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MODIFIED THIAMINE COMPOUNDS*

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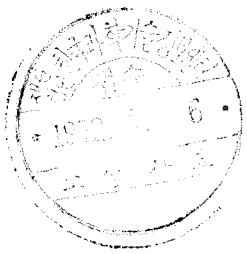

Once beriberi was prevalent in Japan especially among young people in the urban areas from 1915 to 1930. Dr. Umetaro Suzuki, pioneer biochemist in vitamin research, published in 1911 that an active fraction isolated from rice bran to cure bird polyneuritis might be an entirely new factor for animal growth. First he named it "Aberic acid" for its presumable activity to prevent beriberi. Since the date, Japanse scientists have made their continuous efforts o conquer beriberi; first by educational measuees thru undermilling of rice from 1930 to 1940. Undermilling was so effective that the beriberi death rate had been reduced to 10 per 100,000 population in 1940(Fig. 1). The second step was synthetic supply of thiamine to cure beriberi. I don't know exactly when the first synthetic thiamine was produced in Japan but the synthesis was promoted by several groups of pharmaceutical houses. After the War, Japanese pharmaceutical industries had been revived thru the synthetic production of thiamine by improved processes such as thiothiamine process. Tons of thiamine synthesized, gave the successful triumph to eradicate beriberi from Japan, when the synthetic thiamine supply per capita had filled the daily requirement of 1.5mg. The multiplied production of thiamine resulted in reduced price, more vitamin pills and enriched foods. Soon beriberi disappeared from the statistics of death-causes. Increase of enriched rice followed the surplus supply of thiamine and it is a good measure to prevent deficiency diseases among the people (Fig. 1).

After the complete conquest of beriberi. the syntheteic production of thiamine has been tremendously expanded; as the result, thiamine is now a very cheap, easy-to-buy chemical like as glutamate or table-salt. Last year(1970) more than 530 tons of thiamine were produced in Japan; the amount is sufficient to meet one billion people's requirement of thiamine per year. A handful of thiamine is the safe guard of the nation against beriberi and deficiency diseases related with thiamine. As ordinary salst of thiamine is readily soluble in water, easily decomposed by alkaline additives such as bicarbonate and having a odor like rice-bran, more stabilized modified thiamine compounds have been interested and developed by several groups of chemists in Japan: The modified thiamine compounds were discussed in the previous review

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by the author.[1]

Thiamine contains sulfur in the molecule and the sulfur can react as its sulfhydryl radical when the thiazole moiety is opened in alkaline medium. (Chart 1)

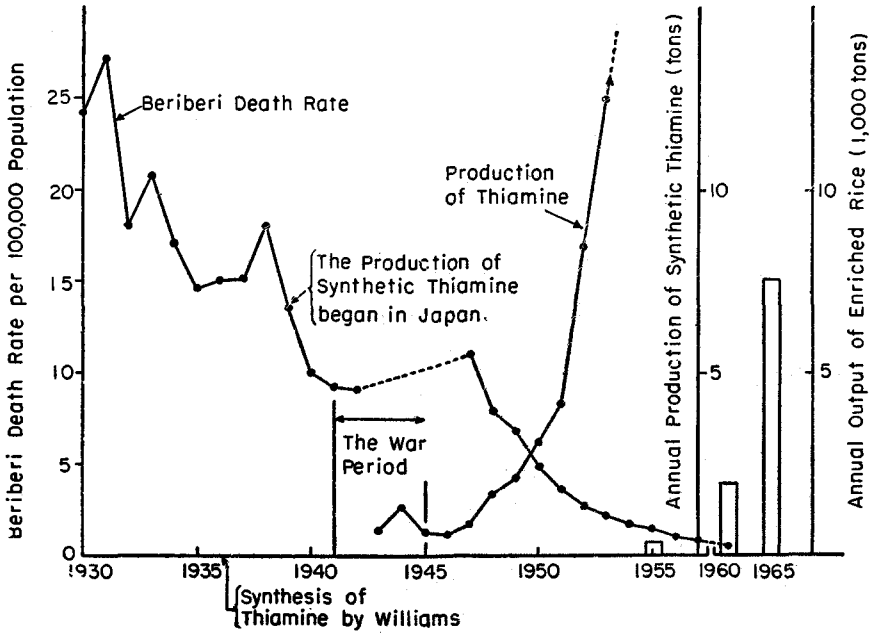
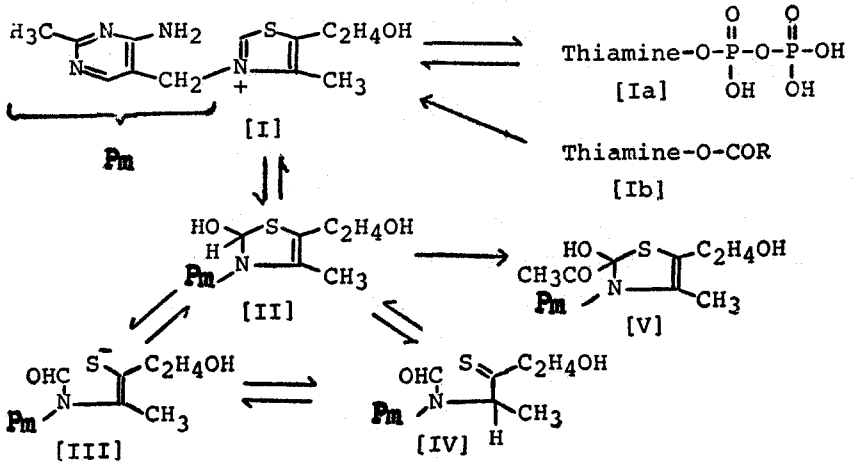


Fig. 1. Death Rate Caused by Beriberi and the Annual Production of Synthetic Thiamine in Japan.

CHART 1. Modification of thiamine molecule



Early in 1940, modification of thiamine to its oxidized compound; thiamine disulfide(TDS) was demonstrated by Zima and Williams. the disulfide, just comparable to cystine, is easily

reduced to thiamine by cysteine and other sulfhydryl compounds. As pairs of reduced and oxidized forms of vitamins such as ascorbic-dehydroascorbic acids are found in nature, the redox system between thiamine and TDS was postulated. TDS pyrophosphate, however, failed to show coenzymatic activity unless reuced to thiamine pyrophosphate. TDS seemed to be improbable to be found in nature, although its occurrence in nature has been claimed by several groups of scientists. TDS is an artifact and easily reduced to thiamine when given to animals.

Another kind of disulfide of thiamine; Allithiamine was isolated in the reaction of thiamine with a garlic extract by Fujiwara[2] in 1952. The compound is not found in Onium plants; this is also an artifact produced by the the thiolexchange of allicin with thiol-thiamine(Matsukawa)[3]. Series of related disulfide compounds were synthesized by Matsukawa [4] and his group. S-Acylated thiamine compounds were introduced by acylation of thiamine instrongly alkaline medium. These new types of modified thiamine compounds developed by Matsukawa were consequently monopolized by Takeda Chemicals; only thiamine propyl disulfide(TPD), its improved form; tetrahydrofurfuryl disulfide

CHART 2. Reaction of thiol-type thiamine in the alkaline medium

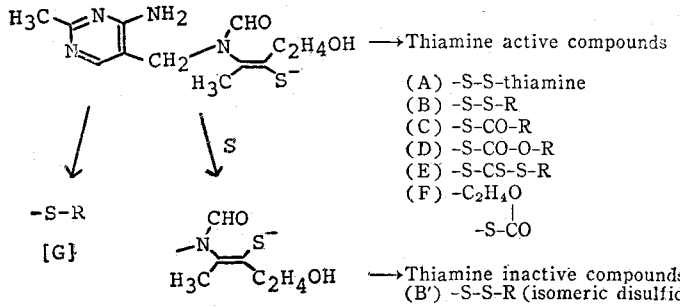


Table 1 The modified thiamine compounds reversible to thiamine *in vivo*.

Type	Compounds	Conversion to thiamine by cysteine
(A) {-S-S-Thiamine, -C_2H_4O-COR}	O-Benzoylthiamine disulfide(BTDS) O-Butylthiamine disulfide O-Nicotinylthiamine disulfide	##
(B) {-S-S-R, -C_2H_4OH}	Thiamine propyl disulfide(TPD) Thiamine hydroxyethyl disulfide Thiamine tetrahydrofurfuryl disulfde(TTFD) Thiamine S-pyridoxyl disulfide Thiamine methyl-6-acetyl-dihydrothioctate disulfide	###
(C) {-S-COR_1, -C_2H_4O-COR_2}	O,S-Dibenzoylthiamine(DBT) O,S-Diacetylthiamine(DAT) O-Acetoglycolyl-S-furoyl-thiamine O-Nicotinyl-S-isobutyroyl-thiamine	##
(D) {-S-COR_1, -C_2H_4O-R_2}	O-Benzoylthiamine monophosphate S-Salicylthiamine monophosphate	+
(E) {-S-CO-OR_1, -C_2H_4O-R_2} (F)	O,S-Diethoxycarbonylthiamine S-Ethoxycarbonylthiamine O,S-Cyclocarbothiamine	+
(G) {-S-R_1, -C_2H_4O-R_2}	S-Benzoylvinylthiamine O-Acetyl-S-acetoxymethylthiamine S-Triphenylmethylthiamine	? (hydrohyzed to thiamine)

(TTFD) and O,S-dibenzoylthiamine(DBT)were introduced on the market.

In 1961, Takeda's monopoly of thiamine compounds was disrupted by several groups of pharmaceutical houses thru the development of various kinds of modified thiamine compounds.

The typical types of compounds are shown in Chart 2 and Table.1

The severe competition among these modified thiamine preparations, however, stimulated an explosive sale of Takeda's preparation;Alinamin(TPD and TTFD) in parllel with promotion of other branded products. These modified thiamine compounds were no more "vitamin," rather they were emphasized as health tonic and therapeutic agent. This was a gold rush for pharmaceutical industries in Japan from 1962 to 1970. Why gold rush? The reason why the modified thiamine compnunds were golden products, is their wide range of clinical application and their acceptance in the national medicare system. The first modified compound;TDS behaves to animals just in the same pattern of absorption as thiamine;no differnce of intestinal absorption between TDS and thiamine. Both thiamine and TDS show the plateau of limited absorption in the oral dose over 10 mg but TPD,TTFD and other new modified compounds were proved to be unlimitedly absorbed from the intestine in response to the increasing doses. Better absorption was simply interpreted as better effectiveness of the preparations and consequently massive dosage of these compounds over 25 mg was recommended for clinical application. The booming sale of modified thiamine compounds(57 millionYen) was marked in 1970 on the market. Massive dosage of modified thiamine compounds was originated from the first trial in Europe; a single dose of 100 mg or daily up to 300 mg was reported to be effective for alcoholic polyneuritis and neuralgia in coping with subclinical thiamine deficiency caused by drinking. The disturbance of peripheral nerve systems is closely connected with thiamine deficiency and improvement of the syndrome may be expected by massive dosage of modified thiamine compounds.

Japanese people like to take vitamin pills; medical doctors had tried injection of thiamine preparations to patients so often that the people were accustomed to take thiamine as health tonic. New modified thiamine tablets of easily absorbable, long-acting property were welcomed by the people. Massive dosage of modified thiamine compounds over 100 mg was also accepted in the medicare system and as the result, there happened an Explosion of the sale of preparations for prescription and over the counter. The modified thiamine compounds are recommended chiefly for the improvement of neuralgia and pains of various origins. They are 100% effective only when subclinical thiamine deficiency is combined. The over dosage of these compounds when the patients are saturated with thiamine, is not rational prescription but quite safe and non-toxic. As compared with cortisone and other pain-relieving agents, massive dosage of thiamine compounds was the first preference for safe medication. This is the reason why these compounds are prescribed so frequently

Modified thiamine compounds, when absorbed from the intestine, are quickly reduced to thiamine and no different from thiamine in blood. Some of the biochemical reactions of modified compounds in vitro can not be observed, when the compound is given to animalsi For example, positive inotropic and negative chronotropic action of TPD or TTFD on isolated atrium is a biochemical reaction of the compound in vitro and no more the compound

in vivo to react with atrium. One of the modified compounds of Type C in Chart 2; S-benzoyl thiamine phosphate was demonstrated to be reduced to thiamine thru a transient intermediate (S-benzoylthiamine) in the process of intestinal absorption and immediately after the absorption at the higher dosage.[5] The model experiments using suspension of erythrocytes in saline showed different pictures of absorption and reaction of three types (A,B and D in Chart 2) of compounds with erythrocytes. Less revertible thiamine compounds can be detected as themselves and thiamine in blood cells while TPD or TTFD is found as thiamine in cells because of its easy reduction to thiamine. As pharmacological agents, further investigation of less active compounds, slowly revertble to thiamine, would be interesting.

Production of modified thiamine compounds in Japen was marked over 270 tons in 1970 and the amount, if consumed in Japan, is too enormous; 7mg per capita per day or 30mg per patient, if used for prescription, to be rational for the therapeutic use of compounds. The booming sale of the compounds is slowly down and some of the patents covered by Takeda's group will be expired soon. Now it is the time to check the compounds again to re-evaluate them from the Standpoints of nutrition.

Why should we modify thiamine? It requires at least one additional step to modify thiamine but the modification means stabilization and improvement of physico-chemical properties of thiamine. The price of thiamne is so cheap that the modification doesn't change the substantial price; it is rather negligibly low in comparison with the price of needed protein or calories. As food additives and pharmaceuticals, acetyl or benzoyl compounds are safely metabolised in the long term tolerance and DBT and O-benzylthiamine disulfide(BTDS) are recommended for enrichment program; they are superior to thiamine in their stability, insolubility, odor lessness and better absorption. I had developed two simple ways of preparing rice-premix using BTDS; the compound is practically insoluble in water but readily soluble in dilute acetic acid. After soaking of rice with BTDS in acetic acid, BTDS is made insoluble on exposure to ammonia. If puffed rice is used, BTDS or DBT can be penetrated into the grains on shrinking of rice with the solution. The dried premix is resistant to washing loss as compared with coated premix of thiamine salt. The commercial producer of the compounds, however, is more interested in emphasizing its therapeutic purpose because of profitable massive dosage as pharmaceuticals than rational use as nutrient.

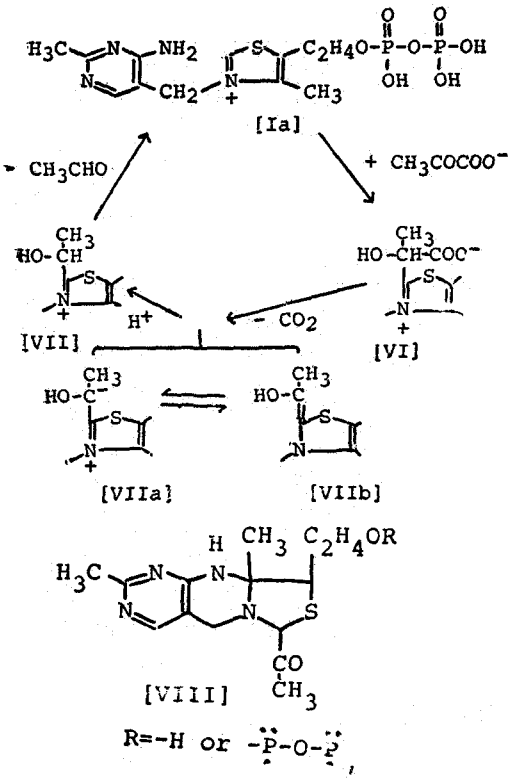
Another example of an acceptable compound may be O,S,-diacetyl thiamine(Compound Type C in Table 1)(DAT). The thiamine levels in goats, milk were elevated quickly as compared with other modified compounds, when DAT was applied to animals by injection. Orally administered to human subiects, there was no difference between DAT and other modified compounds. Infantile beriberi is still found in the developing areas and the modified thiamine compounds should be used for the eradication of thiamine deficiency. As the policy of pharmaceutical houses in Japan, each company had picked up only one featured compound. There are many sleeping compounds, among which some interesting compounds have been left in file. We should awake them from the standpoints of nutrition.

An interesting modification of thiamine is a trans-form of thiol-thiamin in the reaction of

an alkaline solution of thiamine with sulfur.[7] The isomeric thiol-thiamine can be isolated as pure crystals only when converted to its butyl disulfide. (Compound B, in Chart 2) The compound was shown to be inactive as thiamine on animals because of its resistance to the reduction to thiamine in vivo. We can conclude that only the modified thiamine compounds revertible to thiamine in VIVO are active thiamine compounds.

Again returning to the thiamine molecule, two inportant points for its biological activity will be emphasized; first, the hydroxyl side-chain to be phosphorylated and second, sulfur in the thiazolium to be connected with C_2 atom. Matsukawa[8] and some Japanese scientists postulated S-acetylated thiamine pyrophosphate as an intermediate in enzymatic reactions but this was not proven. Later Krampitz[9]

CHART 3. α-Hydroxyethylthiamine as an intermediate of pyruvate decarboxylation

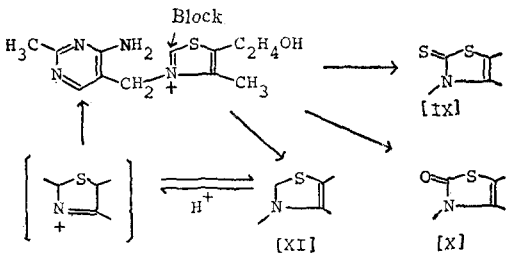


and other groups of scientists demonstrated aldehyde adducts at C_2 as active intermediates in the process of decarboxylation of pyruvates etc (Chart 3).

Hydroxyethylthiamine(HET) was synthesized in its pure form having full activity of thiamine. As HET is pretty stable in acid, the hydroxyethyl radical has been partly remained in the molecule after the metabolic cycle in animals or microorganisms because we were able to detect a small ammount of HET beside thiamine in the urine or the cells when HET was given to human subjecst or Lacto bacillus fermenti. Two interesting compounds related with HET were introduced by Japanese scientists; aceto-pseudo-dihydrothiamine(Compound VIII in Chart 3) by Dr. Hirano and aceto-pseudo-thiamine (Compound V in Chart 1) by Dr, Takamizawa. Both the compounds are easily converted to thiamine by mild acid treatment but they were shown to be less potent or inert as compared with thiamine on animals or Lactobacillus fermenti while HET is fully active as thiamine. Both the compounds in psudobase-form are blocked at C_2 and unable to form reactive carbanion to combine with pyruvate while HET shall be combined with another mol of pyruvate.

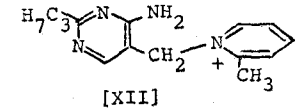
To demonstrate the importance of C_2-position for biological activity, thiamine has been transformed into dihydrothiamine, thiothiamine and oxo-thiamine; (Componds XI IX and X in Chart 4) these compounds are inactive because they are blocked at C_2 by hydrogen, oxygen or sulfur. Although the first two compounds can be converted to thiamine by oxidation, there occurred no biological oxidation of them to thiamine in vivo. These modifications of thiamine are good examples to realize how thiamine has the strict structure specificity keep its biological activity.

CHART 4. Inactivation of thiamine by blocking C_2 of the thiazole moiety

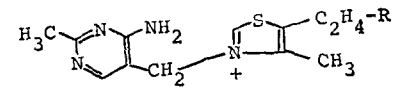


Further modification of thiamine is now directed toward permanent alternation of the molecule; this means complete loss of activity and in some cases to be competitive to thiamine. In the early stage of studies on metabolic inhibitors, such compounds, Analogous to thiamine as 2′-butylthiamine, 4′oxythiamine and pyrithiamine were introduced as antagonists. to thiamine. These classical antagonists have he side-chain to be phosphrylated; the competion agaist thiamine will be observed not only at the stage of absorption but also at the further stage of phosphorylation and coenzymatic action. If the hylroxyethyl side-chain is blocked or modified, the compounds may be antagonistic to thiamine only at the stage of absorption. Since Rogers [10] had developed Amprolium (Compound XII inChart 5) as anticoccidant for poultry, the modification of the sidechain was interested by Japanese scientists; now three kinds of anti-thiamine compounds are available as anticoccidants (Chart5). Dimethalium(XIV) and chloroethylthiamine(Beclotiamine)(XIII) are similar in the mode of competition against thiamine in coccidium and Dimethalium11) is hardly absorbed by chicken. Beclotiamine [12] is not antagonistic to thiamine on animals because the compound is partly converted to thiamine. The two compounds, when used for poul poultry, are quite safe either to chicken or as

CHART 5. Anticoccidant compounds (thiamine analogues and derivatives)

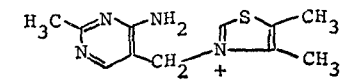


[XII]



R=-Cl [XIII]

R=-SO-CH_3 [XV]



[XIV]

human food. O-benzoylthiamine (OBT) (Compound Ib in Chart 1) is also pretty strong to inhibit the growth of Lactobacillus fermenti at the presence of thiamine and less absorbable from the intestine of rats. OBT was expected to be anticoccidant to chicken but unfortunately it is absorbed easily by chicken and no more anticoccidant. [13] Anti-thiamine compounds are important to clarify the mode of thiamine action in connection with practical use of anti-thiamine compounds and further investigation and development are needed.

In concluding my discussion on modified thiamine compounds, it should be pointed out that the chemical structure of thiamine is too rigid to alter minor parts of the molecule in keeping its full activity. At the first stage of research, synthetic approach to produce natural vitamins in quantity had made it possible to be easy-to-buy chemical to the people. When vitamine are supplied by tons, the second step of research is designed to improve physico-chemical properties of vitamins; the modified thiamine compounds of disulfide and S-acylated types

developed by Japanese pharmaceutical houses are successful modifications of thiamine. Merits of the modified thiamine compounds should be properly estimated as improved thiamine for vitamin supply and not over-estimated as cure-all remedies. As the final step of modification, the permanent alternation of the molecule is expected to be more specific as parmacological ag-ents but less potnent as vitamins. As the starting material, thiamine is very cheap and probably low in ths toxicity after its modification. Biochemists and pharmacologists should explore the secret of thiamine activity to make further advances in developing new pharmacological agents.

Sixty years ago, vitamins had been discove red but the real virtues of vitamins had not been recognized by many of scientists until the first War broke. The War II changed the image of vitamins as synthetic food additives for food processing. Now production of synthe etic vitamins in one factory can cover th-world's demand for vitamins, and synthetic supply of vitamins should be considered in the-education of food and nutrition. Dr. S zenc György, Nobelprize biochemist, isolated ascorbi-acid in pure crystalline form: he joked about vitamins in his recollection. [14] "Vitamin means that one has to eat it. What one has to eat is the first concern of the chef, not the scientist." So he gave his crystals to organic chemists for the elucidation of the c chemical structure. I would say: what one has to eat should be improved by the scientists, not by the chef. Vitamins must be used as nutrition-improver, just as glutamate is popularly used as taste-improver because the both are supplied by tons.

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