DRUG-PYRIDOXAL PHOSPHATE INTERACTIONS

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I. INTRODUCTION

Vitamin B_6 derivatives, in their biocatalytically active form, pyridoxal phosphate, participate at stages in the metabolism of amino acids (Snell, 1953), proteins (Wiss and Weber, 1964, carbohydrates (Krebs and Fischer, 1964), lipids (Mueller, 1964), nucleic acids (Axelrod and Trakatellis, 1964), hormones (Rose, 1978), and other coenzymes (Kutsky, 1973). Pyridoxal phosphate, being the most reactive coenzyme (Snell, 1958) and the most diversified in catalytic functions (Braunstein, 1960), plays an unparalleled role in biochemical transformation of numerous amino acids and in nitrogen-related metabolism in the form of decarboxylation, transamination, deamination, racemization, and desulfhydration reactions.

The importance of these reactions becomes evident when one recalls that many putative neurotransmitters are synthesized and/or metabolized by the aid of pyridoxal phosphate-dependent enzymatic reactions. These include dopamine, norepinephrine, serotonin (Lovenberg et al., 1962), tyramine, tryptamine (Meister, 1965), taurine (Jacobsen and Smith, 1968), histamine (Buffoni, 1966; Kahlson and Rosengren, 1965), gamma-aminobutyric acid (Roberts and Frankel, 1950; Baxter and Roberts, 1958), and even acetylcholine indirectly (Yamada et al. 1956; Williams and Hata, 1959). In recent years these biogenic amines have become the center of considerable interest and the focus of intensive investigations among neuroscientists interested in understanding the etiology of, and in searching for treatment of, neurological, neuroendocrine and psychiatric disorders.

Pyridoxal phosphate also participates in the synthesis of polyamines such as putrescine, spermidine, and spermine by serving as a cofactor for ornithine decarboxylase (Pegg and Williams-Ashman, 1968). In pyridoxine deficiency, the synthesis of polyamines is reduced. In addition, pyridoxal phosphate is involved in the synthesis of sphingosine (Brady and Koval, 1958; Braun and Snell, 1968).

The role of pyridoxal phosphate in neurobiology and metabolism of the CNS has been reviewed (Kelsall, 1969; Ebadi and Costa, 1972; Ebadi, 1978; Ebadi and Govitrapong, 1980; Ebadi, 1981; and Dakshinamurti, 1982). Despite the fact that various classes of neuropharmacological agents alter the level of pyridoxal phosphate, as well as the activities of the vitamin B_6-related enzymes and/or vitamin B_6-dependent enzymes in the brain, the detailed pharmacodynamics have not been presented. Although various stimulants of the central nervous system

reduce the level of pyridoxal phosphate within the CNS and concomitantly cause hyperexcitability and convulsions, the role that vitamin B_6 plays in the maintenance of the integrity of neuronal excitability is obscure. More research on these and similar effects of vitamin B_6 seems indicated.

Nutritionists have established the various effects and complications of vitamin B_6 deficiency, and the biochemists have delineated the mechanisms of pyridoxal phosphate involvement in numerous vitamin B_6-dependent reactions. However, there remains a wealth of knowledge about this coenzyme yet to be discovered by neurophysiologists and neuropathologists who are involved in studies dealing with various neurobiological parameters in health and disease, and by neuropsychopharmacologists who are anxious to study and delineate the pharmacodynamics of various psychoactive agents. It is anticipated that the study of vitamin B_6 in neuropharmacology will prove most revealing and rewarding. In this review, the interactions of pyridoxal phosphate with select neuroendocrine and neuropharmacological systems will be discussed and their health-related therapeutic implications will be summarized.

II. THERAPEUTIC EFFICACY OF DRUGS IN ALTERED NUTRITIONAL STATES

Malnutrition, both clinical and experimental, influences the therapeutic efficacy of drugs. Various nutritional deficiencies may impair drug detoxification mechanisms (reviewed by Williams, 1978), and protein deficiency may alter drug interactions (reviewed by Varma, 1981). Furthermore, malnutrition may alter the tissue responsiveness to drugs. In addition to deficiencies, dietary excess of nutrients including coenzymes will produce adverse reactions (see DiPalma and Ritchie, 1977). Moreover, even proper nutrients and alimentation may alter the bioavailability of drugs (see Toothaker and Welling, 1980; Carr, 1982).

A. Nutrients and Drug Detoxification

Malnutrition may alter the absorption, distribution, binding, excretion, and biotransformation of drugs. Since the biotransformations depend on enzymatic functions, any factors that reduce the supply of amino acids for the synthesis of these enzymes are likely to interfere with drug metabolism. Therefore, either a low protein or a low calorie intake could decrease the availability of amino acids.

Drug biotransformations are commonly classified as Phase I and Phase II reactions. Phase I reactions include oxidations (most commonly), or reductions, or hydrolytic reactions. Phase II refers to various conjugations, i.e., the coupling of a drug, or its metabolites from Phase I, with a conjugating agent that is ultimately derived from nutrients.

In addition to adequate proteins needed for the synthesis of apoenzymes, the following nutrients are required to provide the essential components of the cytochrome P-450 oxidizing system (Williams, 1978):

nicotinic acid – for NADPH
riboflavin – for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)
pantothenic acid – for coenzyme A
glycine – for the heme in cytochrome – P-450
Fe^{2+} – for heme
Cu^{2+} – for converting protoporphyrin IX to heme
Ca^{2+}, Zn^{2+}, Mg^{2+} – for maintenance of membranes
phosphatidylcholine – for maintenance of membrane and ion transport.

Reductive and hydrolytic reactions probably require many of the same components as are needed for microsomal oxidation.

The very conjugating agents that a human uses in Phase II of drug metabolism are derived from one or more of the major nutrient groups, i.e., protein, carbohydrate, or fat. For example:

glucuronic acid is derived from carbohydrate
glycine, cysteine, glutamine, glutathione, methionine, and sulfate are amino acids or derived from amino acids
acetyl radicals are derived from fat, carbohydrate, or protein.

In addition to the conjugating agents, Phase II synthetic reactions require energy, which is supplied by ATP, which in turn is derived from nutrients that yield energy. Likewise, many of the vitamins are needed in carrying out the steps of conjugation reactions. These include pantothenic acid, folic acid, B_{12}, nicotinic acid, and pyridoxine.

It is apparent, therefore, that drug biotransformations are dependent on many nutrients, but those that are most likely to be critical, especially when nutrition is of a marginal quality, are vitamins and proteins.

Dietary protein deficiency results in reduction in the activity of

numerous drug metabolizing enzymes (Kato et al., 1962, 1968; McLean and McLean, 1966; Marshall and McLean, 1969; Mgbodile and Campbell, 1972; Miranda and Webb, 1973; Anthony, 1973; Campbell and Hayes, 1974; Eriksson, et al., 1975; Campbell, 1977; Newberne et al. 1978; Varma, 1980a, 1981). Since metabolites of drugs may possess greater or lesser biological or toxicological activities than the parent compounds, protein deficiency may either increase or decrease therapeutic efficacies and/or toxicities of drugs (Varma, 1981). For example, the metabolism of testosterone and estriol is greatly reduced in patients with anorexia nervosa (Fishman and Bradlow, 1977). The toxicity of some pesticides is greatly increased in protein deficient rats (Boyd, 1972). Hepatotoxic compounds that exert their effects through their metabolites are less toxic in protein deficient animals (Kato et al., 1968; Weatherholtz et al., 1969).

Furthermore, food restriction and protein deficiency do not influence all drug-metabolizing enzymes equally. For example, Sachan and Das (1982) studied drug metabolizing enzymes in feed restricted rats. In these studies, two groups of weanling male Sprague-Dawley rats were fed for 49 days a semi-synthetic diet, one group (restricted group) being fed 50% of the amount consumed by the group fed ad libitum. Compared to the ad libitum group, the animals of the restricted group gained 55% less in body weight and had 60% smaller livers. While serum glucose, urea nitrogen, uric acid, and lipids were lowered in the restricted animals, other serum biochemical parameters were unchanged. The in vitro activities of hepatic drug-metabolizing enzymes were increased two- to threefold in the restricted animals. Feed restriction also caused a significant increase in the activities of NADPH-generating enzymes of liver and adipose tissue. However, the enzymes not concerned with the production of NADPH remained unaffected by feed restriction. It was concluded that feed restriction, unlike starvation, enhanced activities of drug-metabolizing enzymes as well as NADPH-generating enzymes (Sachan and Das, 1982).

The involvement of pyridoxine and its active form, pyridoxal phosphate, in drug-metabolizing enzymes becomes especially apparent when animals are maintained on high protein but low or deficient pyridoxine diets. Vitamin B_6 deficiency has been reported to cause fatty liver (Halliday, 1938). Other studies have shown that rats maintained on a high protein diet without pyridoxine have decreased activity of aminotransferases and marked accumulation of triglyceride and cholesterol

ester in the liver (Okada and Ochi, 1971; Okada and Suzuki, 1974; Suzuki et al., 1976).

Abe and Kishino (1982) studied morphologically fatty liver induced in rats by a high protein diet without pyridoxine. "The microscopic changes were characterized by accumulation of fat in hepatocytes of the centrilobular and midzonal areas. Electron microscopic examination at an early stage showed marked accumulation of small osmiophilic particles in the granular endoplasmic reticulum and vesicles throughout the cytoplasm with similar particles in the spaces of Disse. After 4 weeks, numerous lipid droplets of various sizes were seen in pericanalicular lysosomes in hepatocytes with concomitant increase in the triglyceride level. The droplets gradually formed larger droplets in the cytoplasm. After 8 weeks, myelin figures together with fat droplets were seen in continuity with the endoplasmic reticulum and occasionally crystal clefts were observed within lysosomes of hepatocytes." These findings suggest that development of fatty liver results from impaired lysosomal degradation of lipid (Abe and Kishino, 1982).

Alcoholism may produce pyridoxal phosphate deficiency by dietary restriction and by impaired metabolism. A low concentration of pyridoxal phosphate in plasma (an index of vitamin B_6 deficiency) is often seen in alcoholics with and without impairment of hepatic functions (Hines and Cowan, 1970; Lumeng and Li, 1974). The lowered blood vitamin levels are not caused solely by inadequate intake of the vitamin, but also result from impaired vitamin metabolism. This impairment is believed to be caused by acetaldehyde, the oxidation product of ethanol, which displaces pyridoxal phosphate from protein binding (Fig. 1) and hence renders it susceptible to degradation by phosphatases (Veitch et al., 1975; Lumeng, 1978). Recent studies by Shane (1982) showed that long-term ethanol supplementation in rats that received approximately 30% of their calorie intake as ethanol reduced the hepatic level of pyridoxal phosphate significantly (Fig. 1).

B. Drug Efficacy and Nutritional Status

The efficacy of drugs and the sensitivity of receptor sites to drugs are often, but not always, altered by malnutrition (Drill, 1952; Brodeur et al., 1974; Friedman, 1966; Lowenthal, 1974; and Varma, 1981). For example, dietary protein deficiency in rats (5% low protein diet and 21% protein in control) for 3 weeks was associated with an increase in the plasma half-life, with a decrease in the plasma protein binding, and

with an increase in the acute anti-inflammatory effects and toxicity of oxyphenbutazone (Varma, 1980c) or phenylbutazone (Varma, 1979). The anti-inflammatory effects of dexamethasone were decreased in rats fed protein deficient diets, but these effects could not be attributed to any modification in the pharmacokinetic profile of dexamethasone (Varma and Mulay, 1980). Other studies have shown that dietary protein deficiency had no influence on the inotropic effects of ouabain on isolated papillary muscles and left atria, ventricular fibrillatory doses of digoxin, or the pharmacokinetics of cardiac glycosides (Varma, 1980b). Similar negative results have been shown about the effects of 'starvation' on the sensitivity of the central nervous system to barbital (Brodeur et al., 1974).

C. Drug Efficacy and Disease States

The sensitivity of receptor sites not only may vary in altered physiological conditions but also is modified in disease states (Lowenthal, 1974). In chronic renal diseases, the bradycardic response to the valsalva maneuver is absent (Soriano and Eisinger, 1972), and the amyl nitrite-induced tachycardia is attenuated (Hampers et al., 1967). Similarly, these patients show altered responses to the administration of atropine, a muscarinic cholinergic blocking agent (Lowenthal and Reidenberg, 1972).

In uremia, the otherwise innocuous penicillin may induce encephalopathy (Bloomer et al., 1967). This encephalopathy is characterized by generalized seizures, myoclonus, and coma. The possibility exists that in uremia the integrity of the blood brain barrier is altered, allowing a higher concentration of penicillin to penetrate the CNS. Similarly, in uremia the clearance of penicillin may be altered, leading to a higher concentration of penicillin in the plasma. Furthermore, in uremia, the concentrations of body electrolytes may be altered, causing cerebral irritability (Lowenthal, 1974).

Other examples where disease states may alter the therapeutic efficacy of drugs are refractoriness to pressor effects of angiotensin (Bartter et al., 1962) seen in hyperreninemia; resistance to parathyroid hormone in chronic renal failure (Massry et al., 1973); and digitalis toxicity in hypokalemic states (Doherty, 1973).

D. Vitamin B_6 Efficacy in Hereditary Disorders

Vitamin-dependent genetic diseases include deficiencies of thiamine

(B_1), nicotinamide, cobalamin (B_{12}), calciferol (D) and pyridoxine (B_6). In contrast to the acquired vitamin deficiencies, vitamin dependencies do not respond to physiologic replacement therapy, but only to pharmacologic doses of the missing substance (Rosenberg, 1970). Vitamin B_6 dependency results in convulsions which have been reported to occur as early as 3 hours after birth, can progress to status epilepticus if untreated, and are intractable to the usual anticonvulsant medications.

In vitamin B_6 deficient children, the xanthurenic acid excretion following tryptophan load is abnormal, whereas in vitamin B_6 dependent children it is normal. The tryptophan-nicotinic acid ribonucleotide pathway requires several pyridoxal phosphate-dependent reactions. Vitamin B_6 deficiency is characterized by elevated excretions of kynurenine, 3-hydroxykynurenine, and xanthurenic acid in urine collected after an oral dose of tryptophan (see Rose, 1978, for review).

In vitamin B_6 deficient children, the plasma concentrations and presumably the tissue concentrations of B_6 derivatives are reduced, whereas in vitamin B_6 dependent children they are normal. In vitamin B_6 deficient children, if diagnosed and treated properly, no sequelae develop, whereas in vitamin B_6 dependent children retarded psychomotor development takes place. If not treated early, vitamin B_6 dependency may lead to irreversible mental retardation.

Vitamin B_6 deficiency is associated with malnutrition, or occurs following administration of drugs which deplete the body's reserve of pyridoxal phosphate, whereas vitamin B_6-dependency is a hereditary disorder. Symptoms of vitamin B_6 deficiency always occur after birth, whereas symptoms of vitamin B_6 dependency – such as fetal distress diagnosed through meconium-stained amniotic fluids and respiratory distress – may begin in utero and prior to the onset of B_6-dependent tress – may begin in utero and prior to the onset of B_6-dependent seizures (Weiner, 1976).

Pyridoxine-dependent children are able to synthesize pyridoxal phosphate normally but, due to instability of the pyridoxal phosphate-albumin complex (Fig. 1), are unable to maintain prolonged high levels normally found in plasma after pyridoxal loading (Heeley et al., 1978).

Pyridoxine-dependency may also be associated with other hereditary disorders, such as branched chain aminoaciduria (Scottolini et al., 1978). These abnormalities were also corrected by administration of pyridoxine (Ebadi, 1981, for review).

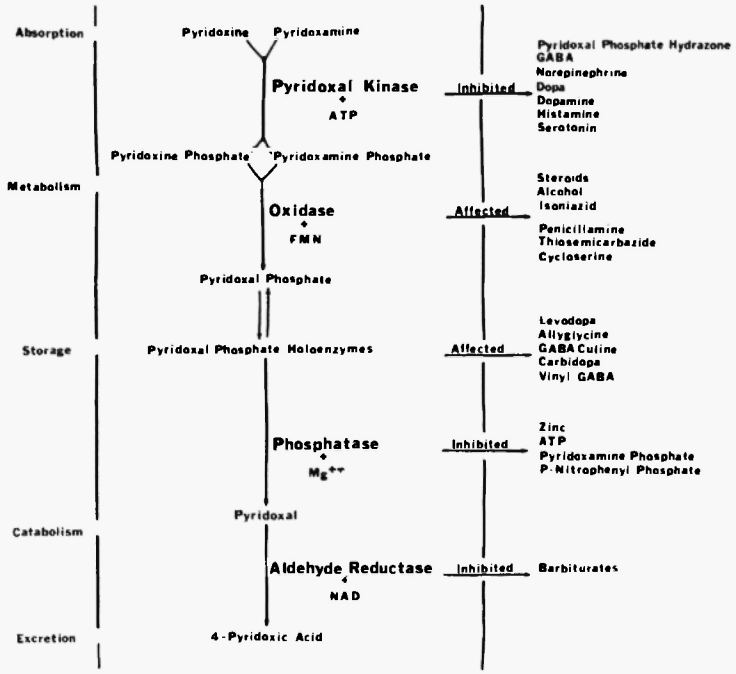

Fig. 1. The pharmacokinetics of pyridoxine and the synthesis of pyridoxal phosphate. Drugs may alter the metabolism and function of pyridoxal phosphate by inhibiting the activity of pyridoxal kinase, such as levodopa (Ebadi and Govitrapong, 1979a); by inhibiting the binding of pyridoxal phosphate to apoenzymes and/or proteins, such as alcohol (Lumeng, 1978; Shane, 1982); by inhibiting the activity of L-aromatic amino acid decarboxylase, which requires pyridoxal phosphate, such as carbidopa (Sandler, 1980); by inhibiting the activity of pyridoxal phosphate phosphatase, such as zinc (Ebadi and Govitrapong, 1979b); and by inhibiting the activity of aldehyde reductase, such as barbiturates (Erwin et al., 1971).

E. Nullification of Therapeutic Effects of Drugs by Vitamins and Vitamin Toxicity

The use of multiple vitamins or single vitamins in enormous doses (megavitamin) has increased in recent years (for review, see DiPalma and Ritchie, 1977). The abuse or injudicious uses of vitamins not only are fraught with vitamin toxicity, but also may result in altered therapeutics. In this section, the nullification of beneficial effects of levodopa by pyridoxine will be cited, and the manifestation of toxicity of vitamins when given in excess will be outlined (Table 1).

Table 1: Select Manifestations of Vitamin Toxicity

Vitamin	Manifestation of Toxicity	References
Vitamin A	Cirrhotic-like liver syndrome including portal hypertension	Russell et al., 1974.
Vitamin D	Cerebral, cardiovascular, and renal damage	Seelig, 1969
Vitamin K_1	Hemorrhagic diseases	Vest, 1966
Vitamin E	Allergic dermatitis	Aeling et al., 1973
Thiamin	Rapid pulse, vasodilation, and cardiac arrhythmias	DiPalma and Hitchcock, 1958
Niacin	Abnormal liver function and jaundice	Berge, 1961
Riboflavin	No major toxicity reported	DiPalma and Ritchie, 1977
Pyridoxine	Sensory neuropathy, convulsive seizures	Krinke et al., 1981, Ebadi, 1981, Ebadi et al., 1983b
Folic acid	Convulsive seizures and hypertrophy and hyperplasia of the renal epithelial cells	Baxter et al., 1973, Preuss et al., 1972
Ascorbic acid	Diarrhea, cysteine or oxalate stone formation in urinary tract	Cochrane, 1965

The widespread use and the efficacy of levodopa in the treatment of Parkinsonism are now established. Aromatic-L-amino acid decarboxylase, a pyridoxal phosphate-requiring enzyme, converts dopa to dopamine, which presumably raises the concentration of dopamine in striatum of dopamine deficient Parkinsonian patients. Since the advent of levodopa, many attempts have been made to increase its therapeutic effects while minimizing its side effects. It was speculated that dopamine deficiency might be due to diminished decarboxylase activity

which theoretically could be corrected by administration of pyridoxine. Subsequently, Duvoisin and coworkers (1969) administered pyridoxine to a group of Parkinsonian patients on levodopa therapy. They found, contrary to expectations, pyridoxine not only failed to enhance the 'dopa effect' in Parkinsonism but also annulled the benefits of L-dopa treatment altogether. Large doses produced a rapid and complete reversal of the 'dopa effect', and small doses produced a gradual and partial reversal. Since the peripheral tissues such as kidney, liver, and small intestine have considerably greater capacity than the brain to decarboxylate dopa, many attributed the paradoxical effects of vitamin B_6 to augmented decarboxylation of dopa in the peripheral tissues, resulting in a reduction in the fraction of administered dopa reaching the brain (Dougan et al., 1975). This interpretation was tested and refuted by Johnson et al. (1976) who reported that pyridoxine administration to Parkinsonian patients on levodopa therapy caused no significant alteration in urinary output of free or conjugated dopa or dopamine. Furthermore, Leon et al. (1971) have shown that the urinary excretion of homovanillic acid and dopamine did not change after pyridoxine. If the effect of pyridoxine were to enhance decarboxylation of dopa, there should have been an increase in these metabolites. These and other investigators (Calne and Sandler, 1970; Evered, 1971; Johnston, 1971; Leon et al., 1971; Pfeiffer and Ebadi, 1972; Sandler, 1973; Johnson et al., 1976) believe that the mechanism of nullification of levodopa by pyridoxine may be more complex than initially appreciated.

The nullification of 'dopa effect' by pyridoxine in Parkinsonian patients is undoubtedly related to unavailability of sufficient dopamine to its receptor sites in the striatum. How this effect is produced continues to remain controversial. Since the highly reactive pyridoxal phosphate undergoes Schiff-base formation with many endogenous amino acids and biogenic amines, including levodopa, one popular theory dealt with formation of tetrahydroisoquinoline derivatives between pyridoxine derivatives and levodopa, or between pyridoxine derivatives and dopamine. These tetrahydroisoquinoline derivatives would undoubtedly not only be pharmacologically inactive but also were thought to inhibit dopa decarboxylase (Tran, 1972). This contention was strengthened by investigations reporting the urinary excretion of tetrahydroisoquinoline alkaloids in levodopa-treated Parkinsonian patients (Cotzias and Papavasiliou, 1971). Furthermore, Sandler (1973)

reported the excretion of tetrahydroisoquinoline alkaloids by Parkinsonian patients who had consumed large doses of L-dopa and ethanol. In addition, Coscia et al. (1977) have reported the occurrence of norlaudanosolinecarboxylic acid in urine of Parkinsonian patients on levodopa with or without carbidopa. Comparative studies with rats on a similar regimen revealed the presence of norlaudanosolinecarboxylic acid in brain as well as in urine (Coscia et al., 1977).

The effects of tetrahydroisoquinoline derivatives on the activity of enzymes that synthesize and metabolize catecholamines have also been reported. Meller et al. (1977) have shown that tetrahydro-β-carboline, a condensation product of indoleamine and glyoxylic acid, competitively inhibited the oxidative deamination of serotonin at micromolar concentrations. Moreover, tetrahydroisoquinoline derivatives have been shown to inhibit tyrosine transaminase (Black and Axelrod, 1969; Fellman and Roth, 1971) and dopamine-β-hydroxylase (Lasala and Coscia, 1979). These studies encouraged us to synthesize tetrahydroisoquinoline derivatives by condensing dopa, dopamine, or norepinephrine with pyridoxal or pyridoxal phosphate and to study their effects on dopa decarboxylase in brain homogenate. These compounds in large concentrations of 50 mM did not inhibit either pyridoxal kinase (Ebadi and Govitrapong, 1979b) or dopa decarboxylase (Ebadi and Govitrapong, 1981).

Previous investigators have implied that tetrahydroisoquinoline derivatives may mediate both the therapeutic effects (Sourkes, 1970, 1971) and the side effects of levodopa (Dougan et al., 1975; Hornykiewicz, 1973; Roberge, 1977). These implications presume that these compounds may serve either as false transmitters having weak dopamine agonistic properties (Sourkes, 1970) or act as antagonists blocking dopamine receptor sites (Dougan et al., 1975; Hornykiewicz, 1973; Roberge, 1977). There is some evidence for both of these implications. Simple aldehyde derivatives of tetrahydroisoquinoline compounds have been shown to interact with catecholaminergic receptors (Cohen, 1971; Cohen et al., 1972). Furthermore, Greenberg and Cohen (1973) reported that perfusion of isolated cow adrenal glands for 1 hour with 23 mM acetaldehyde resulted in synthesis of tetrahydroisoquinoline derivatives. Also, Heikkila et al. (1971) have shown that these compounds can be taken up and accumulated by rat brain synaptosomes. Additionally, they are able to release the previously stored catecholamines and block their uptake. Furthermore, the uptake of tetrahydroisoquinoline deriva-

tives, like catecholamines, was blocked by desmethylimipramine (Cohen et al., 1972). These very interesting observations have never been reported in humans. Whether or not any of the foregoing phenomena apply to levodopa-treated Parkinsonian patients remains to be investigated (Ebadi, 1978).

As pointed out in Nutrition Classics (Anon, 1982), the observation that isoniazid interferes with the metabolism of vitamin B_6 and that the peripheral neuritis associated with the use of isoniazid can be prevented by giving a large dose of pyridoxine (Biehl and Vilter, 1954) was among the first reported interactions between a drug and a vitamin nutrient. This report probably alerted other investigators to seek additional drug-nutrient interactions (Roe, 1981).

F. Anoretic and Oretic Agents in Drug Interactions

Anoretic agents such as amphetamine, amphetamine derivatives, and fenfluramine have been used to control appetite, to restrict food intake, and to correct obesity (Craddock, 1976; Sullivan et al., 1976; and Silverstone, 1975). On the other hand, numerous psychotropic agents including cyproheptadine (antihistamine and serotonin antagonist), benzodiazepines (anxiolytic), haloperidol and chlorpromazine (neuroleptics), amitriptyline (antidepressant), marijuana and tetrahydrocannabinol (hallucinogenic) and barbiturates (sedatives and hypnotics) are oretic in nature, and when taken in therapeutic or effective doses increase appetite and cause weight gain (Sullivan and Cheng, 1978). The influence of acute and chronic feeding modulation by drugs and their effects on the basic process of drug interactions have not been investigated thoroughly. However, studies with albino rats fed semisynthetic diets deficient in vitamin B_6 have shown brain cell alterations suggesting premature aging in that some pyramidal cells of the cerebral cortex, particularly in layers III and V, showed partial to nearly complete dendritic loss (Root and Longenecker, 1983).

III. PHARMACOKINETICS OF PYRIDOXINE VITAMERS

Pyridoxal phosphate is not supplied by diet and must be synthesized in the body. The naturally occurring B_6 derivatives, pyridoxine, pyridoxal, and pyridoxamine are absorbed efficiently and rapidly from the gastrointestinal tract. On the other hand, the phosphorylated derivatives, pyridoxine phosphate, pyridoxal phosphate, and pyridoxamine

phosphate are not transported to a great extent across most cellular membranes. Thus, the phosphorylated derivatives of vitamin B_6 must be hydrolyzed prior to absorption.

Recent studies with transport and metabolism of vitamin B_6 carried out in vivo and in vitro indicate that, similar to bacterial systems, pyridoxal phosphate does not enter rabbit red blood cells, the choroid plexus, or the brain (Spector and Greenwald, 1978; Spector, 1978a, b). In addition, these investigators have shown that 2 hours after intraventricular injection, the major portion of [^{3}H, ^{32}P] pyridoxine phosphate in both cerebrospinal fluid and brain had been dephosphorylated first, prior to entry and rephosphorylation. However, studies with in vivo intestinal disappearance of pyridoxal phosphate in rats seem to indicate that although a major portion of pyridoxal phosphate is dephosphorylated and then transported as pyridoxal, a second mechanism also exists in which pyridoxal phosphate is transported unchanged (Middleton, 1979).

In the body, the absorbed pyridoxine, pyridoxal, and pyridoxamine are converted respectively to pyridoxine phosphate, pyridoxal phosphate, and pyridoxamine phosphate (Fig. 1) by pyridoxal kinase (ATP: pyridoxal-5-phosphotransferase, EC 2.7.1.35). The hydrolysis of vitamin B_6-5′-phosphate is believed generally to be accomplished by nonspecific phosphatases (Saraswathi and Bachhawat, 1963; Snell and Haskell, 1971). However, in mouse liver a nuclear phosphatase that specifically hydrolyzes pyridoxal phosphate, but not pyridoxamine phosphate, β-glycerophosphate, α-naphthyl-phosphate, p-nitrophenyl-phosphate, or glucose-6-phosphate has been reported (Kyaw, 1980).

Pyridoxine phosphate and pyridoxamine phosphate are converted to pyridoxal phosphate by a single enzyme, pyridoxine (pyridoxamine) phosphate oxidase (pyridoxine-5′-phosphate:oxygen oxidoreductase, EC 1.4.3.5) (Wada and Snell, 1961). Pyridoxal is converted to 4-pyridoxic acid by the aid of an aldehyde:oxygen oxidoreductase. Recent evidence indicates that another enzyme, a NAD^+-dependent aldehyde dehydrogenase, is also capable of catalyzing the formation of 4-pyridoxic acid (Stanulovic et al., 1976). Major metabolites of vitamin B_6 are pyridoxic acid and pyridoxic acid lactone (Shane and Snell, 1976).

Pyridoxal phosphate inhibits pyridoxine phosphate oxidase (Wada and Snell, 1961; Merrill et al., 1978; McCormick and Merrill, 1980; Kwok and Churchich, 1981), whereas 4-pyridoxic acid inhibits neither

pyridoxine phosphate oxidase (Wada and Snell, 1961) nor pyridoxal kinase (McCormick et al., 1961).

The transport, synthesis, and interconversions of vitamin B_6 derivatives have been studied following administration of radioactive and non-radioactive pyridoxine (Bain and Williams, 1960; Wada and Snell, 1961; Lyon et al., 1962; Ebadi et al., 1970; Colombini and McCoy, 1970; Bell and Haskell, 1971; Contractor and Shane, 1971; Loo, 1972; McCoy and Colombini, 1972; Tiselius, 1973; Johansson et al., 1974; Spector, 1978a, b; Spector and Greenwald, 1978; Loo, 1980). These studies have revealed that the formation of pyridoxal phosphate in various tissues may follow different pathways (Fig. 1). In liver, pyridoxine is phosphorylated to pyridoxine phosphate which, in turn, is oxidized to pyridoxal phosphate. In contrast, in the brain, pyridoxal phosphate is synthesized from both pyridoxine phosphate and pyridoxal (Colombini and McCoy, 1970; McCoy and Colombini, 1972).

The synthesis of pyridoxamine phosphate in the brain occurs slowly, reaching the level of pyridoxal phosphate between 8 and 24 hours after administration of pyridoxine. The available pyridoxine is not converted to pyridoxamine in significant amounts. Therefore, it must be assumed that the pyridoxamine phosphate is synthesized from and is dependent upon the availability of sufficient amounts of pyridoxal phosphate. The ratio between pyridoxamine phosphate and pyridoxal phosphate is approximately 1:2 (Johansson et al., 1974).

In examining the ontogenetic development of pyridoxal, pyridoxamine, and pyridoxine, one notices that the concentration of pyridoxal remains high through 120 days. The very low level of pyridoxine becomes undetectable after six days whereas pyridoxamine levels decline gradually. The concentration of pyridoxal phosphate reaches the adult level in 5 days, and that of pyridoxamine phosphate remains constant up to 10 days and then increases dramatically. The activity of pyridoxal kinase increases gradually (Loo, 1972, 1980). A positive correlation may exist between the ontogenetic development of pyridoxal phosphate and pyridoxal phosphate-requiring enzymes (see Ebadi and Bifano, 1978, for review).

IV. THE ANTIVITAMIN B_6 EFFECTS OF SELECT COMPOUNDS OF PHARMACOLOGICAL IMPORTANCE

A. Vitamin B_6 and Isoniazid

Isoniazid, the hydrazide of isonicotinic acid, is a highly effective tuberculostatic drug. In tuberculosis patients receiving isoniazid, the urinary excretion of vitamin B_6 and xanthurenic acid after tryptophan load became increased. The administration of vitamin B_6 rectifies both abnormalities. In addition, the administration of isoniazid produces peripheral neuropathy which is initially sensory and may later show signs of motor involvement. The pathological manifestations of neuropathy (reviewed by Aita and Calame, 1972) are characterized by "primary axonal degeneration with subsequent fragmentation of the myelin sheath (Wallerian degeneration) of small to medium-sized fibers, with sparing of large sensory fibers to the muscle spindles, plus concomitant remyelination and collateral sprouting of spared motor axons terminally. This degeneration is more severe distally and may later involve the ventral roots and, still later, the dorsal roots and possibly the posterior columns. The collateral sprouting represents motor fiber regeneration and is at a sufficient rate to compensate for lost fibers; thus, clinically, the neuropathy is initially and usually sensory" (Aita and Calame, 1972).

The incidence of isoniazid-induced peripheral neuritis is increased in malnutrition and in situations where the concentration of hydrazide is increased in the body, such as after chronic administration of large dosages and in patients who are slow metabolizers of this compound.

In addition to peripheral neuritis, other manifestations of the neurotoxicity of isoniazid include generalized seizure, optic neuritis followed by atrophy, muscle twitching, dizziness, ataxia, paresthesias, stupor, and toxic encephalopathy. Furthermore, mental abnormalities such as euphoria, transient impairment of memory, separation of ideas and reality, loss of self-control, and psychosis have been reported following the administration of isoniazid. Although the mechanisms of tuberculostatic and tuberculocidal actions of isoniazid are not known (Youatt, 1969), the antipyridoxine effects of the compound have been established. For example, the isoniazid-induced neurotoxicities may be prevented, arrested, or reversed by the administration of vitamin B_6. Consistent with these views is the observation that the neurotoxicity of isoniazid is increased in malnutrition and in conditions causing secon-

dary vitamin B_6 deficiency, such as pregnancy, hyperthyroidism, infections, and malignancy. Isoniazid may cause vitamin B_6 deficiency by chelating pyridoxal phosphate to form pyridoxal phosphate hydrazone, which in turn inhibits pyridoxal kinase, the enzyme that synthesizes the catalytically active coenzyme. Therefore, isoniazid reduces the tissue concentration of pyridoxal phosphate directly and indirectly (Fig. 1).

In a letter to the editor, Sievers and Herrier (1980) made reference to their earlier work which established the efficacy and safety of using very large doses of pyridoxine hydrochloride intravenously to treat the life-threatening, convulsive effects of acute isoniazid overdoses (approximately on a gram per gram basis) in humans.

A report from the same investigators, however, dealing with a study in dogs (Chin et al., 1981) concluded that somewhat smaller doses of pyridoxine given concurrently with one of several CNS depressants or anticonvulsants could also be effective in preventing convulsions and lethality from an overdose of isoniazid. Since the convulsant effect of isoniazid appears to be related to a decreased concentration of gamma-aminobutyric acid (GABA) in the brain (Wood and Peesker, 1972), Chin et al. (1981) pointed out that pyridoxine reduces the inhibition of glutamic acid decarboxylase caused by isoniazid and thus restores in part the neuronal synthesis of GABA. Depressant and anticonvulsant drugs, on the other hand, act by potentiating the postsynaptic actions of GABA. Consequently, when a moderate dose of pyridoxine is supplemented with a CNS depressant or anticonvulsant, the combination of enhanced GABA synthesis and augmented postsynaptic GABA activity may be enough to control the convulsions induced by isoniazid overdose whereas the depressant or anticonvulsant drug by itself is not adequate. The drugs that were found to act synergistically with pyridoxine included phenytoin, phenobarbital, and diazepam (Fig. 1). Of these drugs, diazepam would appear to be a desirable choice because of its rapid onset of action and broad margin of safety (Chin et al., 1981). Specific recommendations have been made for the intravenous use of pyridoxine and supplementation of this antidote with intravenous diazepam for the treatment of acute isoniazid toxicity (Sievers et al., 1982).

Since both pregnancy itself and isoniazid treatment for tuberculosis put extra demands on the need for vitamin B_6, Atkins (1982) measured the concentration of pyridoxal phosphate in the plasma of pregnant women who were receiving isoniazid (300 mg/day) and also receiving

dietary supplements of vitamin B_6. It was determined that in the compliant, pregnant patient, 52 to 60 mg of vitamin B_6 was enough to maintain elevated plasma concentrations of pyridoxal phosphate above the normal, adequate range. Lower supplementation might be adequate to maintain the normal range, but no attempt was made to establish the minimal dose of vitamin B_6 needed.

Although the antivitamin B_6 effects of isoniazid are well known, there are many other drugs of hydrazine derivation whose chronic effects on the metabolism and functions of vitamin B_6 have not been studied in detail. Among compounds of pharmacological interest, antihypertensive preparations, antianginal agents, cancer chemotherapeutic drugs, immunosuppressive medications, tranquilizers, antidepressants, antiviral substances, antihistamines, antimicrobials, and antiasthmatics may be enumerated (Juchau and Horita, 1972).

Standal et al. (1974) determined the vitamin B_6 status of a normal population and compared it with that of patients who were receiving isonicotinic acid hydrazide. The three experimental groups were (1) healthy individuals; (2) persons receiving isoniazid who had shown positive reactions to the tuberculin test but who did not show active tuberculosis; and (3) patients who were hospitalized with active tuberculosis and were receiving, in addition to isoniazid, ethambutol and pyridoxine daily. Standal et al. (1974) detected vitamin B_6 deficiency in control healthy subjects on 'self-selected diets' and in patients who were receiving isoniazid without pyridoxine who were on a similar diet to those in the control group. These data indicate that the antipyridoxine effects of hydrazine derivatives of pharmacological importance, along with other nonhydrazine compounds such as cycloserine and penicillamine, probably deserve special attention and careful analytical studies (Fig. 1).

B. Vitamin B_6 and Monosodium Glutamate Metabolism

It was concluded by Schaumburg et al. (1969) that a high intake of monosodium glutamate was chiefly responsible for the group of symptoms known as the 'Chinese Restaurant Syndrome'. The symptoms include sensations of warmth or burning, stiffness or tightness, weakness in limbs, pressure, tingling, light headedness, headache, heartburn, or gastric discomfort. Since glutamic oxaloacetic transaminase of erythrocytes (EGOT) is an important enzyme involved in the metabolism of glutamate, Folkers, in cooperation with Kenney (1979), attempted

unsuccessfully to show differences in EGOT activities in subjects who did and did not experience the neurological effects precipitated by monosodium glutamate.

Subsequently, however, Folkers et al. (1981) reported that when they selected 27 subjects who had relatively low basal activities of EGOT from a group of 158 on whom measurements were made, twelve of these responded to monosodium glutamate with the neurological symptomatology, i.e., the Chinese Restaurant Syndrome. Nine of these twelve were given vitamin B_6 orally (50 mg daily), while the other three received placebos, prior to all twelve being challenged again with monosodium glutamate. Eight of the nine showed no symptoms to monosodium glutamate after the pyridoxine supplement whereas all three who received placebo re-experienced the symptoms when given monosodium glutamate. Furthermore, a remeasurement of the EGOT basal activity after 12 weeks of pyridoxine or placebo supplementation revealed that the mean basal specific activity was significantly higher than prior to supplementation in the nine who actually received vitamin B_6, but not in the three who received placebos. The authors (Folkers et al., 1981) interpreted these results as evidence of a deficiency of vitamin B_6 in the tissues of those subjects who responded to monosodium glutamate by developing the neurologic symptoms of Chinese Restaurant Syndrome.

C. Vitamin B_6 and Penicillamine

A commonly used method for showing evidence of a vitamin B_6 deficiency is that which shows an increased urinary excretion of xanthurenic acid after a tryptophan loading dose which can be reversed by administration of pyridoxine. Such tests have indicated that D-penicillamine, used in the treatment of arthritis, can interfere with vitamin B_6 function (Jaffe et al., 1964). Rumsby and Shepherd (1981) recently used a different biochemical method to confirm that penicillamine can produce a deficiency of vitamin B_6, but the deficiency appears not to be of clinical significance. The method employed the measurement of the in vitro activity of erythrocyte alanine aminotransferase in the blood plasma of patients who were receiving penicillamine. Besides an indication that the penicillamine probably reacted chemically with the coenzyme pyridoxal-5′-phosphate, or else inhibited its synthesis, there also appeared to be a decrease in the concentration of the aminotransferase apoenzyme. However, since the deficiency was detectable only

by the in vitro biochemical test, without evidence of overt clinical symptoms, the authors concluded that supplementation with pyridoxine is probably not necessary for patients who are being treated with penicillamine.

V. VITAMIN B_6 AND PICOLINIC ACID IN ZINC ABSORPTION

Vitamin B_6 is involved in the absorption and retention of zinc from the diet (Evans and Johnson, 1981). The authors hypothesized that picolinic acid, a metabolite of tryptophan, is the substance that actually facilitates the absorption of dietary zinc, and vitamin B_6 can be the limiting factor in the production of picolinic acid. It was shown that high concentrations of dietary iron interfere with the absorption of zinc, probably by competition for the available endogenous picolinic acid.

By varying the amounts of dietary iron and by supplementing the diet with various concentrations of picolinic acid, vitamin B_6, and combinations of picolinic acid plus vitamin B_6, Evans and Johnson (1981) found that the interference with zinc absorption by high concentrations of dietary iron could be overcome by supplementations with picolinic acid and/or vitamin B_6. It was also found that the production of picolinic acid in the pancreas was increased by increasing the dietary concentration of vitamin B_6, presumably by increasing the activity of kynureninase, an enzyme required for the metabolic pathway from tryptophan to picolinic acid. This investigation was based on earlier studies that had suggested the competition between iron and zinc for a common carrier ligand in the intestinal lumen (Pollack et al., 1965; Forth and Rummel, 1971; and Hahn and Evans, 1975).

Zinc in physiological doses may play an active role in formation of pyridoxal phosphate. Brain is an unusually rich source of pyridoxal kinase (PL-kinase). A soluble enzyme with optimum pH of 6.5, PL-kinase is preferentially activated by Zn^{2+} (Km 10^{-6} M) and less so by Mg^{2+} (Km 10^{-5} M). Values of Km for pyridoxal and ATP are 10^{-5} M, and 5 x 10^{-5} M, respectively (McCormick et al., 1961). Extensive purification (2000-fold) shifts the pH optimum from 6.5 to 6.0. The order of potency of divalent cations in activating PL-kinase is $Zn^{2+} > Co^{2+} > Mn^{2+} > Mg^{2+} > Fe^{2+}$. McCormick et al. (1961), working with a partially purified kinase (200-fold), stated that Zn^{2+} directly activates PL-kinase. On the other hand, Neary and Diven (1970), working with a substantially more pure enzyme (2000-fold), indicated that the true

substrate for PL-kinase is $ZnATP^{2-}$ and increasing the availability of zinc increases the concentration of $ZnATP^{2-}$ and in turn increases the activity of PL-kinase.

The stimulation of PL-kinase takes place at physiological concentrations of Zn^{2+}. As reported (Ebadi et al., 1981), 0.4×10^{-6} M Zn^{2+} stimulated PL-kinase 2-fold and this concentration of Zn^{2+} is compatible with those concentrations seen in various areas of brain. In addition to stimulating the activity of PL-kinase to synthesize more pyridoxal phosphate, Zn^{2+} seems to inhibit the activity of pyridoxal phosphate phosphatase – the enzyme that catabolizes pyridoxal phosphate (Fig. 1). For example, at 5×10^{-4} M $ZnSO_4$, pyridoxal phosphate phosphatase of pineal gland became totally inhibited (Ebadi and Govitrapong, 1979b).

These studies, when examined collectively, may indicate that pyridoxine may participate in absorption of zinc, and zinc may be involved in the formation of pyridoxal phosphate by activating PL-kinase (Fig. 1). Furthermore, zinc binding proteins identified in brain by our laboratory (Itoh et al., 1983) are inducible by the intracerebroventricular administration of zinc (Ebadi et al., 1983a). These proteins may function as physiological donors of zinc to numerous zinc apometalloenzymes, and also may play a decisive role in preventing neurotoxicity by limiting the rise in concentration of free zinc in the brain.

VI. SPECIES DIFFERENCES IN THE RELATIONSHIP BETWEEN XANTHURENIC ACID EXCRETION AND VITAMIN B_6 DEFICIENCY

A number of drugs interfere with the normal functions of vitamin B_6, either by preventing the formation of pyridoxal-5′-phosphate or by inhibiting various pyridoxal phosphate-dependent enzymes. Examples of such drugs that show evidence of interfering with vitamin B_6 functions in humans include penicillamine, isoniazid, oral contraceptives, hydrallazine, and cycloserine (Fig. 1). Studies by Rumsby and Shepherd (1980) attempted to investigate the effects of certain drugs on the basis of either of two criteria – either that the chemical structure of the drug suggested that it would react with pyridoxal phosphate, or that many patients who had received the drug had shown symptoms of peripheral neuropathy or paresthesia – adverse reactions often associated with vitamin B_6 deficiency.

Among some nineteen drugs screened by the tryptophan load test

for an effect on vitamin B_6 function in the rat (as shown by increased xanthurenic acid excretion), only DL-penicillamine, hydrallazine, and phenelzine were positive (Rumsby and Shepherd, 1980). These effects were reversed by concomitant administration of pyridoxine hydrochloride. The penicillamine was the only drug that caused a decrease in the concentration of pyridoxal phosphate in the liver. The in vitro effects of these drugs on the activity of kynurenine aminotransferase from rat kidney were varied, with penicillamine showing no inhibition, while hydrallazine and phenelzine both showed non-competitive inhibition with respect to substrate. The inhibition by phenelzine was increased as pyridoxal phosphate concentration increased, indicating that the hydrazone formed between phenelzine and pyridoxal phosphate was apparently a more potent inhibitor of kynurenine aminotransferase than phenelzine itself was.

These results in the rat do not exactly parallel findings in the human, since neither D-cycloserine, procarbazine, nor isoniazid caused any increase in the excretion of xanthurenic acid after tryptophan loading in the rat, even though those drugs do cause symptoms of vitamin B_6 deficiency in the human. Thus, Rumsby and Shepherd (1980) concluded that even though the tryptophan load test in rats serves as a useful preliliminary screen for drug-induced vitamin B_6 deficiency, it is not a reliable guide to determine whether a particular drug will produce a similar deficiency in the human. Bender et al. (1982) studied the effects of estrogen administration on vitamin B_6 and tryptophan metabolism in the rat, and found no evidence of any major effects on vitamin B_6. These authors (Bender et al., 1982) suggested that abnormalities of tryptophan metabolism in women receiving estrogens, which have been widely attributed to drug-induced vitamin B_6 depletion, can be accounted for by inhibition of kynureninase by estrogen metabolites.

VII. PYRIDOXAL PHOSPHATE, ENDOGENOUS DEPRESSION, AND STEROID CONTRACEPTIVES

Some, but not all, women taking oral contraceptive medications become depressed. Furthermore, treatment with daily pyridoxine has been shown to be effective in preventing or reversing the oral contraceptive-induced depression (Baumblatt and Winston, 1970; Adams et al., 1973; and Adams et al., 1974). The depression is thought to be due to altered tryptophan metabolism. The abnormality of tryptophan metabolism, which has also been shown to occur during pregnancy,

during estrogen therapy, and in vitamin B_6 deficiency state, becomes corrected by treatment with pyridoxine. The metabolism of tryptophan requires five pyridoxal phosphate-dependent reactions (Rose, 1978, for review). Among these, the conversion of tryptophan to 5-hydroxytryptophan and then to 5-hydroxytryptamine (serotonin) is noteworthy. Serotonin has been implicated in the etiology of depression. The concentration of tryptophan in plasma of depressed patients is reduced (Coppen et al., 1973). Furthermore, the concentration of 5-hydroxyindoleacetic acid, the metabolite of serotonin, is low in cerebrospinal fluid of depressed patients (Ashcroft et al., 1966). In addition, the concentration of serotonin was found to be low in the brains of depressed patients who had committed suicide (Shaw et al., 1967). The oral contraceptive-induced depression seems complex. The experimental evidence gathered by several independent laboratories indicates that the contraceptives may (a) interfere with uptake of tryptophan by the brain, (b) divert tryptophan away from synthesis of serotonin, and (c) inhibit the activity of 5-hydroxytryptophan decarboxylase (Rose, 1978, for review).

VIII. PYRIDOXAL PHOSPHATE AND NEUROENDOCRINOLOGY

The interactions between vitamin B_6 and hormones have been reviewed (Hsu, 1963; Rose, 1978). Among these, only those related to the function of pyridoxal phosphate in CNS function will be discussed.

The secretion of several hormones of the anterior pituitary gland is influenced by dopamine, norepinephrine, and serotonin. Since these amines are synthesized with the aid of a pyridoxal phosphate-requiring enzyme, L-aromatic amino acid decarboxylase, the relations between pyridoxine, pituitary, hormones, and biogenic amines have been investigated (Rose, 1978, for review). Since in man aromatic amino acid decarboxylase seems to be the rate-limiting step in the synthesis of monoamines, the administration of vitamin B_6 is expected to increase the formation of monoamines and influence the secretion of pituitary hormones. Although the understanding of the clinical significance of vitamin B_6 in modulating neuroendocrine functions is far from complete, the following associations may be cited:

Drugs that stimulate the synthesis of dopamine, such as levodopa, or mimic the action of dopamine, such as apomorphine or bromocriptine, decrease prolactin release (Smalstig et al., 1974; Wiggins and

Fernstrom, 1977; Blum et al., 1980). Conversely, drugs that block dopamine receptors, such as pimozide, or interfere with dopamine synthesis, such as α-methyltyrosine, increase prolactin release (Ojeda et al., 1974; Lu et al., 1970).

Pyridoxine in pharmacological doses, like levodopa, suppresses prolactin release in the galactorrhea-amenorrhea syndrome (Delitala et al., 1976; McIntosh, 1976). Furthermore, pyridoxine partially inhibits prolactin release from rat pituitary gland in culture (MacLeod and Lehmeyer, 1974). In addition, pyridoxine has been shown to directly inhibit prolactin release (Harris et al., 1978).

Similar to levodopa, pyridoxine in pharmacological doses also increases the production of growth hormones in man (Bigazzi et al., 1979) with no appreciable changes in luteinzing hormone or follicular stimulating hormone. In addition, chronic vitamin B_6 deficiency impairs the production of growth hormones in animals (Huber and Gershoff, 1965; Makris and Gershoff, 1973; Rodda, 1975). However, pyridixine reduces or nullifies the levodopa-mediated stimulatory effect on release of growth hormone (Mims et al., 1975). Pyridoxine becomes converted to pyridoxal phosphate which in turn stimulates the activity of dopa decarboxylase, metabolizing dopa mostly in the periphery, hence reducing the intraneuronal production of dopamine.

IX. PYRIDOXAL PHOSPHATE AND STEROID RECEPTOR SITES

Vitamin B_6 in its active form pyridoxal phosphate may influence the pharmacodynamics of glucocorticoid, estrogen, progesterone, and androgen receptor sites.

A. Glucocorticoid Receptors

In bringing about their pharmacological responses, steroid hormones undergo multistep processes. Initially, the steroid hormone interacts with a steroid-specific receptor protein in the cytoplasm and forms a cytoplasmic hormone receptor complex which exists in an unactivated state. After activation, the cytoplasmic hormone receptor complex is then translocated on or into the cell nucleus where it interacts with nuclear receptors to form the nuclear bound complex and to initiate the inherent physiological function(s) of the steroid (for review see O'Brien and Cidlowski, 1981). Cake et al. (1978) have shown that in rat liver in vitro, pyridoxal phosphate was an inhibitor of the binding of

glucocorticoid receptor complex to DNA. They suggested that through this action pyridoxal phosphate may function as an endogenous inhibitor of glucocorticoid actions.

Cidlowski and Thanassi (1978), using pyridoxal phosphate, have extracted dexamethasone receptor complexes from thymocyte nuclei in rats. Semicarbazide or hydroxylamine interfered with the extraction, indicating that the native and unaltered C4′ carboxy aldehyde group of the pyridoxal phosphate molecule was necessary.

Other studies by Cidlowski and Thanassi (1979) have elaborated on pyridoxal phosphate-induced alterations in glucocorticoid receptor conformation by stating that this modification occurs at a lysine residue(s) on the various forms of the receptor and causes either conformational changes in the receptors, or the expression of a subunit that is common to and characteristic of all the forms of the nuclear and cytoplasmic dexamethasone receptors found in rat thymocytes. Additional studies by Cidlowski and Thanassi (1981) have shown that the effect of pyridoxal phosphate on glucocorticoid receptors occurs at physiological concentrations. By examining the results of these studies collectively, one gains the impression that pyridoxal phosphate may protect glucocorticoid receptors from degradation. Furthermore, Cidlowski and Thanassi (1981) speculated that pyridoxal phosphate may be involved in the utilization and cytoplasmic recycling of glucocorticoid receptors.

In other studies, Disorbo et al. (1980) have shown that in vitamin B_6 deficient animals the glucocorticoid receptor binding to DNA,cellulose and nuclei from liver cytosol increased. In addition, when compared to vitamin B_6-supplemented, the B_6-deficient animals did not take up as much [^{3}H] triamcinolone acetonide and retained less.

One of the multiple responses to glucocorticoids in the liver is the de novo synthesis of tyrosine aminotransferase (Nickol, 1978). The induction of this enzyme is a direct consequence of the binding of the glucocorticoid receptor complex to nuclear receptor sites. A direct correlation exists between the number of glucocorticoid receptor complexes associated with nuclear receptor sites and the degree of induction of tyrosine aminotransferase.

Disorbo and Litwack (1981), by using a cell culture system, have shown that changes in the intracellular levels of pyridoxal phosphate alter the induction of tyrosine aminotransferase by glucocorticoids. Increased intracellular levels of pyridoxal phosphate demonstrated anti-glucocorticoid effects, whereas a reduction in the level of pyridoxal

phosphate increased the sensitivity of cells to glucocorticoids. It should be stated that the effect of pyridoxal phosphate on glucocorticoid receptor sites is highly specific and variations have been shown between glucocorticoid receptors of thymocytes and those studied in Hela S3 cells (O'Brien and Cidlowski, 1981).

B. Estrogen Receptors

The antagonism between pyridoxal phosphate and estradiol has been recognized. During pregnancy, as the concentration of estradiol is increased, so is the requirement for pyridoxal phosphate (Wachstein, 1964). Furthermore, pyridoxine inhibits prolactin secretion (McIntosh, 1976). Muldoon and Cidlowski (1980) have shown that pyridoxal phosphate modified the estrogen receptor sites in rat uterus. Incubation with pyridoxal phosphate at 4°C changed the estrogen receptor sites from an 8S to a 4S species. The addition of sodium borohydride resulted in a shift of all species binding into the 4S region. Furthermore, pyridoxal phosphate negated the ability of transformed receptor complex to interact specifically with DNA. Similarly, pyridoxal phosphate was capable of disrupting preformed estrone receptor-DNA complex.

C. Progesterone Receptors

Pyridoxal phosphate inhibits the binding of the avian progesterone receptor to ATP-sepharose (Nishigori and Toft, 1979). Similarly, pyridoxal phosphate is able to block the binding of progesterone to nuclei, DNA cellulose, and phosphocellulose; the effect of pyridoxal phosphate is reversible by sodium borohydride. The pyridoxal phosphate-treated receptor shifts to a more acidic isoelectric point and is more stable in nature. The greater acidic nature of the progesterone may be due to the addition of phosphate by pyridoxal phosphate and/or by blocking or masking of a basic region such as lysine residue. The chemical modification of progesterone receptor by pyridoxal phosphate occurs rapidly. Although the basic size of the progesterone receptors is not changed, their ability to aggregate is greatly diminished.

D. Androgen Receptors

The effect of pyridoxal phosphate on the androgen receptors from rat prostate has been studied by Hiipakka and Liao (1980). These studies have shown that the androgen-receptor complex binds to prostate

nuclei. Incubation of the androgen receptor complex and prostate nuclei with pyridoxal phosphate inhibits the nuclear retention of receptor. If the androgen-receptor complex is bound to nuclei before addition of pyridoxal phosphate, the receptor can be removed from nuclei by the addition of pyridoxal phosphate. Other vitamin B_6 derivatives, such as pyridoxine, pyridoxine phosphate, pyridoxamine, and pyridoxamine phosphate, and the vitamin B_6 analog, pyridoxal phosphate oxime, were not inhibitors of 5-α-dihydro-[^{3}H] testosterone-receptor complex binding to DNA cellulose.

X. PYRIDOXAL PHOSPHATE AND GABA RECOGNITION SITES

Pyridoxal phosphate, in addition to modifying receptor sites for glucocorticoids, estrogen, progesterone, and androgen, may be involved in modifying GABA recognition sites, and perhaps other receptors not studied thus far.

Pyridoxine plays a definite but unexplained role in the prevention, as well as the production, of convulsive seizures (Ebadi, 1981 for review). Antimetabolites of pyridoxal phosphate, such as 4-deoxypyridoxine, or compounds which deplete pyridoxal phosphate, such as isonicotinic acid hydrazide, cause seizures in experimental animals which can be interrupted by pyridoxine. Furthermore, the frequency of seizures in epileptic humans increases when they are treated with a tuberculostatic drug such as isoniazid (Irskens, 1964), which is known to inactivate pyridoxal phosphate biochemically by interacting with it to form a hydrazone. The earlier investigators attributed these seizure disorders to the unavailability of pyridoxal phosphate as a coenzyme for glutamic acid decarboxylase. However, recent evidence has shown that convulsions are not always associated with a reduction in the concentration of pyridoxal phosphate (see Hammad et al., 1983 for review). In fact, the known association became even more obscure when Kouyoumdjian and Ebadi (1981) reported that the intracerebroventricular (ICV) injection of pyridoxal phosphate (0.125-1.25 μmole in 20 μl/rat) caused epileptic seizures, including tonic-clonic convulsions. These effects were selective for pyridoxal phosphate, pyridoxal-5′-sulphate and 5′-phosphonoethyl analogue of pyridoxal (Ebadi et al., 1983b) and could not be produced by pyridoxine, pyridoxine phosphate, pyridoxamine, pyridoxamine phosphate, and their metabolite, 4-pyridoxic acid. Furthermore, an antimetabolite of pyridoxine, in its

inactive form (4-deoxypyridoxine) and its active form (4-deoxypyridoxine phosphate) did not cause convulsions.

Since the convulsions induced by pyridoxal phosphate, pyridoxal sulphate or phosphonoethyl-pyridoxal are blocked by GABA and its synthetic analogues, but not by biogenic amines (Kouyoumdjian and Ebadi, 1981), one may postulate that the convulsions may result from subnormal GABAergic transmission, brought about probably, but not necessarily exclusively, by one or several of the following mechanisms: inhibition of the synthesis of GABA, acceleration of the catabolism of GABA, inhibition of the Ca^{++}-mediated release of GABA, stimulation of the Na^{+}-dependent transport of GABA, blockade of GABA recognition sites, blockade of chloride channel, and/or blockade of benzodiazepine recognition site.

It has been proposed that the binding of pyridoxal phosphate to the apoenzyme may regulate the activity of glutamic acid decarboxylase in vivo which in turn may play an important role in controlling the rate of synthesis of GABA (Miller et al., 1978). In our studies, we have shown that the pyridoxal phosphate-induced seizures were not associated with any apparent and significant changes in the activity of glutamic acid decarboxylase or GABA-transaminase at the beginning of the convulsive activities in 14 regions of rat brain analyzed for these enzymes.

One may entertain the possibility that the pyridoxal phosphate-induced convulsions may result from enhanced high affinity uptake of GABA. Ebadi and Klangkalya (1979) have shown that the derivatives of vitamin B_6, namely pyridoxine, pyridoxal, pyridoxamine, PLP, and pyridoxamine phosphate; the B_6 antimetabolite, 4-deoxypyridoxine; and B_6 depletors, cycloserine and isoniazid, in a concentration of 0.1 - 1.0 mM, failed to alter the uptake of labelled GABA into synaptosomes, whereas 0.1 mM of chlorpromazine, which has been reported to inhibit GABA uptake, did inhibit [^{3}H]GABA uptake by 80%. In addition, administration of 100-200 mg of 4-deoxypyridoxine, which reduced the concentration of pyridoxal phosphate significantly, did not alter the uptake of ^{3}H-GABA. On the other hand, pyridoxal or pyridoxal phosphate in a concentration of 1 mM enhanced [^{3}H]GABA uptake in nuclear-free crude homogenates of the rat cerebral cortex.

The hypothesis that pyridoxal phosphate-induced convulsions may result from inhibition of GABA recognition sites is strengthened by in vitro studies showing that pyridoxal phosphate inhibited the binding of [^{3}H]GABA to synaptic membranes isolated from rat brain (Ebadi et

al., 1980) and from cat brain (Tunnicliff and Smith, 1979). In these studies, the inhibitory effect was competitive and selective, occurring with an active coenzyme – pyridoxal phosphate and not with inactive precursors or metabolites. These results suggest that the highly reactive pyridoxal phosphate may undergo Schiff base formation with a component of the membrane, preventing the interaction of GABA with its 'recognition' sites. Consequently, it is reasonable to assume that at least one lysine residue exists at or near the GABA recognition site.

Since the GABA molecule is transported in a conformation different from that in which it activates its receptor (Krogsgaard-Larsen et al., 1975; and Krogsgaard-Larsen, 1980) and since the GABA uptake site and the GABA recognition site may be differentially blocked, then it would seem feasible that pyridoxal phosphate could have opposite effects on the aforementioned systems, stimulating the uptake of [^{3}H]-GABA but inhibiting the binding of [^{3}H] GABA.

The CNS excitation and convulsions induced by pyridoxal phosphate and pyridoxal sulphate should be placed in proper perspective, segregating the physiologic and toxic effects of these compounds. It is very doubtful that pyridoxal phosphate could produce spontaneous convulsions under physiological conditions. First, the lowest convulsive dose of pyridoxal phosphate when given ICV is 0.1 μmole/rat brain, which is considerably higher than the physiological levels of pyridoxal phosphate which have been shown to vary from 3.5 to 5.7 nmoles/g tissue in regions of rat brain (Ebadi and Bifano, 1978). Furthermore, the naturally occurring forms of vitamin B_6 – pyridoxine, pyridoxal, and pyridoxamine – are converted to their phosphorylated derivatives by a single cytoplasmic enzyme, pyridoxal kinase (McCormick et al., 1961). The synthesized pyridoxal phosphate is rapidly bound to various proteins, and any excess is hydrolyzed by ubiquitously occurring alkaline phosphatases and possibly acid phosphatase (Snell and Haskell, 1971). Indeed, the combination of protein binding and enzymatic hydrolysis has been shown to play a definite role in keeping the cellular levels of pyridoxal phosphate constant in liver (Li et al., 1974) and in pineal gland (Ebadi and Govitrapong, 1979b). These studies, along with data gathered concerning vitamin B_6 deficiency-related convulsions, may be interpreted to indicate that a steady state concentration of pyridoxal phosphate is essential for the maintenance of the integrity of the electrical activity of the brain, which may become disturbed both in deficiency state and when excess amounts of pyridoxal phosphate derivatives are administered. The understanding of the mechanisms for the

pyridoxal phosphate-related (deficiency and excess) convulsions and their selective blockade by GABA may provide insight not only about the nature of GABAergic transmission, but also the involvement of GABA in the etiology, the manifestation, and the expression of epileptic seizures. In addition, pharmacological compounds possessing antivitamin B_6 effects (Fig. 1) may alter the integrity of nonsteroid and steroid receptor sites. Consistent with this view is the observation of Dakshinamurti and Paulose (1983) that, in pyridoxine deficient neonate rats, the concentration of GABA is reduced and concomitantly, the high affinity binding of [^{3}H]GABA is increased significantly in the cerebellum.

XI. SUMMARY AND CONCLUSIONS

In this review it has been pointed out that vitamin B_6 and its vitamers can be involved in many interactions with a number of drugs, as well as with the actions of various endocrines and neurotransmitters. Nutritional deficiencies, especially of vitamins and proteins, can affect the manner in which drugs undergo biotransformation, and thereby may also modify the therapeutic efficacy of certain drugs. The differences between nutritional vitamin B_6 deficiency and the hereditary disorder producing pyridoxine dependency are discussed.

In addition to a pyridoxine deficiency being able to adversely affect drug actions, the improper supplementation with vitamin B_6 can in some instances also adversely affect drug efficacy. A decrease by pyridoxine in the efficacy of levodopa used in the treatment of Parkinsonism is an example.

The interrelationships and enzymatic interconversions among pyridoxine vitamers, both phosphorylated and non-phosphorylated, are briefly discussed, particularly regarding their pharmacokinetic properties.

The ways in which the normal biochemical functions of vitamin B_6 may be interfered with by various drugs are reviewed. (1) The chronic administration of isoniazid for the prevention or treatment of tuberculosis can produce peripheral neuropathy which can be prevented by the concurrent administration of pyridoxine. An acute toxic overdose of isoniazid causes generalized convulsions, and the intravenous administration of pyridoxine hydrochloride will prevent or stop these seizures. (2) The acute ingestion of excessive monosodium glutamate will, in some individuals, cause a group of symptoms including among others

headache, weakness, stiffness, and heartburn, collectively known as the 'Chinese Restaurant Syndrome.' These symptoms can be prevented by prior supplementation with vitamin B_6. The beneficial effect is ascribed to the correction of a deficiency in the activity of glutamic oxaloacetic transaminase, an enzyme that is dependent on pyridoxal phosphate.

Some interesting relationships are pointed out between vitamin B_6, picolinic acid, and zinc. It is postulated that the intestinal absorption of zinc is facilitated by picolinic acid, a metabolite of tryptophan. The derivation of picolinic acid from tryptophan depends on the action of the enzyme kynureninase, which is dependent on pyridoxal phosphate; therefore, the adequate absorption of zinc is indirectly dependent on an adequate supply of vitamin B_6. The formation of pyridoxal phosphate, on the other hand, appears to be indirectly dependent on Zn^{2++} which activates pyridoxal kinase.

A state of depression that is induced in some women who take oral contraceptives appears to be reversed by treatment with daily pyridoxine. One hypothesis to explain this effect is that the oral contraceptive is somehow causing a deficiency of serotonin in the brain, and that the vitamin B_6 is helping to overcome this deficiency through the stimulation of 5-hydroxytryptophan decarboxylase by pyridoxal phosphate.

In pharmacological doses pyridoxine has been shown to suppress the release of prolactin from the pituitary and also to increase the production of growth hormone. However, a clear understanding of the clinical significance of these actions attributable to pyridoxal phosphate is still lacking.

Through experiments which showed that pyridoxal phosphate inhibits the binding of glucocorticoid receptor complex to DNA, it has been suggested that pyridoxal phosphate may function as an endogenous inhibitor or regulator of glucocorticoid actions. Likewise, an antagonism has been recognized between pyridoxal phosphate and estradiol, presumably at least partially through modification of estrogen receptor sites by pyridoxal phosphate. Similar modifications of progesterone receptor sites and androgen receptor sites to prevent their binding to DNA in nuclei are also ascribed to the actions of pyridoxal phosphate.

There is evidence for a role of pyridoxal phosphate in the alteration of receptor sites for gamma-aminobutyric acid (GABA). Convulsions

can be brought about not only through a deficiency of pyridoxine, but also by a local excess of pyridoxal phosphate in the cerebroventricular portion of the brain. Regulation of the production of GABA from glutamic acid by the action of glutamic acid decarboxylase does not fully explain these effects of pyridoxal phosphate. Several possible explanations are discussed. It is unlikely, however, that an excess of pyridoxal phosphate could produce convulsions under physiological conditions.

In conclusion, pyridoxal phosphate in physiological concentrations seems to function as an endogenous "down regulator" of a number of receptor sites, including estrogen, progesterone, androgen, glucocorticoid, and GABA. Numerous pharmacological agents and nutrients alter the functions of pyridoxal phosphate by reducing the synthesis of pyridoxal phosphate, by inhibiting the binding of pyridoxal phosphate to apoproteins, and by inhibiting the catabolism of pyridoxal phosphate. These agents and nutrients do undoubtedly modify the functional integrity of these receptor sites and perhaps numerous other receptor sites not yet studied.

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