The Function of Vitamin B_6 in Fat Metabolism[1]

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VITAMIN B_6 is one of the B vitamins which are essential for human nutrition. The requirement of the vitamin for man is believed to be 2–3 mg. per day (1). Structurally related pyridine derivatives, pyridoxine, pyridoxal, and pyridoxamine represent the members of the vitamin B_6 group (Figure 1). Pyridoxine is mainly found in plant tissue whereas pyridoxal and pyridoxamine are largely found in animal tissue (2). The existence of pyridoxal 5-phosphate (3) and pyridoxamine 5-phosphate (4) in natural sources has been proved; the former phosphate is known as codecarboxylase, a coenzyme form of vitamin B_6, which is actively engaged in protein and amino acid metabolism. The various forms of vitamin B_6 and its phosphates are biochemically interconvertible (Figure 1). They are excreted from the body as such (5), as 4-pyridoxic acid (5, 6), and as a complex of unknown structure (7, 8).

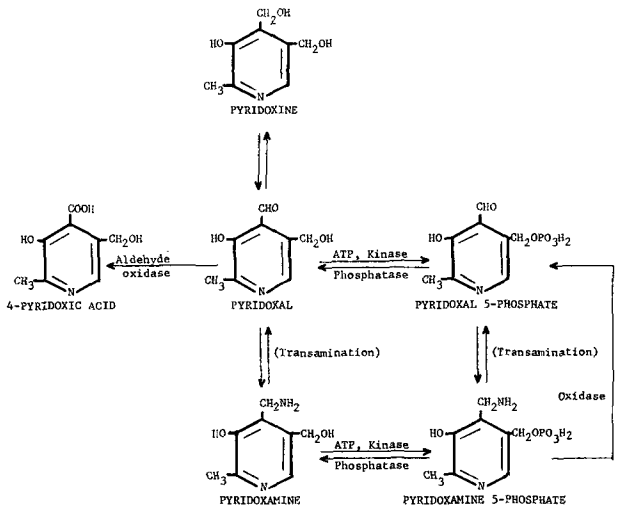

FIG. 1. Biological interrelationship among the members of vitamin B_6 and 4-pyridoxic acid.

Vitamin B_6 and Oxidation of Fats. Vitamin B_6 is known to be unstable in the presence of oxidizing agents (9). Burr and Barnes have suggested that vitamin B_6 might eventually be destroyed when it is brought into contact with oxidized or rancid fats (10). The 4-hydroxymethyl group of the pyridoxine molecule is reactive, and pyridoxine is converted to pyridoxal upon mild treatment with manganese dioxide or potassium permanganate (Figure 2) (11, 12). Further oxidation with the latter oxidizing agent leads to the formation of 4-pyridoxic acid, which is lacking in vitamin activity and constitutes the main excretory end-product of the vitamin in humans (6). By means of spectrophotometric techniques it has been shown that pyridoxal and its structurally related 5-deoxypyridoxal can also exist in the resonance form, a semiquinone structure (13, 14). When pyridoxal or the deoxypyridoxal was treated with hydrogen peroxide in an alkaline condition, the resulting product was an *ortho* dihydroxy compound (Figure 2) (13,

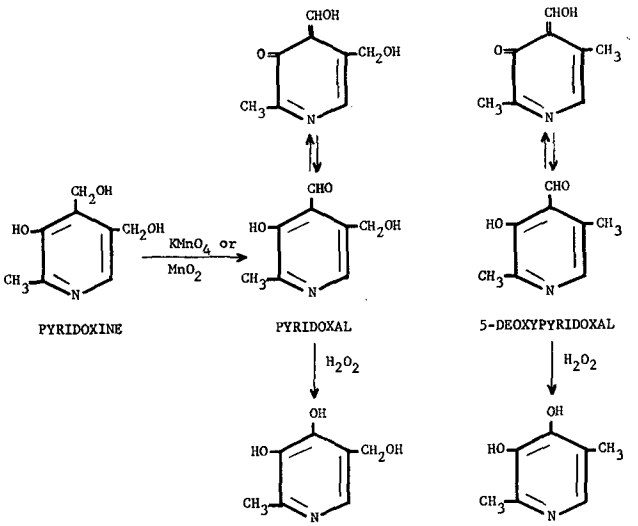

FIG. 2. Oxidation of pyridoxine and its related compounds.

14). It is unknown however whether pyridoxine and pyridoxal undergo such reactions when they come in contact with oxidized fats. Vitamin B_6 contains a hydroxyl group at the 3-position which shows typical phenolic reactions, and structurally it belongs to a group of hindered phenols. It is therefore not surprising to find antioxidative activity in the vitamin. Indeed Hove and Harris reported that pyridoxine, in the form of a free base, stabilized vitamin A in fish oils (15). However an attempt to find possible antioxygenic properties in the fat-soluble derivative of pyridoxine, pyridoxine 5-monopalmitate (16), failed to succeed (17). It was also noted that the 4,5-bis-deoxy analog of pyridoxine, 2,4,5-trimethyl-3-pyridinol, was much less effective than the corresponding benzene derivative, 2,3,6-trimethylphenol, in protecting lard from oxidation at 37°C. (17). This indicates that the nitrogen in the ring probably deactivates pyridoxine as an antioxidant despite the favorable arrangement of the substituents.

A well-known physiological antioxidant, α-tocopherol, has been reported to spare essential fatty acids in rats (15). Because of the properties superficially similar to tocopherol Hove and Harris postulated that vitamin B_6 might also serve as a physiological antioxidant (15). It was noted that, in plant cells, pyridoxine prevented the oxidation of the unsaturated fatty fractions *in vitro* as well as *in vivo* (18). Abnormal excretion of allantoin and creatine by rats which were deficient in both vitamin E and B_6 was eliminated upon administration of either one of the vitamins (19). Such possible antioxygenic properties of vitamin B_6 *in vivo* have been considered as an explanation for the effective cure of radiation injuries with pyridoxine (20). Although no direct evidence has been obtained to show that vitamin B_6 is involved in the biological oxidation-reduction systems, Tulpule and Williams suggested that the vitamin was essential for the utilization of linoleic acid in maintaining the phosphate esterification system which is closely associated with the oxidation of reduced cytochrome c

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(21). Depression in the activity of liver glutamic dehydrogenase in pyridoxine-deficient rats has also been reported (22). How vitamin B_6 contributes to the maintenance of the enzyme function remains obscure. Witting *et al.* observed that the poor growth of rats kept on a diet containing "used" oils could be partially overcome upon increasing the dietary level of pyridoxine (23) although the mechanism of reversal remains unknown.

Vitamin B_6 and Fat Metabolism. It has been claimed that vitamin B_6 plays a vital function in general fat metabolism, including the conversion of carbohydrate and proteins to fats (24, 25), although the studies are open to alternative interpretation. It has been reported that vitamin B_6 is indispensable (26) for the conversion of linoleic to arachidonic acid.

An ingenious method has become available for the preparation of carboxy-labelled essential fatty acids, the synthesis of which is not otherwise readily achieved (27). The principle involves decarboxylation of fatty acids, followed by regeneration of the carboxyl group with radioactive carbon dioxide. By means of tracer techniques Mead and his coworkers investigated the mode of *in vivo* conversion of arachidonate from linoleate or γ-linolenate and other highly unsaturated fatty acids. When linoleate-1-C^{14} was fed to rats, the arachidonate fraction isolated from the tissue contained radioactivity, and 74.7% and 24.5% of the total activity were found to be located at the carboxyl carbon and the 3-position, respectively. Only a trace of activity was detected at the 2-position, and no activity was found in the 4-position or in the rest of the chain (28). This indicated that linoleic acid was a precursor of arachidonic acid. The findings also indicated that a part of the radioactive linoleate underwent β-oxidation *in vivo,* and some of the two-carbon fragments thus freed were then condensed at the carboxyl of the linoleate. Condensation of linoleate and a two-carbon fragment to form an arachidonate skeleton has also been confirmed by using radioactive acetate and unlabelled linoleate (29). Similar tests proved the absence of formation of arachidonate from linolenate (30). Mead *et al.* also prepared γ-linolenate-1-C^{14} and found that this acid served exclusively as a source of arachidonate (31). The results led them to postulate that linoleate was transformed to arachidonate most likely through γ-linolenate. This reaction involved removal of four hydrogen atoms from the molecule and condensation with a two-carbon fragment at the carboxyl end of the linoleate (Figure 3). No evidence however has yet been obtained to show that γ-linolenate is actually formed from linoleate *in vivo.* It is suggested that an intermediate other than γ-linolenate may also be involved in arachidonate formation (31).

Although it is clear that vitamin B_6 is in some way closely connected with the formation of arachidonate and possibly a hexaenoate from linoleate and linolenate, respectively (26), the mechanism is totally unknown. It remains to be investigated whether vitamin B_6 is indispensable for the synthesis or for the maximum function of enzymes involved in metabolism. For the conversion of palmitic acid to palmitoleic acid and of stearic acid to oleic acid, vitamin B_6 is believed to be required as a cofactor (32).

Four sources of evidence have shown a close biological interrelationship between vitamin B_6 and essential fatty acids. a) In rats the skin lesions produced by vitamin B_6 deficiency and those produced by essential fatty acid deficiency show superficial resemblance; b) onset of vitamin B_6 deficiency symptoms is facilitated by removing essential fatty acids from the diet and *vice versa;* c) deficiency symptoms of one of the nutrients can be partially or temporarily cured upon administration of the other, and most efficiently cured with both; and d) changes in the composition of polyunsaturated fatty acids in the tissue take place when vitamin B_6 is removed from the diet (33). The two deficiency symptoms however are histologically quite distinct (33).

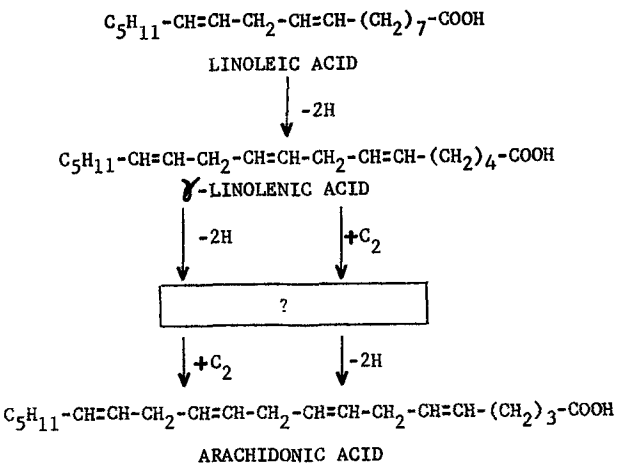

FIG. 3. Postulated conversion mechanism from linoleic acid to arachidonic acid *in vivo* (31).

As early as 1938 Birch postulated that vitamin B_6 might combine with unsaturated fatty acids and phosphoric acid to form a phospholipide type of compound as an essential cell constituent (34). Although the existence of such vitamin B_6 complexes in nature is still unknown, it is of interest that both vitamin B_6 and essential fatty acids are now known to be vitally involved in the maintenance of normal cell functions and the regulation of cell permeability (35).

Sinclair postulated that destruction of cell structure in essential fatty acid deficiency and in vitamin B_6 deficiency was caused by an inability to synthesize phospholipides and an interference with protein metabolism (36). Beaton *et al.* however reported that in the regenerating liver tissues of partially hepatectomized normal as well as vitamin B_6 deficient rats, essential fatty acid deficiency did not affect the synthesis of cellular protein or the maintenance of liver enzyme protein (37). It is of interest to note that either linoleate or pyridoxine is able to protect animals from radiation injury (38, 39). It has also been suggested that a complex of pyridoxine and arachidonate may constitute an essential component in skin tissue (40). Another link of the function of vitamin B_6 to fat metabolism has been amply demonstrated in seborrheic dermatitis. In human subjects vitamin B_6 deficiency, which was often produced experimentally with the aid of an antivitamin B_6, induced seborrheic dermatitis of the "sicca" type. A typical symptom involves abnormal deposition of fatty material on the skin. This can be effectively cured upon topical application of pyridoxine (41).

In conjunction with the effect of vitamin B_6 on the maintenance of cell structure it seems of interest to mention the observation of Holden and Holman. A

strain of lactic acid bacteria, *Lactobacillus arabinosus 17–5,* is rod-shaped with a round end. When the culture of this organism was transferred successively in a vitamin B_6-deficient medium, all the cells began to show a swollen elliptical shape (42, 43). The microorganism might therefore serve as a convenient tool for the study of vitamin B_6 and its relationship to cell structure.

Rinehart and Greenberg observed that the rhesus monkeys which had been kept on a vitamin B_6-deficient diet for a prolonged period of time tended to produce more advanced arteriosclerotic lesions than those found in the control animals (44). They later reported that pyridoxine deficiency in monkeys regularly led to the formation of sclerotic lesions in the blood vessels, which was also accompanied by high blood steroid levels (45). The results obtained by other workers from similar lines of study were not always in agreement with the findings of Rinehart *et al.* (46, 47, 48). It is however not surprising to find some correlations between the deposition of fatty materials in the circulating systems and vitamin B_6, for the vitamin is indispensable for the normal metabolism of fats. If essential fatty acid is directly involved in the mobilization and deposition of cholesterol, as has been postulated (49), it is understandable that vitamin B_6 would indirectly but actively affect the metabolism of the steroid. It may also be implied that vitamin B_6 stimulates the *in vivo* conversion of tryptophan to nicotinic acid, which, in turn, may depress serum cholesterol levels (50). The effect of the vitamin in preventing the deposition of fatty materials, as observed by Rinehart *et al.*, may not be a direct one. They found that the initial lesion was characterized by the accumulation of a mucous substance in the intima and in the media of the arteries involved (51). This material was not fatty in nature and was found to exhibit reactions characteristic of mucopolysaccharides. Deposition of lipide material takes place in the later stage of atherosclerosis. The findings suggest that vitamin B_6 deficiency might impair the functions of enzymes in the cells, and the degenerated cells, in turn, would be susceptible to the deposition of fatty material along the damaged walls.

Conclusion

It is evident that vitamin B_6 serves as a physiological antioxidant. The mechanism cannot be dependent solely on the chemical structure of the vitamin. Despite the favorable arrangement of the substituents, pyridoxine and its derivatives show no, or only slight, antioxidative activity *in vitro*. The inactivation apparently results from the nature of the pyridine ring.

Vitamin B_6 is one of the B vitamins which are required for the normal metabolism of the fats and essential fatty acids. However it still remains to be investigated whether the vitamin is directly associated with fat metabolism or whether the vitamin itself is responsible for the maximum function or the synthesis of enzymes involved.

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