INTRODUCTION

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ANTIVITAMIN-CONCEPT

The exact meaning of the term antivitamin is controversial. According to the classical definition of Woolley (1) and Shaw (2), an active agent should be considered an antivitamin only when the following criteria are fulfilled :

a. Similarity of chemical structure.

b. Similarity of symptoms produced by the antivitamin and by the lack of the corresponding vitamin.

c. Competitive in the effect with respect to the vitamin.

However, a number of compounds that are antivitamins do not fulfil one or more of these criteria. It seemed necessary to extend the definition of antivitamin without the extension of the concept, a great number of substances - nearly all naturally occurring vitamin antagonists - could not be considered antivitamins, e.g. avidin, the antithiamine factors of fern, carp, rice bran, mustard seed and certain antagonists of pyridoxin and vitamin D. Some of these were the very first active agents that were reported showing biological antagonism.

Somogyi (3) subsequently proposed to divide the antimetabolites and antivitamins into two groups : structurally similar compounds i.e. antivitamins as a specific type of antimetabolite, and structure modifying antivitamins i.e. substances, mainly of biological origin, that destroy or decrease the effect of a vitamin as by modifying the molecule itself or forming complexes with vitamin. This classification parallels that used for the inhibitors in enzymology. The structurally similar antivitamins

correspond to the competitive inhibitors, and the structure modifying antivitamins to the noncompetitive inhibitors.

Accordingly, an antivitamin may be defined as a compound that diminishes or abolishes the effect of a vitamin in a specific way.

Mellanby (4), in 1926, was the first to report an antivitamin action of certain cereals to be antagonistic to vitamin D. Similarly, avidin (present in egg white) was demonstrated having antagonistic action against biotin by several workers (5,6,7). Later on, the presence of antivitamin compounds was further established when Green (8) described the presence of antithiamine compound in the viseera of raw fish, and Woods (9) proposed the mechanism of action of the sulfonamides as antagonists of p-amino benzoic acid.

ANTAGONISTS OF THIAMINE

The conception of thiamine antagonists appeared in the literature as early as 1936 when Green (8,10) for the first time described the so-called 'Chastek paralysis' as a thiamine deficiency that appeared in silver foxes eating raw fish. As a continuation of this work Evans et al (11) then demonstrated that the so-called 'Chastek paralysis' of foxes could be prevented by administration of 10 mg of thiamine per day. Similar thiamine dificiency condition was also observed in chick when raw fish was given to them (12) — and even incubation of thiamine with raw carp fish intestine for fifteen minutes resulted in a lose of 50-100% of the biological activity of thiamine. Seelock and Goodland (13) confirmed the enzymatic nature of this toxic factor present in fish. The destructive nature of this factor was inhibited by a number of metal ions such as Cu^{++}, Zn^{++} etc. and certain organic compounds, as for example, iodoacetic acid, cysteine etc. According to Krampitz and Woolley (14) the isolated enzyme from the fish consisted of two parts-one was heat-stable, dialysable and the other was heat labile, non-dialysable. This enzyme was later termed as "Thiaminase" by them. Acute thiamine deficiency was induced in adult sheep by inclusion of low temperature dried, milled bracken rhizomes in diet which was a potent source of thiaminase (15). Autoclaving the rhizomes powder its avitaminosis B_1 capacity could be abolished.

Although antithiamine factors occur predominantly in the viscera of carp (cyprinidiae), these are also found in betel-nuts and in other fresh water and marine fish (16). Thus, antithiamine compounds were recognised by Deutsch and Hasler (17) in 15 of 21 species of fresh water fish (including 4 species of the carp family), by Neilands (18) in fish of the water of Nova Scotia and by Jacabson and Azevedo (19) in fish from the water of Portugal. Twenty species of fish available in Burma, two species of calms, crusteaceaus and their products were analysed for enzymic and thermostable antithiamine activity (20). Viscera had higher antithiamine activity than that of muscle of those fishes. Thiaminase was present in viscera of 4 species of fish tested whereas the heat stable thiamine inactivating factor was present in only two species. It was shown that the occurrence of heat labile thiaminase in fishes was much higher than that of heat stable antithiamine factors still not characterised. The thiamine activity of Crayfish (21) and Skip jack Tuna (22) was destroyed on cooking

and drying as well as on cold storage. Besides, the viscera of the carp fish, its spleen, liver, heart muscle and intestines also possess the antithiamine activity (23, 24).

Sure and Ford (25) detected the decomposition of thiamine by animal tissues as early as 1943. But, for many years there was a general feeling that no antithiamine compounds were present in the organ of warm blooded animals. According to Bojo (26), negative results were obtained from testing the tissues of pigeons, guinea-pigs and cattle. Somogyi (27) was able to recognize antithiamine activity in organs of rabbits and hens, particularly in spleen and heart muscle extracts. In contrast to carp, the intestinal extracts of warm blooded animals activated thiamine only to a very slight degree. Striated muscles from these animals also showed no antithiamine activity.

In an extensive study Bhagvat and Devi (28) detected the presence of thiamine-inactivating factors in different food-stuffs viz. Ragi *(Eleusine coracana)*, rice-polishing, a kind of bean *Phaseolus radiatus*, bazra *(Penniesteum typhodium)*, wheet germ, soyabean *(Glycine hispide)*, cowpea *(Vigna catiang)*, cotton seed *(Gossipium Sp.)*. mustard seed *(Brassica juncea)*, linceed etc. The activity was confirmed by thiochrome method as well as experiments (29) with mosquito larvae, rats and guinea pigs. The nonenzymatic nature of the thiamine inactivating factors present in these food-stuffs was also predicted by them. They showed that the extract obtained with 5% chloroform-water mixture from ragi was resolved into two components on dialysis - one was heat stable, dialysable and the other was heat labile, non-dialysable. Chaudhuri (30) also confirmed the presence of a heat stable thiamine inactivating factor in different varieties of rice and rice-bran.

Weswig and co-workers (31) produced experimental 'fern poisoning' by feeding bracken fern *(Pteridium equilinum)* to rats, just as it occurs in cattle and sheep. High thiamine doses cured the sick animals. Watanabe (32) and Parsons (33)separately demonstrated reduced thiamine excretion in human subjects given 15-20 g bracken fern per day. Haag et al (34) showed that the antithiamine factor of bracken fern was very stable to heat, it was water soluble but was not soluble in ether, petroleum ether, acetone or ethanol. Inactivation of thiamine by bracken fern extract produced rapidly (35). Further studies by various investigators showed that the bracken fern extract inactivates thiamine in vitro (36), that bracken fern has higher activity than other ferns (37-39) and that the thiamine antagonist of ferns is a small, thermostable molecule that, in electric field, migrates exclusively towards the anode (40).

Moore (41) observed the paralysis of legs of swine by feeding a diet containing mostly of rice- bran. Williams (42) studied the effect of commercial byproducts of rice-milling on pigeons and rats and found that rice-milling byproducts were harmful to pigeons and rats when fed a diet cor taining more than 65% of these products. He then predicted the presence of a thiamine-inactivating fac or in rice-milling byproduct. The existence of toxic substances in wheat-germ and other wheat products, which caused nervous disorders was recorded by McCollum (43) as well as Hart et al (44). Rommel and Vedder (45) also observed cotton seed poisoning in swine. A thiamine-inactivating effect was also detected in black berries, blue berries, black currants, red cicerone, red beets, brussel sprouts and red cabbage ; somewhat smaller amounts were found in watercress, green cicerone, kefen, spinach and black cherries (46). Hilker et al (47) made some extensive studies on thiamine-

inactivating factors present in various types of tea such as jasmine, colong, naganium, shui, black tea and instant tea. These workers demonstrated that thiamine-inactivating factors of these teas appeared to be related to the tanin content.

Although the presence of antithiamine compounds in different food-stuffs and other sources was established, the exact mechanism of inactivation of the vitamin is still not clear. The thiamine inactivating factors in the natural sources may also be called antithiamine compounds. According to Chaudhuri and his associates (48), the antithiamine compounds may be classified broadly into two categories, such as :

(a) Synthetic (structural analogues or antimetabolities)

(b) Natural (non-structural analogues and mostly present in different food-stuffs)

(a) Synthetic antithiamine compounds

Most of these antithiamine compounds in this category are structural analogues to the thiamine molecule (49,50). The mode of actions of these compounds towards the inactivation of thiamine are somewhat different from that of natural antithiamines (51, 52). Different types of structural analogues of thiamine molecule behaving as antithiamine compounds are given below.

Pyrithiamine type (53)

Replacement of the thiazole moiety in the thiamine molecule gives an important structurally analogue known as pyrithiamine in which a pyridine ring is attached to the pyrimidine through the methylene bridge. It was found that this compound played an important double inhibitory role on (i) thiamine absorption (54) as well as (ii) thiamine phosphorylation (55) *in vivo*. Several enzymes such as pyruvic decarboxylase from wheat germ (49) and yeast (56, 57) as well as acetoin synthetase (58) were inhibited by pyrithiamine pyrophosphate derivative. Pyrithiamine can also penetrate the brain tissue as a result of which there is the blocking of thiamine pyrophosphate biosynthesis.

In the cell free extract of *S. aureus*, thiaminokinase was strongly inhibited by pyrithaimine resulting in a deficiency of thiamine pyrophosphate in the system (59). Das and Chatterjee (60) prepared a pyrithiamine dependent mutant strain of *S. aureus* and its enzymatic pattern was also studied.

Oxythiamine type (61)

The replacement of amino group at the 6-position of thiamine molecule by a hydroxyl group gives rise to an active antagonist called oxythiamine, which gives deficiency symptoms in mice. Similar to oxythiamine its pyrophosphate derivative is also potent inhibitor of thiamine pyrophosphate requiring enzymes such as wheat-germ carboxylase and acetoin synthetase (62). The conversion of thiamine to thiamine pyrophosphate was less affected by oxythiamine (63). The growth of thiamine requiring bacterial species such as *Lactobacillus fermentii* and *Kloeckera brevis* was affected only at a high dose of oxythiamine (64, 65). Oxythiamine had a greater toxic effect to *S. aureus* in comparison to pyrithiamine (66).

Amproleum type

Several antithiamine compounds belonging to this group (67, 68) have been recorded. Amproleums have similar structure as pyrithiamine. Due to the absence of β-hydroxy chain in these compounds, they can't form their pyrophosphate derivatives. The absorption of thiamine was affected by amproleum in chick (69). In presence of amproleum, thiamine was unable to penetrate the cell membrane, consequently the phosphorylation of thiamine by thiamine kinase was inhibited resulting ultimately in the inactivation of thiamine biologically (70, 71).

Deoxy and ethyl deoxy thiamine

These compounds showed thiamine antagonistic activity (72). The biological activity of these compounds, which are structural analogues of thiamine, were tested on the growth of thiamine requiring L. *fermentii* and *Kloeckera apiculata.*

O-benzoyl thiamine and its derivatives

The antithiamine activity of the structural analogues such as 0-benzoyl thiamine (73), 0-S-dibenzoyl thiamine (74), O-p-nitro benzoyl thiamine and 0-p-methoxy benzoyl thiamine were studied extensively (75) using *L. fermentii* as experimental organism.

Butyl thiamine

If the methyl group is replaced in the pyrimidine ring by a butyl radical, an active antagonist is produced, capable of causing in rats signs of vitamin deficiency (76) which can be reversed by thiamine. This is an interesting phenomenon since inhibition is not caused by substitution with groups of less than 4 carbon atoms. Thus although ethyl homologue shows vitamin B_1 activity, the n-propyl homologue shows little activity either as a vitamin or as an antivitamin.

Phenyl triazinothiamine

The growth inhibition of *Kloeckera apiculata* by phenyl triazinothiamine was not recovered by addition of thiamine-hydrochloride (77). Phenyl triazinothiamine in the broth hydrolysed to pheyl hydrazine which inhibited the growth of the organism.

Imiodazole thiamine and benzoyl imidazole thiamine

The growth of *K. apiculata* in the broth containing thiamine and imidazole thiamine or benzoyl imidazole thiamine was restricted because of the strong inhibition of thiamine uptake by imidazole or benzoyl imidazole compound (78).

(b) Natural antithiamine compounds

Natural antithiamine compounds are further classified into two groups mainly,

1) Natural antithiamine compounds of large molecule

2) Natural antithiamine compounds of small molecule

1) Natural antithiamine compounds of large molecule

These antithiamine compounds are protein in nature (either enzyme-thiaminase or protein)and present in fishes, bacteria as well as in some seeds.

Two types of thiaminase were isolated from different sources, viz.,

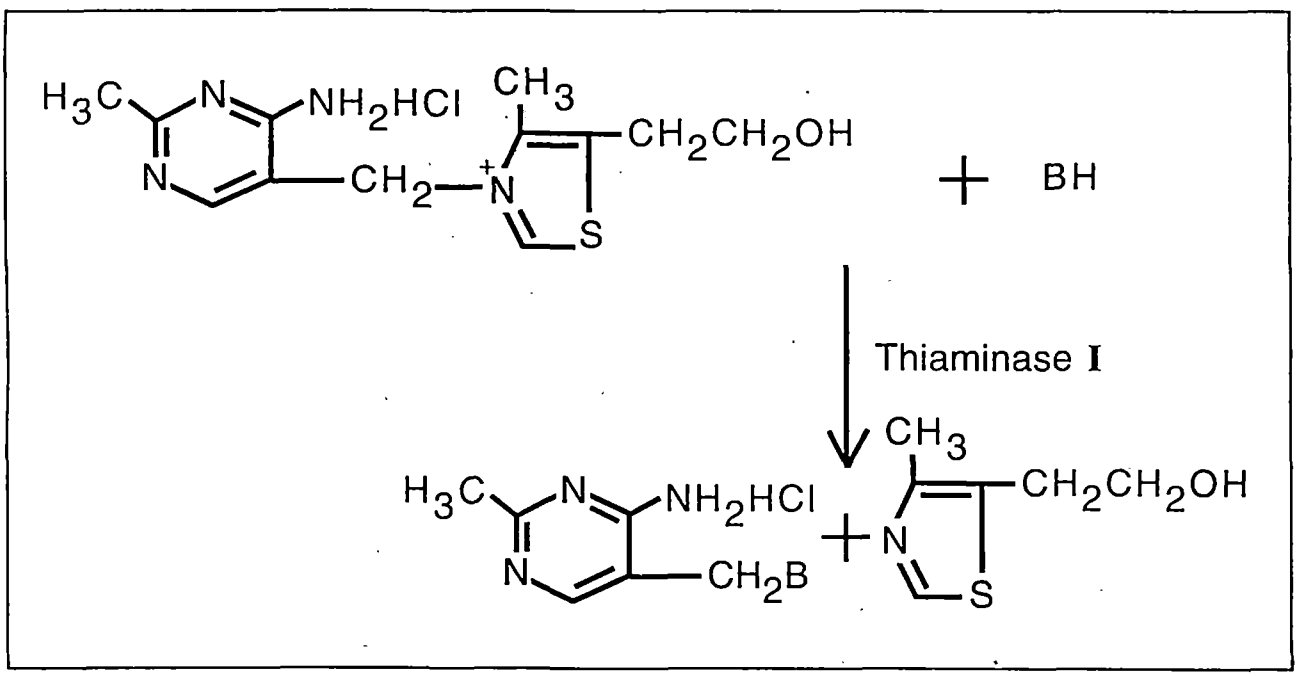
(i) Thiaminase I (79, 80)

(ii) Thiaminase II (81, 82)

(i) Thiaminase I

Matsukawa and Misawa (83,84) isolated one such organism from human feces and named it *Bacillus thiaminolyticus* - this was supposed to contain a 'thiaminase I'. Later, Kimura and Liao (85) discovered another agent in feces that produces ' thiaminase I' : *Clostridium thiaminolyticum*. This enzyme was also isolated from the viscera of fresh water fish, shell fish and bracken fern (86).

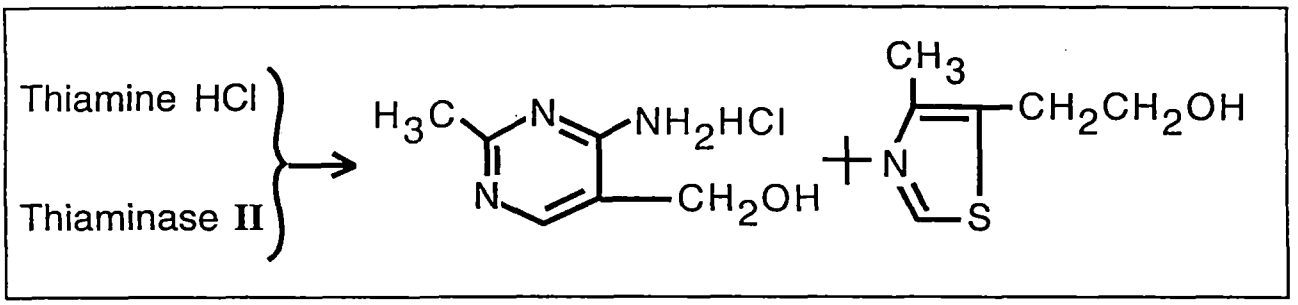
Thiaminase I catalyses a base-exchange reaction, whereby the thiazole moiety of thiamine is replaced by another base (e. g. an amine) and the vitamin activity is lost. The reaction mechanism is given below.



The thiaminase I of *B. thiaminolyticus*, according to Douthit and Airth (87) is predominantly extracellular. The formation of thiaminase I (i. e. the increase of antithiamine activity) was inhibited by the addition of thiamine (88). Further purification and characterization of this thiaminase have been undertaken by Wittlife and Airth (89). These authors demonstrated a method for the determination of thiaminase I activity where aniline was used as a base. The formation of the product as an aniline derivative with pyrimidine moiety was measured spectrophotometrically by an increase of optical density at 248 nm.

(ii) Thiaminase II

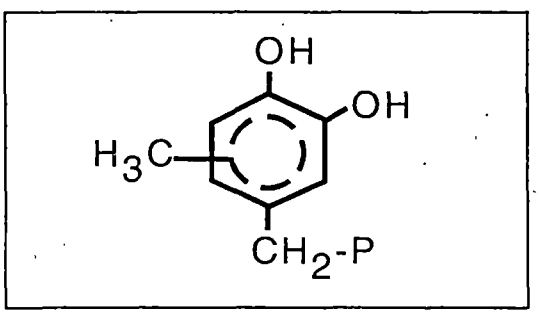
Kimura and Aoyama (90) isolated from hay and soil one type of thiamine degrading bacterium viz. *Bacillus aneurolyticus* which produces thiaminase II. It is also present in some yeasts, bacteria and fungi (91). Thiamine is hydrolysed in presence of thiaminase II without any base requirement according to the following reaction.



Somogyi et al (92, 93) isolated from carp viscera a protein other than the enzyme thiaminase with antithiamine activity and designated it as substance K having molecular weight in the range of 75,000 - 100,000. It contained a thermostable part similar to hemin.

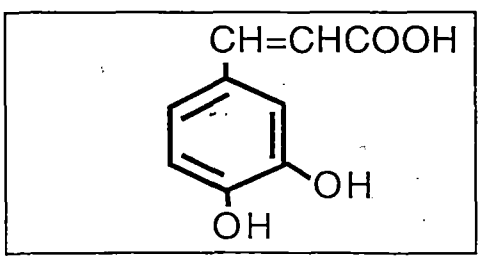
(2) Natural antithiamine compounds of small molecule

The existence of natural antithiamine compounds of small molecule was established in the literature when Chaudhuri (94), for the first time, isolated from rice-bran a heat stable simple organic molecule having antithiamine activity. De and Chaudhuri (95) identified it as a glucoside and named it 'compound X'. Subsequently the active principle of this glucoside (compound X) designated as 'Fraction A' which was separated and partly characterized as ortho dihydroxy phenolic compound (96) is represented below,

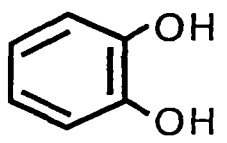


where P-an aliphatic side chain containing hydroxyl and carboxylic acid groups. The effect of fraction A and compound X on transketolase enzyme system and on the growth of *S. aureus* was also studied by these workers (loc. cit).

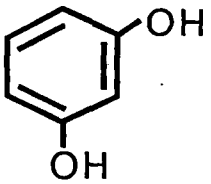
Later on, Somogyi and Beruter (97) isolated an antithiamine factor from fern *(Pteridicum aquilium)* and characterised it as caffeic acid, the structure of which is given below.



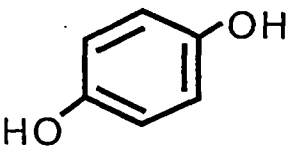
In the investigation of the mechanism of thiamine inactivation by caffeic acid, Davis and Somogyi (98) proposed that the reaction consists of two phases. The first phase was reversible, independent of pH and temperature whereas the second one was mostly an irreversible phenomenon. Somogyi and Bonicke (99) then carried out the experiments with several different phenolic compounds and postulated that ortho and pare dihydroxy phenolic compounds had maximum and moderate antithiamine activity respectively whereas meta dihydroxy phenolic compounds had no antithiamine activity as represented below.



Ortho—



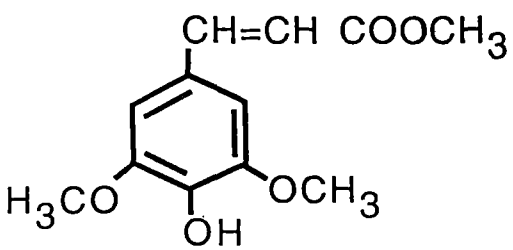
Meta—



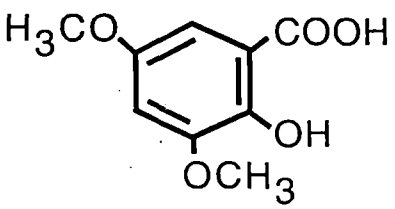
Para—

Hilker (100) also isolated caffeic acid from blue berries by a different method. Bonicke et al (101)showed that antithiamine compounds isolated from coffee were caffeic acid, chlorogenic acid and pyrocatechins.

Chaudhuri and his associates isolated two new antithiamine compounds characterised as methyl sinapate (102) and 3, 5 dimethoxy salicylic acid (103) from mustard seed *(Brassica Juncea)* and cotton seed *(Bombex melabericum)* respectively. The structure of these compounds is given below.



Methyl sinapate



3, 5 - dimethoxy salicylic acid

These workers also studied the effect of methyl sinapate (104) and 3, 5-dimethoxy salicylic acid (105, 106) on thiamine pyrophosphate requiring enzyme and on the growth of thiamine dependent bacteria.

Antithiamine activity of *Phaseolus radiatus* (a kind of bean) was reported in literature by Bhagvat and Devi (28). But attempts were not made to isolate and characterise the thiamine - inactivating factor(s) present in it. The present work was thus aimed to do isolation, characterisation and biochemical studies of the antithiamine factor(s) present in *Phaseolus radiatus*.