Interconversions of Vitamin B_6 in Mammalian Tissue

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A review of interconversions of vitamin B_6 in mammalian tissues is presented. Orally administered (PN) pyridoxine is rapidly absorbed from the intestinal tract. Intravenously administered PN is rapidly transformed to the several forms of B_6 in liver, brain, and carcass. The amount retained and pattern of interconversion is characteristic for each tissue. The enzymes involved in the interconversions have been studied but little data are available on factors which control the rates of interconversions. Chronic administration of marijuana to mice alters the distribution of retained phosphorylated forms of vitamin B_6 in brain.

Vitamin B_6 is the generic term used for the three phosphorylated and three nonphosphorylated forms of this vitamin. Pyridoxal phosphate and pyridoxamine phosphate are essential for the metabolism of amino acids in transamination, decarboxylation, elimination, and replacement and dehydratase reactions (Braunstein, 1960). The functions of vitamin B_6 can be assessed either by *in vitro* studies of enzyme reactions (Snell and Ayling, 1968) or by observation of animal or human species where vitamin B_6 is deprived (Greenberg, 1964) or a dependency state exists.

A number of vitamin B_6 functions can be demonstrated in the vitamin B_6 dependency states. Vitamin B_6-dependent seizures (Scriver, 1960) occur shortly after birth and are resistant to usual anticonvulsant medications but are controlled by pyridoxine given daily at 10 to 20 times the usual daily requirement. The manifestations of the disease are thought to be due to decreased synthesis of γ amino butyric acid which is catalyzed by the PLP dependent enzyme glutamic decarboxylase. A decreased binding of PLP to this enzyme is thought to be the basic defect in this disease. Another example of vitamin B_6 function is illustrated by the disease pyridoxine responsive anemia. Individuals with this condition have a microcytic, hypochromic anemia on a normal diet (Harris and Horrigan, 1964), are unresponsive to other forms of therapy, but respond to 10 to 20 mg of pyridoxine per day with return to normal hemoglobin values. Pyridoxal phosphate catalyzes the formation of δ-aminolevulinic acid from glycine and succinyl CoA as a step in heme synthesis. It is thought that defective binding of PLP to δ-aminolevulinic acid synthetase is the defect in this condition. Other examples of vitamin B_6 dependency states are cystathioninuria and xanthurenic aciduria.

Although an extensive literature exists on the enzymatic reactions catalyzed by pyridoxal phosphate and pyridoxamine phosphate, little work has been carried out on the enzymes involved in the metabolism of vitamin B_6 and even less on the rates of interconversions of vitamin B_6 and factors which influence the metabolism and distribution of vitamin B_6. A number of drugs such as amphetamine (Bilodeau, 1965), chlorpromazine (Ebadi, 1970), reserpine, isoniazid (Vilter, 1964), birth control pills (Brown *et al.*, 1969) and marijuana, have been shown to affect either the concentration of vitamers or the enzymes of vitamin B_6 metabolism. The present paper reviews data on the absorption of pyridoxine (PN) and the enzymatic reactions involved in vitamin B_6 interconversions. In addition, detailed studies are reported on the interconversion of ^{14}C-PN to pyridoxine phosphate (PNP), pyridoxal phosphate (PLP), pyridoxamine phosphate (PMP), pyridoxal (PL), and pyridoxamine (PM) in liver, brain, and carcass of normal male Swiss–Webster mice and the effect of marijuana or B_6 depletion on the process.

ABSORPTION AND EXCRETION OF PYRIDOXINE

The absorption of PN has been studied (Booth and Brain, 1962). These authors administered ^{3}H-PN orally in doses varying from 0.05 to 5.0 mg, alone or accompanied by intraperitoneal nonradioactive PN. The amount absorbed was determined either by the percent of administered ^{3}H-PN excreted or the percent of ^{3}H-PN not absorbed at varying levels of the gut. When 0.05 mg of ^{3}H-PN containing 1 μCi of radioactivity was administered, an average of 34% of administered ^{3}H-PN was excreted, primarily in that form.

When animals were killed at intervals between 7.5 and 60 min after the administration of 0.05 mg ^{3}H-PN and the percent of ^{3}H-PN remaining in the intestinal tract was calculated, it was found that the absorption of pyridoxine was remarkably rapid. Within 15 min 74.7% and by 60 min greater than 90% had been absorbed. From a study of the amount of radioactivity remaining in the gut at varying levels and times, it was concluded that: virtually no absorption occurred in the stomach; the greater part of PN was absorbed in the jejunum; a smaller amount of PN was absorbed in the ileum than jejunum but the ileum had the capacity to absorb PN; virtually no PN was absorbed in the colon but this portion of the gut was capable of absorbing some PN. Their data favor the concept that absorption of PN occurs by passive diffusion rather than active transport.

When 1.0 mg of ^{3}H-PN containing 20 μCi was administered to human volunteers (Brain and Booth, 1964), it was found that approximately 17% was excreted. When large amounts of cold PN were administered simultaneously the amount of ^{3}H-PN excreted rose to an average of 50%. Absorption appeared to be maximal between 60 and 90 min after administration and was virtually complete by 12 hr. It was found that conditions which contribute to malabsorption, such as idiopathic steatorrhea, as well as resection of major sections of the small intestine had no effect on the amount of PN absorbed.

It has been shown (Contractor and Shane, 1968) that after oral administration of 100 mg of PN to humans, the highest concentration in blood appeared 2 hr later. Large increases in the concentration of pyridoxal phosphate, pyridoxal, and 4-pyridoxic acid occurred but no significant increase in concentration of PMP or PM was noted.

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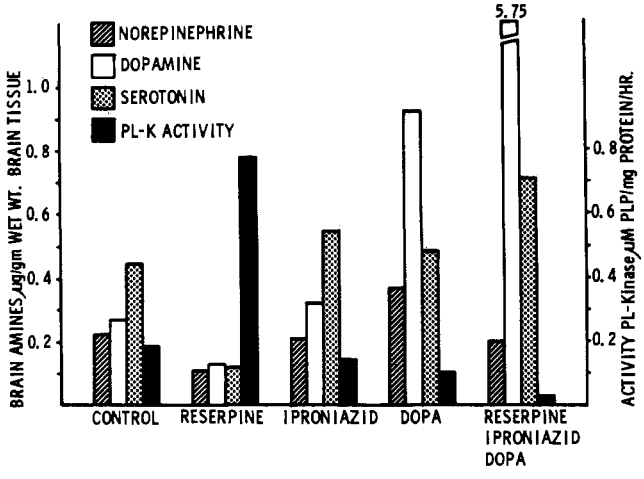

Figure 1. Relationship of brain amine concentration to PL kinase activity. New Zealand white rabbits were administered reserpine 5 mg/kg I.V. or iproniazid 100 mg/kg I.V. or DOPA 150 mg/kg I.V. at varying times prior to decapitation. Brain amines extracted and measured fluorometrically; brain PL kinase activity measured by method of McCormick and Snell

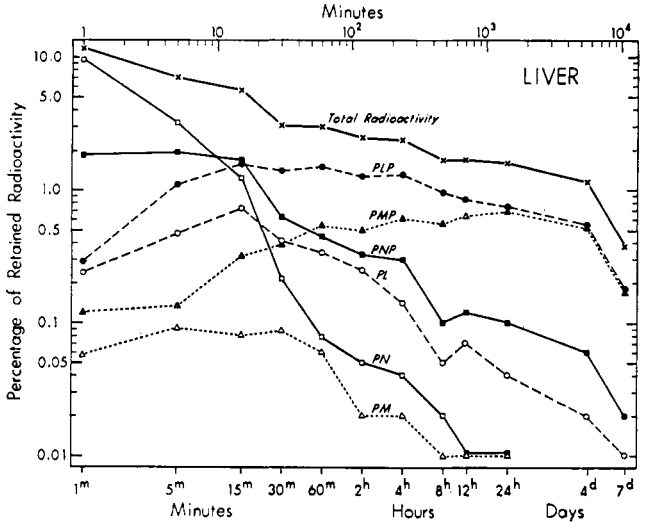

Figure 2. Retention and distribution of ^{14}C-pyridoxine in liver. Swiss–Webster mice were injected I.V. with ^{14}C-PN and then sacrificed at times as shown. Liver was homogenized, B_6 vitamers extracted, separated and radioactivity determined as described. Results expressed as individual vitamer percent retained of total injected radioactive ^{14}C-PN at times indicated

Johansson and coworkers (Johansson *et al.*, 1966) administered ^{3}H-PN to human volunteers to study the turnover of vitamin B_6 in man. From the radioactive substances excreted in urine, data on B_6 turnover were calculated. Isotope retention was characterized by an initial rapid fall in the first day and then a slow decline with a half-life of 13 to 38 days of the administered radioactive ^{3}H-PN.

ENZYMES WHICH CATALYZE VITAMIN B_6 TRANSFORMATION

Pyridoxal Phosphokinase. Ingested pyridoxine may be either phosphorylated to PNP or oxidized to PL. Pyridoxal is phosporylated directly by the same kinase, pyridoxal phosphokinase, that phosphorylates PN. McCormick, Gregory and Snell (McCormick *et al.*, 1961) were the first to report detailed studies of pyridoxal phosphokinase. Their work showed that in mammalian tissue PL was the preferred substrate but PN was also readily phosphorylated. The enzyme occurred in all tissues with the highest activity in the supernatant of the particle-free cell fraction.

The author's studies of the properties of PL kinase stemmed from the observation that when an animal was depleted of the amines norepinephrine, dopamine, and serotonin by reserpine, the activity of brain PL kinase increased but when amine levels were increased by administration of monamine oxidase inhibitors such as iproniazid, a decrease in enzyme activity occurred (Ebadi *et al.*, 1968). These results are shown in Figure 1. This inverse relation between the concentration of brain amines and PL kinase activity suggested that amine concentration may control the rate of PLP synthesis in brain. Pyridoxal phosphokinase from rabbit brain has been highly purified and the activity of the enzyme in the presence of added amines studied (Henn and McCoy, 1971). When amines such as dopamine, norepinephrine, or serotonin were added at 1 m*M* concentration to the incubation mixture of the purified enzyme in the presence of excess PL, inhibition of 68–75% of normal activity was observed. These results showed that *in vitro* as well as *in vivo* increased concentration of amines inhibited pyridoxal kinase.

Pyridoxine Oxidase. Pyridoxine may be oxidized to pyridoxal or phosphorylated to pyridoxine phosphate which, in turn, is oxidized to pyridoxal phosphate. Present data suggest that two distinct enzymes catalyze these two oxidation reactions. Marino and coworkers (Marino *et al.*, 1960) purified pyridoxine oxidase from rabbit liver and studied the properties of the enzyme. The purified enzyme did not oxidize PNP, the pH optimum was 5.7 to 5.9, and was not activated by addition of FMN or metals. There was no report of inhibition of enzyme activity by adding pyridoxal or pyridoxamine. Wada and Snell (Wada and Snell, 1961) purified pyridoxine phosphate oxidase which catalyzes the oxidation of both PNP to PLP and PMP to PLP. These authors noted that the oxidation of PMP was another pathway in addition to amine oxidase for the disposition of the amine group of amino acids and that the oxidation of PMP was a mechanism for the net conversion of PMP to PLP. Their data showed that purified PNP oxidase did not convert PN to PL; the enzyme was FMN dependent, was inhibited by its product PLP, and used either PNP or PMP as substrate at pH 8.0. At this pH, PNP was bound preferentially to PMP. In crude tissue extracts of liver, PNP was oxidized much more rapidly than PN.

INTERCONVERSIONS OF ^{14}C-PYRIDOXINE

Although data were available on the individual steps of B_6 transformation, little information was available on the rate of transformation and distribution of PN was determined simultaneously in brain, liver, and carcass. For these reasons studies were initiated which used ^{14}C-PN rather than ^{3}H-PN which, depending on the method of preparation, has a 10 to 60% loss of the tritium label during transformation. In the present studies, male Swiss–Webster mice were given intravenously 0.5 ml of a solution containing 5.3 μCi and 115 μg of ^{14}C-PN. They were sacrificed at time intervals of 1 min to 7 days by immersion in liquid nitrogen, dissected while frozen, and B_6 vitamers were extracted in 7% TCA. The concentrated extracts were applied to a thin layer cellulose plate and B_6 vitamers separated by electrophoresis (Colombini and McCoy, 1970a). Using this technique it was possible to separate all six forms of the vitamer found in brain, liver, and carcass (Colombini and McCoy, 1970b).

Table I. Percentages of the Recovered Radioactivity and Distribution of the B_6 Vitamers in the Mouse Brain

Tissue	Experiment	Total dpm	% of injected radioactivity	% PN	% PM	% PL	% PNP	% PMP	% PLP
Brain	Controls	9,571	0.16	3.17	2.17	17.98	13.03	14.17	49.57
	Red Oil (Acute)	5,825	0.09	9.18	7.35	10.36	12.75	15.47	44.89
	Red Oil (8 days)	13,795	0.22	4.14	0.70	8.73	39.73	21.39	25.32

Total radioactivity injected as ^{14}C-pyridoxine = 6,093,000 dpm.

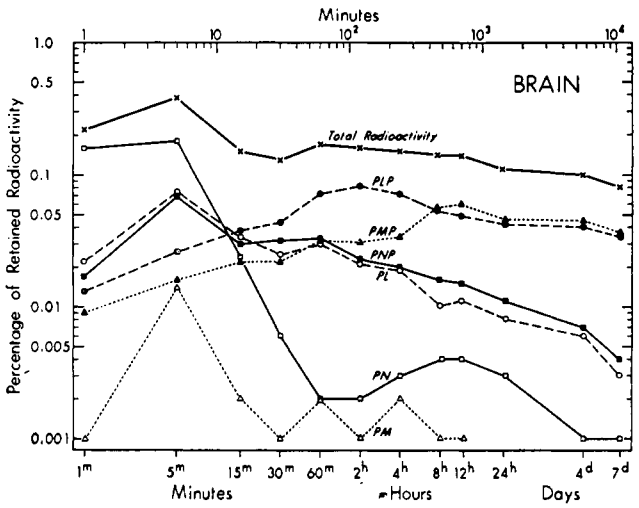

Figure 3. Retention and distribution of ^{14}C-pyridoxine in brain. Conditions of administration of ^{14}C-PN, extraction of B_6 vitamers and measurement of retained radioactivity as described in Figure 2

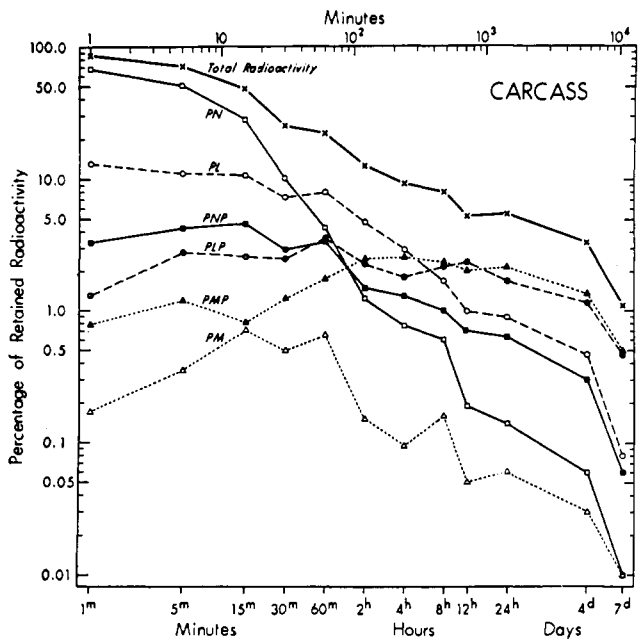

Figure 4. Retention and distribution of ^{14}C-pyridoxine in carcass. Conditions of administration of ^{14}C-PN, extraction of B_6 vitamers and measurement of retained radioactivity as described in Figure 2

RETENTION AND DISTRIBUTION OF VITAMIN B_6 IN LIVER

The shortest time interval between ^{14}C-PN injection and sacrifice was 1 min. The total retained radioactivity was 12.2% distributed as 9.6% PN, 1.8% PNP, 0.3% PLP, and 0.25% PL (Figure 2). These results indicated that phosphorylation proceeded more rapidly than oxidation and that PLP was formed within the first minute after PN administration. By 5 min after injection the total retained radioactivity had decreased to 7.5%, of which 3.4% was PN, 2.0% PNP, 1.2% PLP, and 0.5% PL. These data again indicated that phosphorylation proceeds more rapidly than oxidation as the amount of PNP was greater than PLP which is derived from both PL and PNP. By 60 min the total radioactivity retained was 3.4%, of which 1.7% was PLP, 0.5% PMP, 0.4% PNP, 0.3% PL, and 0.07% PN. These results illustrated that the principal reactions occurring after PN administration were a conversion to PLP and a continued interconversion of PLP to PMP. Of the 2% of total radioactivity retained at 24 hr, 1.5% was almost equally divided as PLP and PMP.

RETENTION AND DISTRIBUTION OF VITAMIN B_6 IN BRAIN

The brain, unlike liver, did not reach peak retention of radioactivity until 5 min after injection of ^{14}C-PN. Initial conversion of PN differed in brain compared to liver. Whereas phosphorylation was the dominant initial reaction in liver, oxidation appeared equally active in brain as indicated by the equal percent of retained radioactivity in PL and PNP at 1 and 5 min (Figure 3). The maximum total radioactivity retained was much less in brain (0.4%) compared to liver (12.2%) but that radioactivity which was retained by brain was lost more slowly than that of the liver. At 60 min the distribution of retained radioactivity was 0.08% PLP and almost equal amounts (0.03%) of PMP, PNP and PL while at 24 hr there were almost equal amounts of PLP and PMP (0.05%) and approximately 0.01% of both PNP and PL. The distribution of retained radioactivity predominantly as PLP and PMP reflected the distribution of actual amount of vitamin B_6 in brain which is predominantly PMP and PLP (Bain and Williams, 1960).

RETENTION AND DISTRIBUTION OF VITAMIN B_6 IN CARCASS

It was an unexpected finding that 1 min after ^{14}C-PN injection 86.7% of the total radioactivity injected was found in carcass (Figure 4). The radioactivity was distributed as 68.3% PN, 12.9% PL, 3.3% PNP, and 1.3% PLP. Fifteen minutes after injection, there was still retention of ^{14}C-PN distributed as 30% PN, 11% PL, 4.8% PNP, and 2.6% PLP. These results indicated that in contrast to liver, the predominant initial pathway of PN metabolism in carcass is oxidation to PL and that PL kinase activity is decreased compared to liver. At 24 hr the total radioactivity was 7% of the injected dose compared to 1.6% in liver and 0.1% in brain. These data further support the proposal that the carcass is a storage site for vitamin B_6 in the body. Although like liver, the predominant forms at this time were PMP and

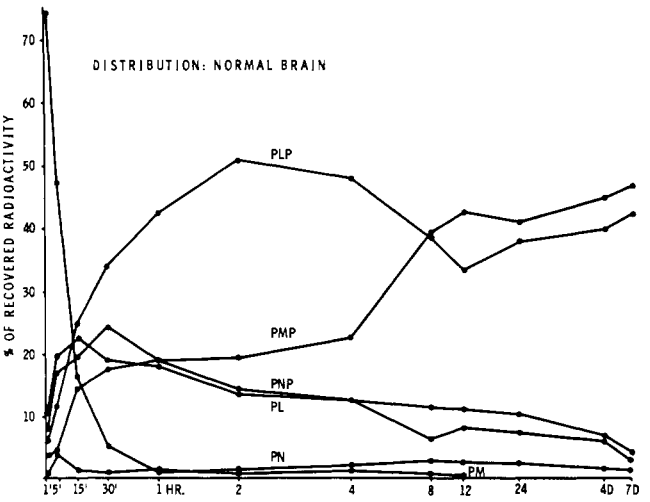

Figure 5. Distribution of retained radioactivity in normal brain. Swiss-Webster mice were injected with ^{14}C-PN and treated as described in Figure 2. Results are expressed as distribution among B_6 vitamers of radioactivity retained by normal brain

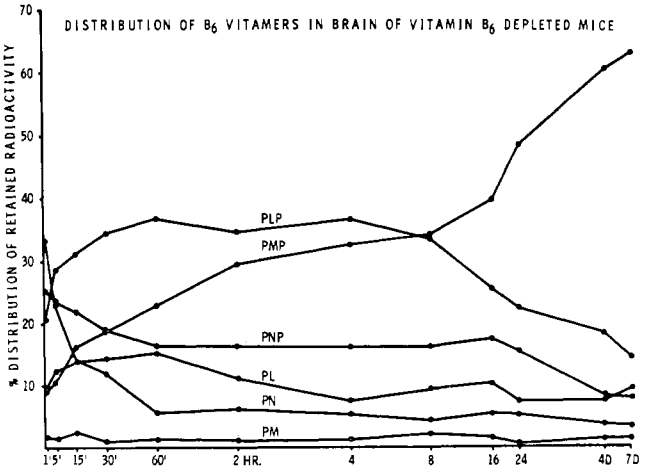

Figure 6. Distribution of retained radioactivity in brain of vitamin B_6 deficient mice. Conditions of administration and results expressed as in Figure 5

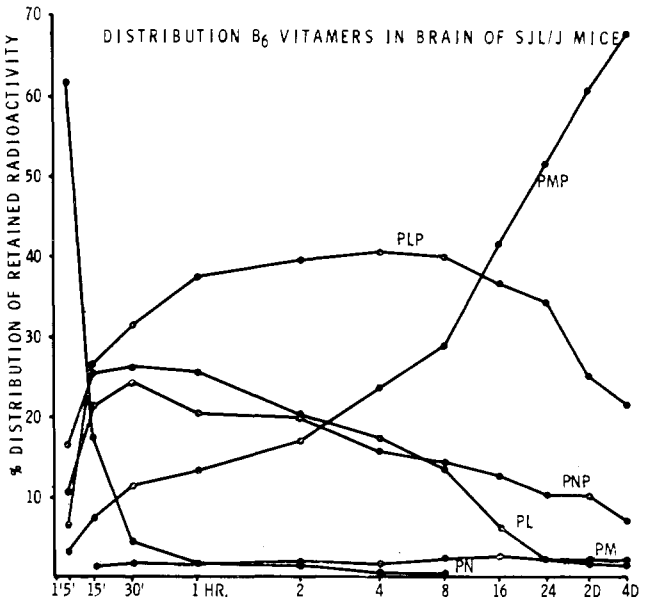

Figure 7. Distribution of retained radioactivity in brain of convulsogenic mice. Conditions of administration and results expressed as in Figure 5

PLP, a larger proportion was present as PL, PNP, and PN than in liver.

EFFECTS OF MARIJUANA ON PN TRANSFORMATION

The data on transformation of vitamin B_6 in liver, brain, and carcass of normal mouse indicated that PN was rapidly transformed to PLP and PMP and retained in these forms. The described technique was used to study the effects of various drugs on vitamin B_6 metabolism in brain. The red oil of marijuana was administered as a single dose of 500 μg/kg or daily for 8 days at a dose of 300 μg/kg intraperitoneally to Swiss-Webster mice and contained 20% Δ^9-tetrahydrocannabinol. Continued red oil administration resulted in increased retention of radioactivity when the animals were sacrificed 30 min after ^{14}C-pyridoxine administration but the distribution of retained radioactivity was markedly altered (Table I). The percent of radioactivity retained as PLP and PL was decreased while that of PNP and PMP was increased. This suggested that prolonged administration of the red oil of marijuana inhibited the activity of pyridoxine oxidase in brain.

PN TRANSFORMATION IN BRAIN OF B_6 DEPLETED AND CONVULSOGENIC MICE

The distribution of retained radioactivity was studied in young adult male Swiss-Webster mice after receiving for 40 days a vitamin B_6 deficient diet (Nutritional Biochemicals Corporation). The metabolism of vitamin B_6 in mice that had received the deficient diet was compared to normal and audiogenic seizure-prone mice strain SJL/J obtained from Jackson Laboratories, Bar Harbor, Maine. Normal, B_6 depleted and seizure-prone mice all received 0.5 ml of ^{14}C-PN containing 5.3 μCi and 115 μg of PN. The results are expressed as the percent distribution of total retained radioactivity at times indicated, among all B_6 vitamers. In normal mice the highest percent of retained PLP occurred 2 hr after injection of ^{14}C-PN, whereas in depleted and convulsogenic mice the maximum percent retention occurred at 1 hr (Figure 5). This finding suggests a more rapid conversion of retained radioactivity to PLP in these mice compared to normal. The percent distribution of retained radioactivity in brain for the first 8 hr (Figures 5, 6, and 7) after administration of radioactive ^{14}C-PN was similar for normal, B_6 depleted, and convulsogenic mice. By 24 hr, however, the percent of radioactivity retained as PMP was greater in both SJL/J and B_6 depleted mice compared to control animals (Figures 6 and 7) while the percent retained as PLP was lower. These studies together with a study in progress of effects of amphetamine on B_6 metabolism suggest that where the metabolism of vitamin B_6 is increased, the percent retention of PMP is increased. The mechanism of this observation is not clear at this time.

CONCLUSION

The pathways for the normal metabolism of vitamin B_6 are known and some data exist on the rate of transformation of PN to the phosphorylated forms. Although considerable information is available on properties of the enzymes which catalyze phosphorylation (White and Dempsey, 1970) and oxidation (Wada and Snell, 1961) of the vitamers, there is limited data on control mechanisms for pyridoxal kinase and very little data on control mechanisms operating on pyridoxine

phosphate oxidase and aldehyde oxidase. Although the total body half-life of administered ^{3}H-PN has been calculated (Contractor and Shane, 1968), there is no data on turnover of vitamin B_6 in brain or liver. However, techniques are now available for this work to be done. The work on interconversion and distribution of vitamin B_6 in liver, brain, and carcass of normal mice (Colombini and McCoy, 1970b) has laid the foundation for further work of the overall effects on B_6 metabolism of drugs such as amphetamines or reserpine known to alter single steps in the metabolism of this vitamin. It is hoped that soon data will be available on the turnover of each of the B_6 vitamers and of the factors which control B_6 turnover in brain, liver, and carcass.

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Normal and Pathological Conditions Which May Alter the Human Requirement for Vitamin B_6

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Factors which may affect the production and assessment of vitamin B_6 deficiency in humans are presented. Although the tryptophan load test has been widely used to assess vitamin B_6 deficiency, this test used alone is difficult to interpret because interrelated hormonal and metabolic factors play an important role in affecting the results obtained. Other methods of measuring vitamin B_6 nutrition, such as the determination of blood and tissue levels of the vitamin B_6 vitamers, and the measurement of erythrocyte aminotransferases, should be used in conjunction with the tryptophan load test. Although the abnormal tryptophan metabolism observed in pregnant women and in women using oral contraceptives is probably only in part due to a vitamin B_6 deficiency, it seems reasonable at this time to suggest that such women receive supplemental pyridoxine. However, serial studies will be necessary to establish the long-term effects of such supplementation.

Since the codiscovery of vitamin B_6 by György, Edgar, and Chick (Robinson, 1951) numerous functions for this versatile nutrient have been defined. The chief function of this vitamin is in the metabolism of amino acids (Williams, 1964), but it is also involved in lipid and carbohydrate metabolism (Wiss and Weber, 1964; Mueller, 1964). It is beyond the scope of this report to acknowledge the many investigators who have made significant contributions to the knowledge of requirements and functions of this important vitamin; these have been reviewed in recent years (Robinson, 1951; Wiss and Weber, 1964; Snell, 1958; Storvick and Peters, 1964; Harris *et al.*, 1964).

Conditions Which May Lead to Vitamin B_6 Deficiency or Dependency. In discussing conditions which may alter the human requirement for this vitamin, it will be helpful to consider a number of conditions which might possibly lead to vitamin B_6 deficiency or dependency. Some of these factors are listed in Table I. Inadequate dietary intake is the first and most obvious possible cause for a deficiency. Although this vitamin is widely distributed in a variety of foods, the content of foods eaten by a weight conscious population may be rather marginal. That is, foods such as beans, peas, cabbage, nuts, cereals, etc., contain reasonable amounts of this vitamin while meats, eggs, and milk are not such good sources. Hence, someone eating a high protein diet, which actually increases the need for the vitamin (Natl. Acad. Sci., 1968), may have a marginal intake.

Even with adequate intake, there may be a tissue deficiency if there is impaired delivery of the vitamin. We may list defective intestinal absorption, defective cellular and intracellular transport, as well as impaired activation of the vitamin by oxidation to pyridoxal and phosphorylation to the active coenzyme, pyridoxal phosphate (PLP). Examples of these mechanisms are rare, and in some instances not clearly established.

Conditions of deficiency may also arise if there is excessive loss of the vitamin, such as enhanced renal clearance of the vitamin due to impaired renal function. Also, if there is

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