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Vitamin B_6 Antagonists of Natural Origin

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Vitamin B_6 deficiencies resulting from the ingestion of natural antagonists fed at the natural level are rarely observed. The only well documented case is in chickens from the ingestion of linseed meal which contains linatine, a hydrazino peptide. D-Cycloserine, a cyclic hydroxylamine derivative with antibiotic properties, also produces B_6 deficiencies when administered at therapeutic levels. Other potential antagonists include L-canaline, obtained by the enzymatic degradation of canavanine (*Canavalia* sp.), 4-hydroxymethylphenylhydrazine, a constituent of agaritine (*Agaricus* sp.) and methylhydrazine produced from gyromitrin (*Gyromitra esculenta*). Several dietary substances produce a stress on the vitamin B_6 supply or induce neurological disorders that respond to pyridoxine therapy including L-dopa, choline, mimosine, β-cyanoalanine, high level of dietary protein, and thermally processed high protein foods.

Compounds having antivitamin B_6 activity have been used for experimental and chemotherapeutic purposes for many years. For the most part, these have been synthetic compounds that are either structural analogs of the B_6 vitamers or carbonyl trapping agents. Of the many structural analogs studied, 4-deoxypyridoxine is perhaps the most potent and widely used agent for *in vivo* studies with higher animals, especially when used in conjunction with a vitamin B_6-deficient diet. Under these conditions, classical vitamin B_6 deficiency symptoms can be induced which respond dramatically to pyridoxine (PD).

There are many reports in the literature of the induction of vitamin B_6 deficiency through ingestion of hydrazines, either by accidental poisoning or by use of drugs. Convulsions associated with therapeutic doses of isonicotinyl hydrazide are readily relieved or prevented by timely administration of PD. Hydrazines, hydroxylamines, and semicarbazides of the type RNH_2 are all capable of forming stable hydrazones, oximes, and semicarbazones, respectively, with pyridoxal (PL) and pyridoxal phosphate (PLP). Derivatives of carbonyl trapping agents, both hydrazines and hydroxylamines, have been isolated from food and feedstuffs obtained from diverse genera. Although the carbonyl trapping agents generally occur in nature in the form of unreactive derivatives, enzymes having hydrolase or transferase activity capable of releasing the reactive carbonyl trapping agent are widely distributed in nature, including the digestive tract of higher animals. The release of these agents by digestive processes offers the possibility for inactivation of a portion of the vitamin B_6 in the metabolic pool.

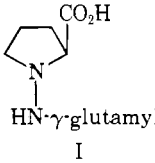
Other factors which may place a stress on the vitamin B_6 supply are the conditions of processing, especially thermal processing of high protein foods, and a high protein diet. Although these latter conditions are not true examples of *in vivo* vitamin antagonism, the results are much the same—clinical conditions which are relieved by administered PD.

The existence of a vitamin B_6 antagonism is sometimes suggested by certain drugs and neurotoxins which affect the central nervous system and produce convulsions similar to those induced by B_6 deficiency. In some of these cases, partial relief is obtained by use of PD, even though evidence for a vitamin B_6 deficiency is not substantiated by laboratory tests.

Recent reviews have discussed some of the conditions which may alter nutritional requirements for vitamin B_6 (Brown, 1972) and methods for the biochemical assessment of the nutritional status of vitamin B_6 (Sauberlich *et al.*, 1972). For the present article, a natural vitamin antagonist is defined as a substance of natural origin which raises the vitamin B_6 requirement or induces an adverse physiological condition which responds favorably to vitamin B_6 therapy. Included are substances having chemical characteristics of vitamin B_6 antagonists which are produced from inactive precursors by means of simple enzymic transformations which might be encountered in higher animals. The general topic of vitamin B_6 antimetabolites was reviewed by Rosen *et al.* (1964).

CARBONYL TRAPPING AGENTS

Linatine. The only clear example of a vitamin B_6 deficiency resulting from a natural feedstuff or food was given by Kratzer *et al.* (1954), who found that the poor growth shown by chickens on a diet of linseed meal was counteracted by the addition of PD to the diet. Subsequent studies by Klosterman *et al.* (1967) showed that linseed meal contains about 100 ppm of linatine (I). Hydrolysis of linatine produced 1-amino-D-proline (DAP) (II), an asymmetrically substituted secondary hydrazine which is probably responsible for the *in vivo* toxicity of linseed meal diets. DAP condenses readily with the carbonyl of PL and PLP to form stable hydrazones.



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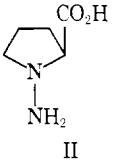

II

Although first observed in the flax seed, linatine has been found to occur in all parts of the immature flax plant, *Linum usitatissimum* (Nugent, 1971). Acute doses

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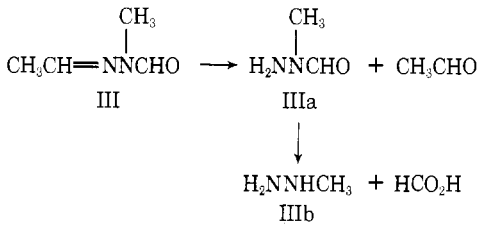
of linatine or DAP produce behavior patterns and convulsions in chicks that are indistinguishable from those of classical vitamin B_6 deficiency and hydrazine poisoning. The adverse effects in chicks are completely alleviated by prompt administration of any of the B_6 vitamers. The toxic effect has not been observed in mature poultry or mammals.

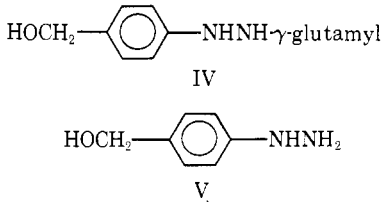
Linatine, DAP, and other α-hydrazino acids also show bactericidal properties. DAP was more potent than its optical isomer and several hydrazino acids against 20 microorganisms (Parsons *et al.*, 1968). The *in vivo* site of DAP activity has not been established. DAP inhibits a variety of PLP-requiring enzymes from *E. coli*, including glutamic aminotransferase, tryptophanase, tyrosine decarboxylase, and glutamic decarboxylase. Spectrophotometric evidence was obtained for the formation of a PLP–DAP complex when DAP was added to the respective holoenzymes. Addition of PLP reversed the inhibition of the decarboxylases and tyrosinase, but not the aminotransferase (Sleeper, 1972). Linatine does not show inhibition of these enzymes *in vitro* because the reactive hydrazino function is masked by the γ-glutamyl group.

Gyromitrin and Methylhydrazine. *Gyromitra esculenta* (*Helvella esculenta*) is one of the desirable wild mushrooms. A mild reaction is occasionally experienced after eating this species. List and Luft (1968, 1969) found that extracts of *G. esculenta* contained gyromitrin (III), *N*-methyl-*N*-formyl hydrazine (IIIa) and methylhydrazine (IIIb). They postulated that IIIa and IIIb were produced from gyromitrin by hydrolysis during maceration or cooking.



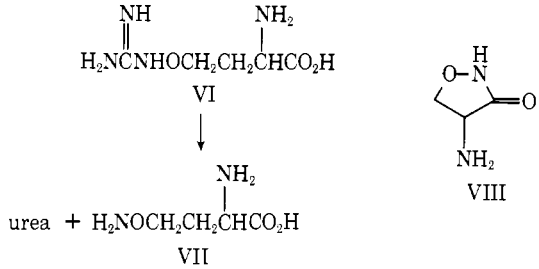
When the mushroom tissue is cooked in an open kettle, methylhydrazine is lost by steam distillation and no toxicity is observed. When cooked in a closed container or when incompletely cooked, gyromitrin or the hydrazines IIIa and IIIb are not lost, and under these conditions mild hydrazine poisoning may be observed. PD is commonly used as an antidote for methylhydrazine poisoning and is recommended for the illness that sometimes results from ingestion of *G. esculenta* (Simons, 1971).

Agaritine. *Agaricus bisporis*, the edible mushroom of commerce, was found to contain 0.3% of agaritine (IV) the γ-glutamyl derivative of 4-hydroxymethyl phenylhydrazine (V) (Levenberg, 1964).



The free hydrazine (V) forms hydrazone derivatives with typical carbonyl reagents, including PLP. Enzymes having γ-glutamyl transferase activity are widely distributed in nature, including *Agaricus bisporis* (Levenberg, 1964), and will release free 4-hydroxymethyl phenylhydrazine (V) from agaritine. Toxicological studies have not been reported for agaritine or V nor has there been any report of the production of a vitamin B_6 deficiency from eating *A. bisporis*. However, since V is a good carbonyl trapping agent, it is conceivable that the consumption of larger quantities of mushrooms over an extended period of time could result in a vitamin B_6 deficiency, similar to that resulting from linatine (I) in linseed meal.

Canavanine and Canaline. Several Leguminosae, especially *Canavalia* species, contain substantial amounts of canavanine (VI) (Turner and Harbourne, 1967). Although canavanine shows low toxicity for mice, 2 g/kg (Tschiersch, 1962), recent studies (Rosenthal, 1970; von Topfer *et al.*, 1970; Whiteside and Thurman, 1971) show that the principal metabolic pathway for the degradation of canavanine in many Leguminosae is by the action of canavanase with the production of L-canaline (VII) and urea.



Canaline was found by Rosenthal (1970) in the cotyledons of the germinating jack bean, *Canavalia ensiformis*. Canaline is also produced from canavanine by homogenates of liver (Kitagawa and Yamada, 1932) and hog kidney (Walker, 1956) by hydrolysis or transamidination with ornithine. Canaline is a substituted hydroxylamine and forms oximes with PL or PLP. Rahiala *et al.* (1971) demonstrated that canaline is a potent inhibitor of a variety of PLP-dependent enzymes and presented spectral evidence for the stoichiometric formation of a canaline–PLP complex. Although no clear examples of vitamin B_6 deficiency symptoms have been reported from the ingestion of foods containing canavanine or canaline, the demonstration of the enzymic conversion of canavanine to canaline raises the distinct possibility for this.

Cycloserine. Several Actinomyces produce D-cycloserine (VIII), an antibiotic that is a substituted hydroxylamine. Cycloserine is the next lower homolog of canaline (VII) but exists mainly in the lactam form. The administration of D-cycloserine at therapeutic levels in the treatment of tuberculosis often produces toxic effects, including convulsions, which are relieved by administering PD (Epstein *et al.*, 1959). Cycloserine forms a stable complex with PLP and acts as an inhibitor of a variety of PLP-requiring enzymes (Roze and Strominger, 1963), but was only about 1% as effective as canaline in this respect (Kekomaki *et al.*, 1969). The reaction between PLP and cycloserine proceeds much more slowly than the reaction between PLP and canaline, presumably because of the tendency of cycloserine to remain in the cyclic form (Rahiala *et al.*, 1971). The final product of the reaction of cycloserine with PLP is the substituted oxime (Khomuton *et al.*, 1963) as is the case with canaline.

Although cycloserine does induce a vitamin B_6 deficiency in humans and animals, its effect as an antibiotic is observed as the inhibition of bacterial cell wall biosynthesis (Perry, 1972).

The overall metabolic effects of substituted hydrazine and hydroxylamines may be much more complex than that of merely reducing the available PL or PLP. McCormick and Snell (1961) found that hydrazones and oximes of PL, including PL–canaline, were strong inhibitors of pyridoxal phosphokinase and were bound by the enzyme 100 to 1000 times more firmly than PL, the normal substrate, but the corresponding complexes were without effect on this enzyme. Conversely, Furst and Gustafson (1967) found that the PLP hydrazones of methylhydrazine and 1,1-dimethylhydrazine showed toxicities for mice

which equalled or exceeded the toxicity of the parent hydrazines. Oehme *et al.* (1969) proposed that hydrazine–PLP antagonism in whole animals results from two factors, reduction of the free reactive group (PLP) and a specific antagonism. In the case of cycloserine inhibition, *in vitro* inhibition of PLP-requiring enzymes is commonly observed, but the effects are especially potent with D-alanine metabolizing enzymes of bacteria (Oehme *et al.*, 1969; Roze and Strominger, 1966), suggesting that either cycloserine or the cycloserine–PLP complex is adsorbed to the substrate binding site (Khomuton *et al.*, 1963). It is possible that synthetic hydrazine derivatives used in chemotherapy, *e.g.*, isonicotinyl hydrazide, can also function in this dual manner.

MISCELLANEOUS AGENTS

There is a limited number of natural compounds which induce adverse effects when administered *in vivo* which are at least partially alleviated by the B_6 vitamers. With some of these, the *in vitro* formation of PLP complexes can be demonstrated as well as the *in vitro* inhibition of PLP-requiring enzymes. Since only partial remission of adverse effects is usually observed, the biological effects must be considerably more involved than can be explained by a simple vitamin B_6–antagonist relationship.

L-Dopa. The therapeutic use of L-dopa in the treatment of Parkinsonism is sometimes accompanied by adverse effects which are relieved by administering PD intravenously (Cotzias, 1969; Jameson, 1970), although the usual clinical evidence for vitamin B_6 deficiency is absent. Duvoisin *et al.* (1969) found that PL could either reverse the undesired side effects of L-dopa or negate the desired good effects of L-dopa therapy. Although Kurtz and Kanfer (1971) found that PLP levels in rat brain were lowered by L-dopa treatment, the explanation of the L-dopa–vitamin B_6 interaction is probably a matter of maintaining the proper level of dopamine in the brain tissue. In the presence of excess L-dopa, administering PL, PD, or PLP will activate L-dopa decarboxylase, which lowers L-dopa levels to the desired range. The activity of L-dopa decarboxylase must be regulated carefully to prevent excessive decarboxylation of L-dopa and return of the Parkinson syndrome. Fellman and Roth (1971) found that PL and PLP form tetrahydroisoquinoline derivatives with L-dopa. These products were found to be competitive inhibitors for certain PLP-requiring enzymes. Tran and Laplante (1972) presented spectral evidence for the binding of PLP by L-dopa melanine to form a complex which was inhibitory for rat liver L-dopa decarboxylase. Although the picture is not clear, there is both chemical and biological evidence for a dopa–PLP interaction. This interaction has some practical considerations as a result of the findings by Bell and Janzen (1971) and by Daxenbichler *et al.* (1972) that seeds of certain species of the genus Mucuna (Leguminosae) contain L-dopa in excess of 6%. *Mucuna deeringiana* (velvet bean) is widely grown for forage. The roots and seeds are used for the treatment of nervous disorders in parts of Asia.

Mimosine. The chemical and biochemical properties of mimosine, 3-hydroxy-4-keto-1(4*H*)-pyridinealanine, have been summarized (Fowden *et al.*, 1967; Hylin, 1969). The amino acid is found in the seed and foliage of *Mimosa* and *Leucaena* (Leguminosae) and is toxic to animals. Mimosine forms a complex with PLP (Lin *et al.*, 1965) and inhibits a number of PLP-requiring enzymes. The toxic effects of mimosine in the rat are partially overcome by PD, but other evidence suggests an interference with the metabolism of aromatic amino acids, especially tyrosine (Lin *et al.*, 1967). It appears that while mimosine may inhibit PLP-requiring enzymes, the overall effects are more complex.

β-Cyanoalanine. The adverse effects of β-cyanoalanine, a neurotoxin obtained from Vicia species, are partially reversed in the rat by PL (Ressler *et al.*, 1964). Although this suggests an antagonism with PL, Tate and Meister (1969) found that β-cyanoalanine was a noncompetitive inhibitor of L-aspartate-β-decarboxylase. β-Cyanoalanine was bound at the aspartate binding site on the enzyme and formed a stable ketimine which could not decarboxylate as aspartate normally would.

Choline. The vitamin B_6 nutritional status of the chick was found to be dependent upon the level of choline in the ration (Saville *et al.*, 1967). When fed a standard diet fortified by the addition of 1 g of choline/lb, the chicks showed poor growth and developed typical vitamin B_6 deficiency symptoms. The addition of PD to the diet alleviated the deficiency symptoms and restored good growth. While it is unlikely that the additional choline would lead to a loss of PLP, the additional PLP required for the degradation of the excess choline could have been drawn from other metabolic processes which normally supply vital metabolites such as γ-aminobutyric acid, needed for smooth functioning of the central nervous system. The adverse effects of excess dietary choline could result from the introduction of a stress on the metabolic pool of PLP rather than a specific antagonism.

Proteins. A marked loss of vitamin B_6 activity is often observed upon storage of condensed milk products that had been sterilized by heating. Bernhart *et al.* (1960) showed that in raw milk most of the vitamin B_6 activity was present in the form of PL, but after heat sterilization most of the PL was converted to pyridoxamine, with retention of most of the original vitamin B_6 activity. However, on storage, the vitamin B_6 activity is lost with the formation of a sulfur-containing pyridoxyl complex. Wendt and Bernhart (1960) found that the product was bis-4-pyridoxyl disulfide, the same product as was obtained by the reaction of PL with cysteine. Bis-4-pyridoxyl disulfide had only 12–23% of the vitamin B_6 activity of PL for rats on a molecular basis. Srncova and Daidek (1972) found that the loss of vitamin B_6 activity in heat-processed milk accompanied the loss of available sulfhydryl groups. PL and PLP can also react with other free amino acids (Heyl *et al.*, 1948) and proteins (Anderson *et al.*, 1966) to form Schiff's base structures, but these complexes usually show good vitamin B_6 activity, presumably as a result of the dissociation of the complex with release of PA or PLP.

The dietary requirement for vitamin B_6 is somewhat proportional to the daily protein intake in man (National Academy of Science, 1968) and in chickens (Kirchgessner and Friesecke, 1963) because of the extensive involvement of the vitamin in amino acid metabolism. Although not an example of true metabolic antagonism, the stress placed on the vitamin B_6 supply is best counteracted by increasing the level of PD in the diet.

DISCUSSION

Although a number of vitamin B_6 antagonists have been obtained from plants and fungi, there is only a single example of a clinical B_6 deficiency resulting from the ingestion of these materials at the natural level of abundance. This occurs in young poultry fed a diet containing linseed meal. In general, the known B_6 antagonists occur in relatively low levels or in foodstuffs that are consumed only occasionally or in small quantities. Under these conditions, the endogenous B_6 supply is apparently adequate to counteract the possible harmful effects of small amounts of antagonists in the food. It is conceivable that the consumption of larger amounts of ordinary mushrooms, which contain hydrazine derivatives, over several days could well produce a temporary B_6 deficiency unless offset by supplementary PD. This effect would probably be aggravated if one of the other factors which increase the B_6 requirement is also present as for example L-dopa or an unusually high protein diet. The possible synergistic effects of the natural B_6 antagonists have not been investigated.

The demonstrated existence of a variety of B_6 antagonists in diverse genera suggests that these and related substances may have widespread distribution in nature, if only in low or trace levels of abundance. Unusual or exotic dietary practices could produce the combination of circumstances which result in a clinical B_6 deficiency.

There are no reports of the natural existence of vitamin B_6 antagonists which are structural analogs of the B_6 vitamers.

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