic type. Although the classic disease is rare in advanced societies today, the important question is whether it is seen in marginal expression and, if so, how common it is. The symptoms of the fully developed condition are so numerous that a review of this nature could not deal with them; nor should it, since older texts are plentiful and clinical and laboratory characteristics well described.

There is much clinical evidence that marginal malnutrition is in virtually epidemic form today in the well developed countries, where prevailing attitude denies such a disaster. Thus the discerning and alert physician who knows the symptomatology of the classic nutritional deficiency diseases is recognizing them every day, and this includes minor expressions of beriberi. It is possible to use beriberi as a model from which we can understand how the body behaves under hypooxidative conditions. To summarize the effects of the disease, it can be stated that it represents a prototype for autonomic dysfunction.[15] Autopsy findings clearly showed autonomic neuropathy[15] and different stages during life were associated with parasympathetic or sympathetic dysfunction, according to the state of nutritional deprivation or its reversal after therapy began.[16] Autonomic dysfunction may be seen in other nutritional deficiency states, arising perhaps because of inefficient use of oxygen in the nervous system.

Experimental thiamine deficiency has been induced in human subjects.[17] Depression, weakness, parasthesiae, dizziness, backache, hypotonic or painful musculature, cardiac palpitations, pericardial pain (pseudoangina) on exertion, insomnia, anorexia, nausea, vomiting, weight loss, hypotension, bradycardia at rest and tachycardia with sinus arrhythmia on exertion were all seen after severe deprivation for several weeks. Moderate, prolonged thiamine restriction without caloric deprivation resulted in emotional instability, irritability, mood changes, quarrelsome behavior, poor co-operation, fearfulness progressing to agitation and numerous somatic symptoms. The investigators noted that neither severe nor moderate deprivation of this one vitamin produced the classic syndrome seen in beriberi. Such symptoms as these are common in the U.S.A. today, particularly in children and adolescents, but are frequently considered to be the normal behavior of youth and excused as such. A vast industry has been built around the cult of sugar, and "soft" drinks are encouraged in excess in preference to "hard" alcohol. Society condones the huge intake of sugar as a source of "quick energy" and fails to recognize that it becomes a drug under these circumstances. It is apparent that an important cause of widespread lack of discipline and irrationally abnormal behavior can be traced to nutritional sources. This 20th century paradox has been emphasized[18] by showing that such behavior could be correlated in some youngsters with an abnormal red cell transketolase and that the response to dietary change could be monitored by this relatively simple laboratory test.

The generic symptoms of beriberi may be considered in reference to increased naked calories, particularly from refined carbohydrate, which may be ingested easily in the form of fruit drinks, carbonated beverages, milk, and many other readily available and extremely alluring liquids, many of which are addictive. Thiamine requirement is dependent upon calories ingested, particularly those from carbohydrate. Hence thiamine

analysis of diet may appear to be adequate unless this ratio is considered. This was illustrated in a report[19] of a patient receiving intravenous fluids as the sole source of nutrition. Though the fluid included 24 mg of thiamine a day, Wernicke encephalopathy was found at autopsy, a disease which is known to be closely associated with thiamine deficiency.

Two brothers were reported[20] with nausea and vomiting after mild exertion. Calf tenderness, edema, pericardial effusion increased cardiac output, tachycardia, abdominal pain, loin pain, elevated pulse pressure and acidosis were all typical of acute beriberi. The conclusion was that this disease was a familial unknown form of cardiomyopathy. A nutritional history was not given and it is suggested that this illustrates the clinical myopia in considering the possibility of severe nutritional disease as a differential diagnosis in modern developed societies.

Dependency

Vitamin dependency is regarded as a structural abnormality affecting the binding of a cofactor with an enzyme complex. A number of examples of thiamine dependency have been reported in which pyruvic dehydrogenase has been found to be defective.[21] One report[22] concerns a child who responded to large doses of thiamine hydrochloride and who has now been treated preventively for more than 14 years. It is useful to emphasize some of his symptomatology which amply illustrates the effects of inefficient energy metabolism. He had intermittent episodes of cerebellar ataxia before thiamine was started. They were always associated with physical stress such as an infection or an injury, and this and other symptoms were typical of childhood beriberi. A routine daily dose of 600 mg of thiamine appears to prevent symptoms most of the time, but environmental stress, injury, or infection may precipitate early symptoms and he must increase the daily dose of the vitamin to 1200 or 1800 mg temporarily. For example, he entered an air conditioned store from a 90 degree external temperature. He became unconscious and developed asthmatic wheezing, although there was no previous history of asthma. This patient's brother has the same biochemical defect, illustrating the genetic component.

Selye[23] proposed that diseases arise as a failure to adapt to environmental stress. A student of his found that he could reproduce the general adaptation syndrome in rats by experimental induction of thiamine deficiency. Selye always exposed his experimental animals to significant starvation before inducing the general adaptation syndrome, since he recognized the importance of inadequate nutrition in failing to adapt to stress.

Maple syrup urine disease occurs in infants as a result of a failure to decarboxylate the three essential branched chain amino acids, leucine, isoleucine, and valine. A thiamine dependent form of the disease has been described,[25] a fact which is not surprising since the enzyme requires TPP as cofactor.

A retarded girl with hyperuricaciduria was reported.[26] Her metabolic abnormality responded to a supplement of thiamine, probably by increasing pyruvic dehydrogenase activity and hence decreasing abnormal activity of the hexose monophosphate shunt which would result in overproduction of uric acid. Thiamine responsive megaloblastic anemia has been described,[27] even though thiamine has no known direct hematopoietic action. Another example of possible dependency on this vitamin is provided by case reports of two children with repeated episodes of febrile lymphadenopathy. Both revealed laboratory abnormalities in thiamine metabolism and were clinically and metabolically responsive to supplementation with thiamine. An unusual feature was elevation of serum folate and B12. In one of the children this was shown repeatedly to fall after the supplement was commenced, and to rise again after it was discontinued. It was suggested that this represented reversible inactivation of folate and B12. The supplemental thiamine may have improved ATP synthesis since this is required in the formation of S-adenosyl methionine in the process of transmethylation. One of the children also excreted in urine the factor reported to inhibit the formation of TTP in brain.[7]. The only clinical condition that has hitherto been associated with this substance is Leigh's disease,[6] a

lethal disease which occurs in infants and children and produces a Wernicke like encephalopathy. The child did not have any of the stigmata of Leigh's disease during the period of surveillance. Whether the "stress factor" was recurrent activation of bacterial or viral infection is irrelevant in constructing a therapeutic plan since it was the host mechanism that was significantly at fault. It is suggested that the biochemical evidence of TTP deficiency and the therapeutic response may point to the critical role of the brain stem and lower brain in organizing appropriate body defense mechanisms, including that required to meet infection. This was what was proposed by Selye and such a case report strongly supports his concept. Since febrile lymphadenopathy in children is a common condition, it is of some importance to seek ways and means of identifying biochemical abnormalities which provide clues to host response dysfunction of this nature. The present approach assumes bacterial or viral infection, resulting in administration of antibiotics. The state of nutrition is seldom considered. It is possible that recurrent illness of this nature is a host response to forms of stress other than infection, and might easily be referred to as a disease of adaptation.

A middle aged woman, who had been unconscious for a month, suddenly stopped breathing.[16] She was successfully resuscitated, and further studies revealed that she had edematous beriberi. After administration of thiamine she began to recover slowly. As this recovery proceeded and consciousness returned it was found that she had developed severe anemia. Urine was examined by chromatography and ethanolaminuria was detected. This suggested a defect in the transfer of a methyl group in the endogenous synthesis of choline, and serum folate was then found to be abnormally low. A supplement of folate was started and was followed by a reticulocyte response and the anemia became corrected. After a further two years she developed hyperpigmentation of the skin on her forearms and progressive neurologic deterioration. This strongly suggested indication for B12 supplementation and after an initial injection of 100 micrograms she experienced transient fever and muscle pains, thereafter beginning to make a slowly progressive neurologic recovery. Her complex condition was thought to be exacerbated by her intractable addiction to cigarette smoking.

Naturally Occurring Inhibitors of Thiamine

The ecology of thiamine is complex. Naturally occurring inhibitory enzymes have been recognized in raw fish, ferns and bacteria.[29] Anti thiamine substances have been identified in tea and betel nut.[31] One of the earliest reports of such a phenomenon was concerning Chastek paralysis.[32] Foxes were fed with raw fish which resulted in their ingestion of thiaminase and which caused a lethal paralysis. Paralysis of the recurrent laryngeal nerve in horses causes a condition known as "roaring disease" from the peculiarity of the noise that they make when attempting to neigh. Evidence that this may be caused by ingestion of similar substances from plants such as ferns has been reported.[33] In man, beriberi frequently affects the left recurrent laryngeal nerve rather than the right since it is the longer nerve of the two. One of the well known features of beriberi is that it affects the most used and the longest nerves first. This was epitomized in a tailor who developed "thumb drop" as the first sign of the disease. The thumb was used to thrust the awl through the cloth and this represented an occupational stress in such individuals.

Amprolium, used as a coccidiostat in chicken feed, is a thiamine antagonist[34] and thiaminase is found in shellfish. Both are potentially toxic agents which might affect human nutrition, thus causing symptoms which would be very hard to trace to the actual source. It is predictable that if such a situation should present itself to a modern physician it would be explained as a form of encephalitis due to unknown infection.

Two enzymes have been studied in detail. Thiaminase I (thiamine: Base 2-methyl-4-aminopyrimidine-5-methenyl-transferase, EC 2.5.1.2.) and thiaminase II (thiamine hydrolase, EC 3.5.99.2.) At least three different species of bacteria are known to produce these enzymes. Bacillus thiaminolyticus is aerobic and found in human colon. Clostridium thiaminolyticum is anaerobic

and found in the small intestine. These organisms produce thiaminase I. Bacillus aneurinolyticus is aerobic, found in colon, and produces thiaminase II. All three species produce spores.

Thiaminase I attacks the methylene bridge between the thiazolium and pyrimidine rings of the thiamine molecule and becomes highly activated in the presence of certain base compounds. After the molecular cleavage, which takes place by a base exchange reaction, the pyrimidine ring is attached to the base and creates a thiamine analogue which substitutes for the biologically active vitamin, thus inhibiting its function. The significance of this ecologic relationship is obscure, but it is of potential significance that activation of the mechanism in the bowel is possibly dependent upon nutritional factors. Murata and associates[35] showed that thiaminase I becomes highly active in the presence of nicotinic acid, thyoglycolic acid and cysteine. The production of bacterial thiaminase I increases in the absence of thiamine in the culture medium, suggesting a regulatory role of the vitamin in the metabolism of the bacteria.[36]

Veterinary work has thrown some important light on thiamine metabolism. Polioencephalomalacia, also called cortical necrosis, occurs in cattle and sheep, and is a rapidly fatal encephalopathy caused by "effective" thiamine deficiency. Ruminal contents of affected animals become infected with Clostridium sporogenes which produces thiaminase I.[37] In the presence of nicotinic acid or its amide, the enzyme becomes active and forms N-(2 methyl-4 aminopyrimidyl (-5) - methyl-3-carboxypyr-idinium) chloride hydrochloride, a compound which may itself have potent anti-thiamine action.[38] The disease has been induced experimentally in a calf by treatment with amprolium.[39] Of potential importance is that Clostridium thiaminolyti-cum, found in human small intestine, has been recognized as a subgenus of C1 sporogenes[40] and produces thiaminase I. Thus, in heavy infestations with the organism in man there may be secondary neurologic symptoms produced by this mechanism, a study that has never been performed, to our knowledge.

Of potential interest is the peculiar disease known as Bangungut[41] which occurs in parts of the far east. An affected person, usually a male, goes to bed after a typically Filipino heavy meal. During the night he develops a restless delirium from which he never recovers consciousness, hence the descriptive title, which means "poisonous or toxic dream". It may be of significance that the meal usually consists of food which includes a great deal of rice and a sauce called pati which is made from fish. It is possible that thiaminase I might be responsible for this rapidly lethal condition since fish do synthesize the enzyme. Though speculative, such thinking represents the rising interest in the importance of nutrition in disease, but perhaps even more, emphasizes the need for comprehensive knowledge and awareness of man's place in ecology.

Laboratory Studies for Abnormal Thiamine Metabolism

It has already been suggested that evaluation of blood thiamine may be misleading, since a high concentration may not be consistent with dietary excess. In fact, there is some evidence that non-specific stress may dephosphorylate the vitamin, causing it to accumulate in serum in its free form.[13]

One of the best and simplest tests is red cell transketolase (TKA) combined with thiamine pyrophosphate percentage uptake, or effect (TPPE). Transketolase requires TPP as cofactor. After a baseline activity is measured, it is repeated following addition of TPP in vitro. If the baseline activity accelerates, it reveals TPP desaturation of the enzyme. After prolonged deficiency,[42] or because of structural defects in the enzyme,[43] TKA activity may be Suboptimal. An increase in TPPE of greater than 20 percent is unequivocal evidence of red cell TPP deficiency. After administration of large doses of thiamine given over a moderately long period of time to patients with abnormal TKA and TPPE, symptoms disappeared. The TKA returned to normal and TPPE decreased, in some cases to zero.[18] The symptoms were functional in each case and involved aggressive and sometimes violent behavior, illustrating the association of behavior with the quality of nutrition.

Unfortunately, TKA and TPPE do not

provide information on the activity of TTP. Its deficiency in the central nervous system may be responsible for symptoms even when these laboratory studies are normal. The only test of TTP deficiency at present available is finding a urinary inhibitory substance which is reportedly indicative of SNE.[7] As already indicated in this review, this test has been found to correlate with thiamine responsive conditions that do not demonstrate SNE clinical characteristics.[28 44] Raised concentrations of folate and vitamin B12, already mentioned, have been reported in two thiamine dependent children.[28] This may be a useful indicator of other biochemically induced nutritional deficiency, since it suggests inefficient or defective transmethylation.

Increased concentrations of creatine in urine occur in beriberi.[45] Creatinuria disappeared in patients treated only with bed rest and before thiamine became available. Creatine, formed in liver and kidney, is transported in blood and 90 percent of tissue concentration is in muscle. The other 10 percent is found in brain and testes. Its absorption probably requires an active transport mechanism and it is then "trapped" as creatine phosphate to provide a reservoir of readily available ATP. Creatinine is formed directly from creatine phosphate[46] and represents the turnover of these important compounds. Hence the ratio of creatine to creatinine in urine demonstrates the efficiency of this turnover. If there are large amounts of both creatine and creatinine present, it can be assumed that production is good and the normal absorption and trapping mechanism efficient. If both are in relatively low concentration the production is decreased. In both of these cases the ratio of creatine to creatinine would be normal. If the trapping or absorption is abnormal the ratio of creatine to creatinine increases in urine and is therefore a useful indicator of the abnormality of these mechanisms.

One of the earliest means of detecting thiamine deficiency was by the demonstration of increase in bisulfite binding substances in urine. Since TPP has a central role in decarboxylation, keto acid production increases. Animal experiments[47] showed that thiamine deficiency caused increased excretion of methylglyoxal. The glyoxalase pathway to D-lactic acid has two enzymes which are found in animal systems, though it may be that this represents an ancient and now atavistic mechanism in energy metabolism. Under anaerobic conditions it is possible that activation of this metabolic system occurs. Accumulation of lactic acid under such circumstances might be of no energy value since it would be in the D form. Detection of urinary methylglyoxal might be useful, but would probably be found only in severe prolonged thiamine deficiency.

Thiamine as a Therapeutic Agent

Current consensus of medical opinion holds the view that there is no value in using doses of vitamins greater than those considered to be physiologic, even if a deficiency can be clearly demonstrated by laboratory studies.[48] Exceptions to this are made in the treatment of specific inherited vitamin dependency conditions. Little consideration has been given to the concept of such a dependency being acquired, as was demonstrated in experiments with rats. Zinc deficient rats produced immunologically crippled offspring that could not be zinc reconstituted by providing them with an adequate dietary intake.[49]

Because of its poor absorption and endogenous activation, physiologic doses of thiamine may be totally ineffective in chronic deficiency states. Japanese investigators have shown that 20 mg of thiamine hydrochloride will result in recovery of as much as 13 mg in the stool.[50] There is no known storage mechanism for the vitamin in the body, so dietary sources must be continuously adequate to meet prevailing environmental circumstances. It is suggested that thiamine deficiency could result in a vicious cycle. Increasing failure of absorption would increase the deficiency, resulting in even worse absorption. Thiamine deficient animals excrete in urine an increased amount of creatine relative to creatinine,[51] and this may be a reflection of membrane dysfunction, as already discussed.

The dose of the vitamin for therapeutic purposes remains an open question, but there is ample reason to give very large doses with complete clinical safety, particularly when biochemical evidence of

deficiency is present, or even where a theoretical reason exists. For example, it has been used successfully in treating a child reported to have defective activity of pyruvic carboxylase.[52] The authors postulated that increased effectiveness of this biotin dependent enzyme resulted from positive stimulation of acetyl CoA from increased activity of thiamine stimulated pyruvic dehydrogenase. This then caused increased anaplerotic glu-coneogenesis and relieved hypoglycemia in the patient. It is possible that stimulation of the carboxylase with biotin might have produced the same result, but this was not attempted in this case.

A middle aged woman had complained for many years of symptoms considered to be neurotic in character. Tenderness in both gastrocnemius muscles, a physical sign seen in beriberi, suggested an underlying biochemical defect. Urine contained a large amount of TTP inhibitory substance[7] and her symptoms were appreciably improved with megadoses of thiamine hydrochloride. One month later the urine test for inhibitor was negative (Lonsdale, D. Unpublished observations 1982). Perhaps other similar cases are common and detection of an underlying biochemical abnormality becomes a matter of considerable therapeutic importance.

Allithiamine and Its Derivatives

Garlic is a time honored remedy for many conditions, including infections. It has been a remarkable fact throughout history that good clinical observations have frequently been borne out by the later approval of science. Garlic bulbs were hung around the patient's neck to treat sore throats, and poultices of mashed bulbs were applied to the feet. There is every reason now to suspect that this was a valid approach. Garlic contains an enzyme which attacks the thiazole ring of thiamine when the bulb is cut with a knife. Another sulfur atom is bound to the existing sulfur to create a disulfide bond and the thiazole ring remains open. The resulting compound is known as allithiamine, which possesses a remarkable ability to pass through cell membranes. Fujiwara[50] found that when an aqueous solution of thiamine was added to an extract of various plants, including garlic, and with pHadjusted to 8 and heated at 60 degrees centigrade, there was a marked loss of the characteristic thiochrome reaction. Thiochrome is a reagent used to detect the presence of thiamine and it was thought that the resulting solution would have lost its biologic action. Surprisingly, it was found to exert a powerful thiamine activity when administered to animals and the addition of reducing agents, such as cysteine, caused the solution to recover its thiochrome reaction. This led to the discovery of allithiamine, the prefix being intended to indicate its formation in plants of the allium species and not the allyl radical which occurs in the molecular structure.

A Vitamin B Research Committee of Japan undertook intensive research of this important discovery and experiments by members of this committee led to synthesis of a whole new series of alkyl derivatives of thiamine. The most important of these are thiamine propyl disulfide (TPD) and thiamine tetrahydrofurfuryl disulfide (TTFD). In general the thiamine alkyl derivatives are basic compounds, sparingly soluble in water, forming water soluble salts with one mole of acid, and readily reduced to thiamine by cysteine and other reducing agents such as glutathione. When administered to healthy male subjects under fasting conditions, it was shown that allithiamine (TAD), methyl allithiamine (TMD) and TPD all were better absorbed than water soluble thiamine hydrochloride (THCL). Thiamine excretion increases as much as eight fold after administration of these derivatives. After oral administration of 20 mg of TPD, 3 mg of thiamine could be recovered from stool.

By radioactive labelling of the sulfur in TPD it was shown that the prosthetic fragment remained outside the red cells and became bound to albumen in the serum, whereas thiamine concentration inside the red cells rose quickly in the first hour. From this it was deduced that TPD penetrates into blood cells quickly and it appears that the disulfide bond fractures at the cell membrane. The thiazole ring is assumed to close inside the cell, thus introducing an active molecule of the vitamin to the interior of the cell. Obviously this is an advantage in any situation where energy dependent cell membrane function is impaired, as has already

been suggested in thiamine deficiency.

The action of TPD has been well studied. Of potentially great importance was the discovery that it gave partial protection against cyanide poisoning in mice. After pretreatment with 1 mg of TPD intravenously, potassium cyanide was administered in a dose of 150 *u*g per 10 g body weight by stomach tube. The death rate of the TPD pretreated animals was 17.7 percent compared with the controls of which 70.6 percent died within 24 hours. It was reportedly effective in the prevention of trichlorethylene and lead poisoning. When TPD was infused into the lumen of dener-vated dog jejunum, peristalsis was observed, an effect that was not seen with THC1. This suggests that the fat soluble derivative has a cholinergic action which appears to be different from the water soluble vitamin.

Synthesis of a number of S-acyl derivatives of thiamine resulted in testing of three similar compounds; O. S-diacetyl thiamine (DAT), S-dibenzoyl thiamine (DBT) and S-benzoyl thiamine monophosphate (BTMP). These compounds are readily absorbed from the intestine, but appear to lack the ability to penetrate into cells like the disulfide preparations. Neither do they have a protective action against cyanide toxicity. It was concluded that the difference was due to the disulfide bond which the S-acyl molecule does not possess. The S-acyl homologues are reduced to thiamine by an action which takes place in liver and kidney, whereas the disulfides are easily reduced in the presence of cysteine or glutathione.[50]

These vitamins introduce a vast new dimension in thiamine pharmacotherapy which has great promise in the treatment of a number of human disorders which are presently untreatable, or only treatable by means of potentially toxic agents. The author has held an independent investigator license for a number of years in order to study TTFD. Some of the clinical experience supports the principle claimed by the Japanese investigators. Its cell membrane penetrability is demonstrated by the fact that a drop of TTFD solution, placed on the tip of the finger, can be tasted within 30 seconds. In attempting treatment in more than 90 patients there was no evidence or directly harmful effects due to toxicity. Brief functional symptoms in the early stages of treatment occurred in a few patients, but they appeared to be the same symptoms that had afflicted the patient before administration began, only exaggerated in some cases. For example, four infants were treated because they were experiencing repeated episodes of life threatening nocturnal apnea.[53] One of these infants was injected intravenously with 25 mg of TTFD solution. While the infusion was being injected, she had a brief apnea similar to the ones observed during sleep. Her ultimate recovery from recurrent apnea, like the other three infants, was considered to be due to improved biochemical efficiency in brainstem. This conclusion was supported by serial examinations by means of brainstem auditory evoked potentials.

Other apparently harmful effects were probably due to its use as though it were a conventional drug. If given singly in an attempt to identify true efficacy, it behaves in a fashion similar to any supplementary nutrient. It appears to create demands for any one or more of the vitamins and minerals which constitute the nutrient "team". For example, a middle aged patient was treated for diabetic neuropathy with 150 mg a day of TTFD by mouth, since there is a published precedent for its use in diabetic neuropathy. No additional vitamins were administered. Heightened facial color and improved color in lip vermilion suggested increased arterial oxygen tension. After several weeks of treatment, he returned with severely compromised circulation in both lower legs, which threatened ultimate gangrene. A high potency multivitamin was started and the circulatory danger was no longer present within 48 hours. The metabolic response of this patient was monitored by repeated sampling of urine and measurement of creatine and creatinine. The ratio of creatine to creatinine was consistently lower when he was receiving TTFD, though this could only be observed by comparison of this ratio when receiving or not receiving the agent. If this interpretation is correct, and there certainly appears to be clinical evidence that it is, then it suggests the great difficulty of determining whether an observed vitamin or mineral deficiency is primary or secondary. This is obviously an important consideration

in the interpretation of hair analysis, for example. It may frequently be true that the mineral balance is severely compromised by a vitamin defect which has not even been considered from a clinical standpoint. An example of this metabolic riddle has already been discussed in relation to elevated concentrations of serum folate and B12[28].

Animal studies have been used to study TTFD. Audiogenic seizures occur in the weanlings of DBA/2J mice at about 19 days of age and spontaneously disappear at about 26 days of age. Experimental evidence[55] suggests that audiogenic seizures in rodents are cholinergic in origin. Experimental administration of TTFD to weanlings of the DBA/2J strain resulted in increased audiogenic sensitivity and prolongation of the time to natural remission.[56] This suggests confirmation of the evidence that TTFD enhances or stimulates cholinergic neuronal activity.[50]

Other animal studies have been performed on isolated heart preparations. Atrial contractions in guinea pig heart that had been arrested with high concentrations of potassium could be restored with TTFD.[57] In a potassium free solution, atrial contractions were arrested within 30 to 90 minutes and this action could be delayed in the presence of TTFD. When atrial contractions were arrested with quinidine they could be restored with TTFD. This effect was observed only weakly with water soluble THCl. This suggested that either the disulfide bond in TTFD or its prosthetic group had a powerful effect on potassium influx and efflux into excitable membrane.

A number of thiamine derivatives were shown to have a positive inotrophic effect on toad heart,[58] the strongest being TPD and TTFD. Both had a positive inotrophic and negative chronotropic effect on isolated guinea pig atrium with little or no similar effect from THCl.[59]

Isolated guinea pig small intestine was treated with TTFD.[60] Decreased muscle tone and frequency of contraction of smooth muscle was observed, but individual contractions were noted to be increased in amplitude. Repeated doses of TTFD partially protected rats and mice from damage induced by whole body irradiation[61] and exerted a modifying effect on carrageenin induced rat paw edema after pretreatment by intraperitoneal injection.[62] Large doses of TPD modified the progressive neuropathy induced in rabbits by acrylamide, and accelerated regeneration in experimentally severed sciatic nerve.[63]

A number of clinical benefits have been reported, most of which is in the Japanese literature. Non specific muscle pain,[64] diabetic neuropathy,[54] nerve deafness[65] and paralytic ileus[66] have been investigated. These clinical experiments need to be repeated and investigated further, since they strongly suggest that the clinical efficacy of these agents has to be identified better. The evidence indicates that nerve function is improved, probably by serving a vital part in energy metabolism.

The widespread deficiency of thiamine reported in psychiatric patients[67 ^ 69] gives rise to the question whether this is a primary causative factor or a secondary result of malnutrition resulting from appetite disorder. There is nevertheless frequent benefit experienced by such patients and there seems to be no reason to withhold the vitamin, particularly when there is laboratory evidence that such deficiency exists.[69] Baker and associates[70] tested TTFD and TPD in normal human subjects and alcoholics comparing them with the effect of THCl and TPP. The differences were shown to be significantly in favor of the fat soluble compounds, apparently related to increased efficiency and rapidity of absorption.

Indications for use of allithiamine derivatives have not been well established. It seems clear that their use goes beyond considering a simple dietary deficiency and that laboratory guidance can be the strongest indicator. Their remarkable safety and lack of toxicity makes a clinical trial reasonable if thiamine dependent chemistry is implicated, irrespective of the organ system involved or the superficial diagnosis. Unfortunately, TTFD is not FDA approved and there has been no evidence of interest from American drug manufacturers.

Summary and Conclusions

It is becoming increasingly apparent that vitamins and trace elements are powerful therapeutic tools. Any physiologically essential nutrient, if increased in dose, becomes a drug, and its potential toxicity is a function of dose and length of exposure time. For

example, selenium has a narrow range before becoming toxic, whereas thiamine, like oxygen, has a wide range. Large doses of both oxygen and thiamine have potential toxicity. Vitamins and trace elements are members of a complicated nutrient "team" and adverse effects can be observed from secondary deficiency of nutrients other than the one that is administered singly. This is one of the present hazards of the current wave of self administered nutrients.

As our knowledge of vitamin therapy increases, it is becoming clearer that the basic common denominator of health is the appropriate and efficient use of intracellular oxygen. As the vital oxidant it is useless in mammalian metabolism without thiamine and all the other catalysts which govern the process of oxidation. Peters[71] showed that pigeon brain brei used oxygen at the same rate in thiamine deprived and thiamine fortified tissue, until glucose was added. The acceleration of respiration in the thiamine fortified brei was in stark contrast to the lack of response observed in thiamine deficient cells. Peters called this the catatorulin effect. He noted also that thiamine was in heavier concentration in the lower brain of pigeons and this appears to be true for the distribution of TTP in human brain. Since 20 percent of the oxygen needs of the body are due to brain function, behavior and adaptive organization of the organism are vitally related to the presence of thiamine and the other agents which result in redox. Thiamine deficiency in nerve tissue has serious consequences since automatic brain control centers in the hypothalamus and brain stem must maintain vital and endocrine coordinating mechanisms which function even during sleep. An abundance of caloric nutrients, without the capacity to oxidize them appropriately, compromises normal function of these cells. A diet may appear to contain adequate vitamin concentrations unless considered with reference to total calories.

It may well be that a currently adverse nutritional deficiency in America is encouraged by the ingestion of large quantities of high caloric fluids, particularly since they are often regarded as sources of quickly generated energy. It is obvious that health education must emphasize these fundamental facts since nutritional consequences appear to make society as a whole pay heavily.

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