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VITAMIN B_6 METABOLISM

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INTRODUCTION

The metabolism (110), methods of analysis (51), and human requirements (17) for vitamin B_6 have all been reviewed recently. Renewed interest in vitamin B_6 has resulted from the development of improved methods that are sensitive enough to allow experimentation with physiological concentrations in laboratory animals. These enzymatic, chromatographic, and isotopic methods have made it possible to examine metabolic and transport processes in whole animals, perfused organs, and cell suspensions. The scheme of reactions below, which describes the metabolism of the B_6 vitamers in animals, was postulated more than ten years ago. Recent experimentation has established the validity of the scheme and its almost universal applicability to animal cells.

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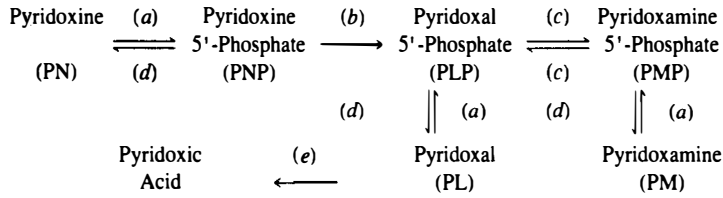

The enzymes involved are (*a*) PL kinase, (*b*) PMP (PNP) oxidase, (*c*) aminotransferase, (*d*) phosphatase, and (*e*) PL oxidase; aldehyde dehydrogenase.

Much of the fascination with vitamin B_6 is due to the simplicity of the chemistry of the interchangeability of the various vitamers, their specific binding to nonenzyme proteins in the circulation, and the regulation of coenzyme concentrations in spite of the fact that there is little or no selective transport of the nonphosphorylated forms through membranes. The use of inhibitors to impair all PLP-dependent enzyme catalysis or suicide inhibitors to impair selectively only one such reaction has attracted the attention of those who seek a means of investigating amino acid metabolism. Study of the vitamin B_6 group has permitted workers to locate free amino groups in proteins and to identify the active site for enzymes that require PLP or an amino group with which PLP will form a covalent bond.

This review does not discuss the coenzyme functions of PLP and PMP; the list of vitamin B_6–dependent enzymes has become relatively stable in recent years, and the reaction types requiring PLP as a coenzyme are well understood. This review focuses instead on the nutritional aspects of vitamin B_6 function and metabolism.

DIGESTION AND ABSORPTION

The availability of [^{3}H]B_6-vitamers with high specific activity and the availability of improved analytical methods have permitted experimentation that has clarified the processes of digestion and absorption of physiological quantities of the naturally occurring forms of vitamin B_6; PLP, PMP, and PN. As described below, the hydrolysis of PLP to PL and PMP to PM occurs in the intestinal lumen and is catalyzed by intestinal phosphatases. PL, PM, and PN are then absorbed.

Digestion and absorption are properly dealt with together because these processes are not easily separated in a system approximating the in vivo situation. Earlier reports based on experiments with whole animals (12), everted sacs (95, 74), isolated loops (72), and intestinal rings (112) all indicated that PN is absorbed by passive diffusion. The use of intact intestinal tissue either as everted sacs or as rings, i.e. where the circulation has been interrupted, allows only tentative conclusions regarding in vivo absorption. The use

of physiological quantities of the B_6 vitamers placed in the lumen of the vascularly perfused rat intestine appears to provide an appropriate system for studying both digestion and absorption. Absorption of the nonphosphorylated forms is rapid (PL, 40%; PN, 23%; and PM, 18% in 10 min). The complications added by the presence of other tissues, including erythrocytes, makes interpretation of results obtained with whole animals equivocal.

Using the intestinal perfusion system developed by Windmueller et al (119), Buss et al (15) demonstrated that transport of PN both into the mucosal tissue and transmurally into the perfusate was proportional to dose over a 10,000-fold range of concentration, which confirmed the conclusion that PN transport occurs by passive diffusion.

Use of this system with PL and PLP as substrates established that absorption of PL is rapid, nonsaturable, and unaffected by 4-deoxypyridoxine (64). PM is also absorbed by passive diffusion (35). The distribution of ^3H in the labeled products observed in the mucosal tissue and the perfusate when [^3H]PLP or [^3H]PMP was the lumenal substrate gave strong support to the idea that both substrates are largely or completely hydrolyzed before absorption. Unlike the results with PL and PM, 1000-fold dilution with unlabeled PLP or PMP reduced the label in the perfusate and mucosa, presumably because the phosphohydrolase was saturated by these high concentrations of PLP or PMP. With small doses, the percentage of ^3H from labeled PL, PLP, PM, PMP, or PN in the mucosal cells after 10 min was 13–17% for each of the five vitamers. However, only 2–4% of PM or PMP label and 7% of PN label was in the perfusate compared to 25% for PL or PLP. Middleton (73) used lumenal perfusion of a segment of the jejunum in vivo and confirmed that PLP can be absorbed slowly without hydrolysis when a high dosage is given (64). Most of the PMP is also hydrolyzed to PM prior to absorption (35). The alkaline phosphatase activity in the lumen is associated with the membrane (70).

Studies of the bioavailability of vitamin B_6 as it occurs in foods, particularly after processing and storage, have revealed complex relationships which have been discussed by Gregory & Kirk (32). Bioavailability is defined as the percentage of vitamin B_6 activity for *Saccharomyces uvarum* in the food that is available to an experimental animal receiving the food as a source of the vitamin. In certain foods, especially after heat processing, the bioavailability can be as low as 40–50%. Gregory & Kirk (31) reported that the most likely cause for the loss of vitamin B_6 during thermal processing and storage is the reduction of the Schiff's bases formed between PL or PLP and the ϵ-amino groups of lysine residues in the proteins. The ϵ-pyridoxyllysine residues formed in this way accounted for about half of the PLP that was degraded during the storage of dehydrated model systems containing PLP. Further, processed foods contain these phosphopyridoxyllysine residues, which have low vitamin B_6 activity for bacteria and variable activity for rats. Gregory (29)

reported that bovine serum albumin–bound phosphopyridoxyllysine has about 50% of the molar vitamin B_6 activity of PLP for rat growth, feed efficiency, and maintenance of liver PLP concentration. In animals, this phosphopyridoxyl protein can have a stimulating or inhibiting effect, which depends upon the ratio between its concentration and those of other forms of vitamin B_6 in the diet. Giving low doses of phosphopyridoxyllysine to rats accelerated the onset of vitamin B_6 deficiency and accentuated the vitamin B_6 deficiency symptoms (29). This inhibiting effect may account for the unexpected and thus far unexplained epidemic of convulsive seizures observed thirty years ago in infants who were fed nonfortified, heat-sterilized, canned infant formula (18). PMP (PNP) oxidase will convert a variety of N(5'-phospho-4-pyridoxyl) amines to PLP (41). Pyridoxyl lysine is metabolized to PLP by the sequential action of PL kinase and PMP (PNP) oxidase (28).

METABOLISM AND TRANSPORT

From the results discussed above, it is clear that PL, PN, and PM are the forms in which most dietary vitamin B_6 reaches the circulation. All three vitamers are rapidly transported into organs and tissues. It is recognized that this uptake is promoted by the metabolic trapping as the 5'-phosphate esters in all tissues that have been examined. In some cases the compound concentrated intracellularly was not identified and the process was incorrectly described as active transport.

Spector & Greenwald (102–104) concluded that brain slices and the isolated choroid plexus concentrate extracellular PN by a mechanism that depends on pyridoxal kinase. This conclusion was based on the correlation between phosphorylation and accumulation of PN. The concentration of 4'-deoxypyridoxine that inhibited pyridoxal kinase activity for each type of nervous tissue was the same concentration that inhibited accumulation of PN in intact tissue. Further, PN itself was not found in the tissue. These studies suggested that the choroid plexus is the locus for the transport of PN from blood to the cerebrospinal fluid (102).

Results from studies of perfused liver indicate that PN is rapidly taken up by passive diffusion, followed by metabolic trapping as PNP (63). Results from rat hind-limb muscle perfusion support diffusion and metabolic trapping of PN by this tissue as well (15). In perfused muscle, no conversion of PNP to PLP was observed. In perfused kidney, PN accumulated against a concentration gradient even when the PN concentration was high enough to saturate the PL kinase (36). While this suggests active transport into kidney, it is more likely that PN accumulates in the tubules, because tubular secretion of this compound occurs when it is present at high concentrations in the blood. Pyridoxal, which is not excreted by tubular secretion, was taken up by the kidney less rapidly and accumulated only as a result of phosphorylation (36).

Liver and intestine are very active in their metabolism of B_6 vitamers. When PN is taken up by these cells, it is rapidly acted on by PL kinase and then converted to PLP by pyridoxine phosphate oxidase (15, 63). These two enzymes plus phosphohydrolase provide a means of converting dietary PN to circulating PL, which can then serve as a source of the coenzyme PLP in all tissues that contain pyridoxal kinase, whether they contain PNP oxidase or not.

Erythrocytes present an interesting spectrum of phenomena in terms of their handling of the various vitamin B_6 forms. PN and PL are taken up by simple diffusion (65). Both are phosphorylated by the kinase. In human erythrocytes the PNP oxidase forms PLP; however; the erythrocytes of rats and some other species exhibit no oxidase activity (65) and the PNP has no known fate other than hydrolysis back to PN, which finds its way to the plasma and then to other tissues for conversion to PLP.

The early investigations did not measure initial rates of transport into erythrocytes. These long-term incubation experiments were complicated by metabolic trapping of PN as the phosphate ester. Use of the rapid mixing technique with high specific activity [^{3}H]PL or [^{3}H]PN made it possible to determine the uptake rate during the first 1–3 min after mixing and to characterize the transport process (65). At high concentrations of PN (16–160 μM) that saturated the kinase, the uptake was complete in 3–5 min and the concentration in the erythrocyte approached that in the medium. Low concentrations led to what appeared to be concentrative uptake but which proved to be metabolic trapping. The uptake of PL by erythrocytes from isotonic buffer gave concentrations of ^{3}H in the cells that far exceeded the concentration in the medium even at PL concentrations sufficiently high to saturate the kinase. This high-capacity, concentrating process proved to be the result of specific binding of PL to the N-terminal valine of the α-chain of hemoglobin (37). This binding of PL by hemoglobin is considered again below. The rapid mixing experiments demonstrated that although PN and PL are both taken up by simple diffusion, their transport is remarkably sensitive to temperatures between 13° and 20°, presumably because of the effect of temperature on the membrane fluidity (65).

It is generally accepted that PLP is not taken up by erythrocytes (100), but there have been several reports to the contrary. Solomon & Hillman (101) reported that PLP enters erythrocytes without prior dephosphorylation. This was reported earlier (106) on the basis of the observation that the ratio of ^{32}P to ^{14}C in PLP inside the cell was the same as that of the double-labeled PLP to which they were initially exposed. Caution must be used in interpreting the results of PLP transport into erythrocytes in view of the report that PLP is a competitive inhibitor of phosphate and phosphoenolpyruvate transport via the Band 3 protein in erythrocyte membranes (34). Further, transport of PLP into erythrocytes is inhibited by known inhibitors of anion transport such as stilbenedisulfonate compounds (76). Kinetic studies confirm that Band 3 protein

mediates PLP transport. The binding of PLP to the anion transport site could have been mistaken for transport in observations such as those mentioned above. The direct transport of PLP into erythrocytes does not occur when they are suspended in a medium containing plasma albumin (2) because PLP is tightly bound to this protein. The inconclusive nature of these types of experiments results in part from the lengthy (1–2-hour) exposure of the red cells to the substrates.

It has been suggested that enzymatic hydrolysis of PLP in various tissues may control the intracellular level of this coenzyme (52). This hypothesis is supported by the results of Govitrapong & Ebadi (27), who showed that PLP phosphohydrolase activity is about 200-fold higher than PL kinase activity. Middleton (71) observed that low PLP levels were elevated by incubation with PN in jejunal everted sacs from vitamin B_6–deficient rats. Everted sacs from control and repleted rats did not show a change in PLP levels, although uptake and phosphorylation rates of PN were equal to those of the deficient sacs. PLP was elevated in control sacs incubated with PN in the presence of 80 mM phosphate buffer. The increase in PLP concentration that occured when phosphatase was inhibited by phosphate suggested that phosphatase regulates PLP concentration by hydrolyzing the excess PLP. Such regulation had been suggested for erythrocytes (2, 58). Evidence for this type of control in the liver was provided by Li et al (52), who found that PN uptake and conversion to PLP did not increase the PLP levels in the livers of rats that had been given normal vitamin B_6 intakes. These results for liver, red cells, and intestine may be explained by the binding of PLP to intracellular proteins in a complex that is not hydrolyzed by phosphatase. Thus, the true control of intracellular concentration of PLP would be exerted by the concentration of the PLP-binding proteins in these cells, and when these proteins were saturated, the newly synthesized PLP would be hydrolyzed. It is also possible that product inhibition of PNP oxidase plays a role in regulating PLP concentrations in the tissues (69).

Lumeng et al found that PN is rapidly metabolized to PLP in isolated rat hepatocytes (60). The newly formed PLP was not freely exchangeable with endogenous PLP; it was converted to PL and pyridoxic acid and released into the medium. Human plasma PL levels can increase 12-fold in response to PN supplementation (60). These results and the observation that PLP in plasma is almost entirely bound to albumin (3) and hence is not readily available to cells suggest that PL may be the major source of vitamin B_6 for most tissues and organs. The binding of PL to hemoglobin (37) and the resulting accumulation of this vitamer in the erythrocyte may provide another means for PL to function as a transport form in the blood. In humans given physiological doses of this vitamer, PL is rapidly cleared from plasma and oxidized to pyridoxic acid (121). Further evidence that PL has a transport role was provided by the observation that PL was the major labeled metabolite released from rat livers

perfused with [^{3}H]PN (63). Although circulating PLP may not be the direct source of vitamin B_6 for many tissues, it accounts for 60% of the plasma vitamin B_6 and arises from the liver (56, 59). Because the PLP is bound to plasma albumin, its effective metabolic concentration and its turnover may be much lower than those of PL. The binding of PL to serum albumin (K_f, formation constant = 340/M) is not as tight as that of PLP ($K_f = 10^5$/M to 10^6/M) (19, 37). Both are bound to hemoglobin, and PLP ($K_f = 2.2 \times 10^3$/M) (7) is bound more tightly than PL ($K_f = 730$/M). The primary binding sites on serum albumin for PL and PLP are distinct, as are the sites on hemoglobin (38). The competition for PL by albumin and hemoglobin leads to a concentration in the red blood cell that is four- to five-fold greater than that in the plasma. If PLP were freely diffusible, its concentration in erythrocytes would be kept very low by the competition from serum albumin. The action of the erythrocyte kinase and phosphatase no doubt determines the erythrocyte PLP concentration.

In spite of rather extensive investigations, views of the processes by which the three forms of vitamin B_6 and their 5'-phosphate esters are interconverted have not changed materially since the subject was reviewed by Snell & Haskell (100). Pyridoxal kinase (ATP:pyridoxal-5'-phosphotransferase; EC 2.7.1.35) catalyzes the phosphorylation of all three forms; PL, PN, and PM. This enzyme appears to be present in all mammalian tissues. The kinases purified from liver, brain, and erythrocytes differ from each other in pH optima, metal ion requirements, and molecular weights (39). Pyridoxamine (pyridoxine)-5'-phosphate oxidase (EC 1.4.3.5) is an FMN-dependent enzyme that catalyzes the oxidation of PMP, PNP (80, 117), and a variety of N-(5'-phospho-4-pyridoxyl)-amines (41) to PLP. It is subject to product inhibition (69). It has been proposed that the activity of this enzyme in rat liver and kidney could be used as an index of riboflavin status (84); it can be assayed by a sensitive radiometric method (49).

The vitamin B_6 phosphate esters are hydrolyzed in the intestinal lumen and in various tissues by nonspecific phosphohydrolases (100). Lumeng & Li (57) made a careful study of the phosphatase in liver that hydrolyzes phosphorylated B_6 vitamers. Most of the hydrolytic activity in liver cells was present in the plasma membrane and had specificity and kinetic behavior similar to those of alkaline phosphatase. Its K_m at pH 8.2 for PMP was 25 μM and for PLP, 12 μM. The K_m at pH 7.4 for PLP was 2 μM. The existence of a phosphatase with high specificity for PLP was demonstrated (118) in phosphasome granules of human neutrophils.

Pyridoxic acid, the major vitamin B_6 excretory product, is formed from PL by the action of aldehyde oxidase (92) or by the action of an NAD-dependent aldehyde dehydrogenase (105). While it is acted on by dehydrogenases from many tissues (105), PL is a substrate for the aldehyde oxidase only in the liver (25). Dehydrogenase activity is found in the mitochondria, cytosol, and microsomes in many tissues, and these enzymes have low substrate specificity.

Under physiological conditions, it appears that the dehydrogenase is more involved in forming pyridoxic acid than is the oxidase.

STORAGE

Sixty percent of the vitamin B_6 present in rat muscle and 75–96% of that present in mouse muscle are associated with glycogen phosphorylase (44). Since this enzyme comprises nearly 5% of the soluble protein of muscle, it might serve as a physiological repository of vitamin B_6. Black et al (10) observed that the phosphorylase content of the gastrocnemius muscle of the rat increased in response to high intakes of vitamin B_6 but that this elevated level of phosphorylase did not decrease when the animals received a vitamin B_6–deficient diet for the following eight weeks (11). When the rats were starved, however, muscle phosphorylase activity decreased.

Phosphorylase requires PLP for catalytic activity, but the function of PLP appears to be different from those of other PLP-dependent enzymes (97, 108, 122). PLP binds to Lys-679 in rabbit muscle phosphorylase with the 5'-phosphate adjacent to the substrate binding site, which suggests that the phosphate is involved in the catalysis (107, 109). Starch phosphorylase from plant leaves does not contain PLP as a prosthetic group (45, 46).

EXCRETION

Although the major vitamin B_6 excretory product, pyridoxic acid, is formed when the pyridoxal concentration is high, the quantity excreted is indicative of intake, not of body stores. When 100-mg doses of PL, PM, or PN were given to humans, the major portion of each dose was found unchanged in the urine after 36 hours (82). When physiological doses were given, most of the excretion of pyridoxic acid occurred in the three hours following the dose (121). With increasing doses of PN up to 10 mg, the percentage of the dose that was recovered as urinary pyridoxic acid decreased from 63% to 35% of the dose (121). The low-percentage excretion of pyridoxic acid at the higher PN dosage may reflect the loss of PN through the kidney by tubular secretion (35). Only about 2% of an intravenous dose of PN was found in the bile (54).

ASSESSMENT OF NUTRITIONAL STATUS

Many procedures for evaluating vitamin B_6 status in humans have been proposed and evaluated (51). These include measurement of various PLP-dependent enzymes in erythrocytes with and without added PLP, estimation of B_6 vitamers in blood, and measuremant of metabolite excretion levels in response to overloading doses of methionine and tryptophan. Many techniques

for quantitating B_6 vitamers in tissues have recently been described in the literature. They include the use of monoclonal antibodies (115), HPLC cation exchange columns (16) and other HPLC methods (111, 113), and microbiological assays (30) with radiometric modification (33). Lumeng et al (61) concluded that plasma PLP correlates well with the PLP content of the skeletal muscle, the major repository for vitamin B_6. Plasma PLP represents a direct and versatile indicator of the vitamin B_6 status of the rat; aspartate and alanine aminotransferase holoenzyme activity in rat erythrocytes were found to be useful but insensitive indicators of vitamin B_6 status. Liver and kidney cytosol aspartate aminotransferase activity also decreased when rats were fed a vitamin B_6–deficient diet (78), but activity was not restored by addition of PLP. The total antigenic activity of this enzyme in the livers of vitamin B_6–deficient rats was maintained at normal levels, which indicated the presence of a form of transaminase with little or no enzyme activity (78). Both the intracellular location and tissue of origin of aspartate aminotransferases are important variables in the correlation of enzyme levels with vitamin B_6 status (43, 48). Meisler & Thanassi (67) reported that PN kinase activity in rat liver and brain decreased when the animals were fed a vitamin B_6–deficient diet but the activities of PNP oxidase and phosphatase remained unchanged. These results reveal the complexities of correlating enzyme activity with vitamin B_6 status.

Recently Ramsay et al (83) analyzed samples of maternal and cord blood and placental tissue at partruition from low-income Kenyan women. The indexes for vitamin B_6 status included glutamate pyruvate aminotransferase of erythrocytes, its stimulation by added PLP, and placental diaminoxidase. Based upon the stimulation of erythrocyte aminotransferase, the observed incidence of vitamin B_6 deficiency was 35% for maternal blood and 15% for cord blood. The placental analyses indicated that 24% of the mothers were deficient in vitamin B_6. The correlation between the results obtained using the maternal erythrocytes and the placenta aminotransferases as indexes of deficiency was very high ($P < 0.03$). Diamineoxidase levels were lower in the placentas of vitamin B_6–deficient mothers.

REQUIREMENTS

The vitamin B_6 status of certain segments of the population has been considered in detail (17). Among these are the pregnant or lactating woman and the infant. The concern for the infant was heightened by the occurrence of seizures in infants who were fed a commercial infant formula in which vitamin B_6 had not been preserved during processing and storage (9). Breast-fed infants have plasma PLP levels as low as one-tenth of those of formula-fed infants (62). As lactation proceeds, it may require more than four weeks for the vitamin B_6

levels in the milk to reach the plasma concentrations. These findings suggest that during the early weeks of life, breast-fed infants may be at risk for vitamin B_6 deficiency (42). This suggestion is supported by the correlation between the vitamin B_6 status of pregnant and lactating women and that of their infants (24, 85, 91).

Results obtained with pregnant and lactating rats indicated that more than adequate vitamin B_6 intake by the mother during pregnancy did not insure optimum status in the pups during the suckling period. Levels above the National Research Council recommendation for lactating rats were necessary to achieve maximum physical and neuromotor development (1). Plasma PLP levels do not reflect vitamin B_6 intake in pregnant and lactating rats as well as whole blood levels do (98, 99).

Long-term use of oral contraceptives (i.e. for more than 30 months) prior to pregnancy has been reported to result in low vitamin B_6 concentrations in maternal plasma at five months of pregnancy, at delivery, and in the milk (86). Numerous studies have shown that women using oral contraceptives excrete increased amounts of tryptophan metabolities after a loading dose, which can be corrected by administration of pyridoxine (81, 88). This suggested that users of oral contraceptives have an increased requirement for this vitamin. Leklem et al (50) and Brown et al (14) reported that a daily intake of 2 mg of vitamin B_6 was adequate to maintain normal levels of plasma PLP, erythrocyte alanine, and aspartate aminotransferase activity in oral contraceptive users. This suggested that the elevated excretion of tryptophan metabolites is of limited use in detecting vitamin B_6 deficiency. The data of Donald and Bosse (13, 22) support this view. More recent reports (5, 6, 120) indicate that the abnormalities in tryptophan metabolism in women receiving estrogens can be accounted for by the inhibition of kynureninase by estrogen metabolites.

Biochemical evidence of vitamin B_6 deficiency in the elderly who are in good health has been presented (53, 87), and dietary surveys have indicated that the intake of this vitamin is low in the aged (116). This report (116) indicates further that the biochemical parameters do not correlate well with dietary intake in the elderly. Age-related changes in the metabolism of vitamin B_6 may play a role in causing the low tissue levels of the vitamin in the elderly. Fonda et al (26) reported that senescent mice have increased PLP phosphatase activity and hence decreased protection of liver PLP compared to younger mice.

Low plasma PLP levels in alcoholics indicate that their incidence of vitamin B_6 deficiency is high even in the absence of liver disease and hematologic disorders (58). Plasma PLP levels in rats ingesting ethanol drop regardless of dietary vitamin B_6 intake (114). Ethanol also reduces the net rate of PLP formation in livers from vitamin B_6-deficient rats and lowers the PLP content of the livers of rats with sufficient vitamin B_6. These effects of ethanol are

thought to result from displacement by acetaldehyde of PLP from intracellular proteins to which they are bound, which allows the freed PLP to become a substrate for phosphatase (55). A more recent report (96) indicates that chronic ethanol ingestion has little effect on the total body stores of B_6 vitamers in the rat.

CLINICAL ASPECTS

Large doses of vitamin B_6 have been found to be helpful in the treatment of certain conditions. Seizures occur, seemingly because of an increased requirement for vitamin B_6 within the central nervous system. Without early treatment with large doses (10–25 mg) of vitamin B_6, continued seizures and neurologic damage may result (93). The most frequent cause of these conditioned deficiencies appears to be defective binding of PLP to the apoenzyme glutamate decarboxylase. The result of lack of this enzyme is a deficiency of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. In addition, PLP enhances GABA transport into the central nervous system, and this could influence the seizure threshold (23). Recent case studies indicate that PN-responsive seizures present a wider clinical spectrum than was once thought, which suggests that the physician should consider a diagnosis of pyridoxine-responsive seizures in assessing any infant with intractable epilepsy (4).

Six other vitamin B_6–responsive genetic conditions have been identified (75). These conditions include a vitamin B_6–responsive anemia, xanthurenic aciduria, cystathioninuria, homocystinuria, hyperoxaluria, and gyrate atrophy of choroid and retina. A mutation altering the structure of a particular apoenzyme and modifying its interaction with PLP is thought to be the cause of the cystathionuria, the cystinuria, and the xanthurenic aciduria.

Other clinical conditions also respond to large doses of vitamin B_6. Sickling of sickle erythrocytes is inhibited by PL and PLP, so they could be used as antisickling agents in the extacorporeal treatment of sickle-cell patients (40, 47). Pyridoxal phosphate lowered the oxygen affinity of normal cells but had no effect on the binding of oxygen by sickle cells. The oxygen affinities of normal and sickled erythrocytes were increased equally by PL. The opposing effects of PLP and PL on the affinities of hemoglobin for oxygen may be explained by the fact that they bind at different sites on the hemoglobin. PL binds to the N-terminal valine of the α-chain (37), while PLP binds exclusively to the N-terminal valine of the β-chain (8) of deoxygenated hemoglobin.

Pyridoxylated hemoglobin has also been utilized as a potential oxygen-carrying resuscitation fluid in blood volume replacement therapy. Hemoglobin is polymerized to increase plasma half-life, but the high affinity of the polymer for oxygen limits oxygen delivery to the tissues. As mentioned above, PLP

binding can lower the affinity for oxygen and thus provide hemoglobin with near-normal oxygen-carrying capacity (20, 94).

Although the literature contains many reports that vitamin B_6 deficiency inhibits the growth of rodent tumors (68), high levels of PN have been found to inhibit rat hepatoma cells in culture (21). The authors suggested that these findings indicate a potential use of vitamin B_6 as an antineoplastic agent and proposed that PN causes cell death by inhibiting DNA polymerase. Other findings with Morris hepatomas indicate that PNP oxidase and PN kinase activities are significantly lower than in control livers (66, 77), which suggests that the tumor cells may not be able to synthesize PLP from PN.

Even though some of the above conditions may respond to vitamin B_6 therapy, care must be taken in administering pharmacologic doses. When dogs were given high doses of PN, sensory nerve conduction velocities decreased and myelin loss occurred in individual fibers of the dorsal nerve roots (79, 89). Sensory neuropathies in humans taking megadoses of vitamin B_6 have also been reported recently (90).

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