PHARMACOLOGICAL ASPECTS OF VITAMIN B_6

PETER HOLTZ AND DIETER PALM

Pharmakologisches Institut der Universität Frankfurt/M., Germany

TABLE OF CONTENTS

The discovery and isolation of vitamin B_6 are closely related to the recognition of pellagra as an avitaminosis by Goldberger and Lillie (179). The disease is seen chiefly in the poorer populations of the southern parts of Europe and the Americas, whose food consists of a low-protein, corn-rich diet. Typical manifestations can also be induced experimentally by means of deficient diets. The "black tongue" of the dog has been considered analogous to the glossitis seen in man. The rat develops a localized dermatitis (acrodynia) on the paws, ears, and snout which is, to a certain extent, comparable with the characteristic skin lesions seen in man: dermatitis and browning of the skin, particularly in areas exposed to light. Further investigations revealed a relationship between the development of human pellagra and the low tryptophan content of zein, the protein of corn. This is of interest, since nicotinic acid and nicotinamide were recognized by Elvehjem *et al.* (142) as the "pellagra preventive factor" in man.

It is realized today that tryptophan can be metabolized in animal and man to nicotinic acid. While nicotinamide was established to be identical with the "anti-black-tongue factor" in the dog, another compound was recognized as the preventive or curative factor in the pellagra-like dermatitis of the rat (63, 193). "Adermin" was first prepared from yeast extracts by Kuhn and others and identified chemically as 2-methyl-3-hydroxy-4,5-bis(hydroxymethyl)py-

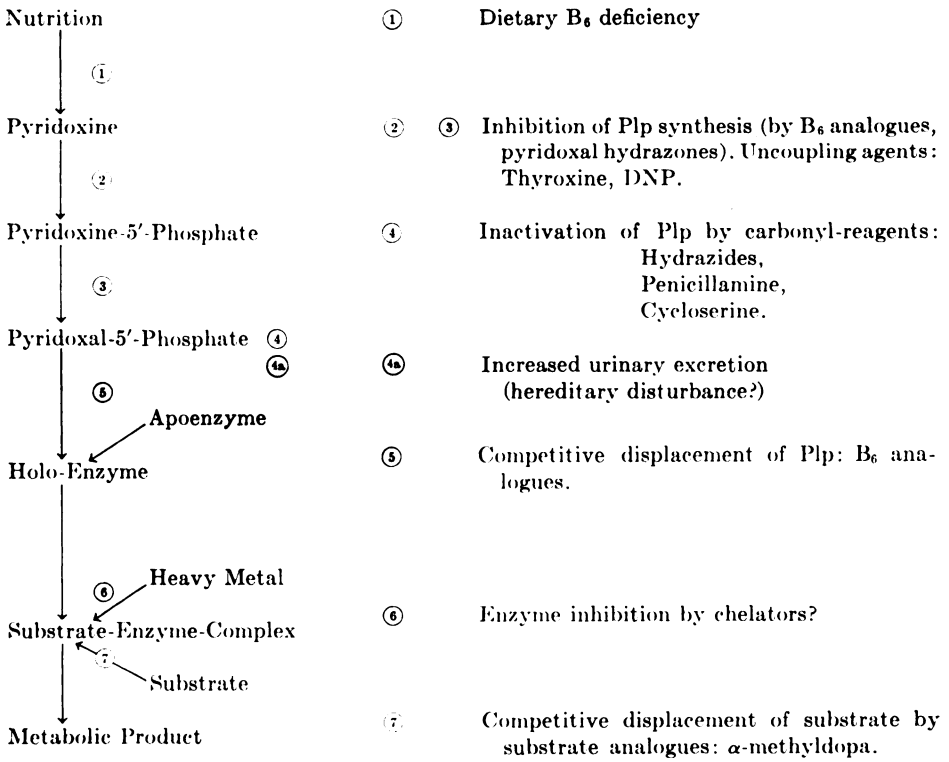


FIG. 1. Possible mechanisms of interference with the function of vitamin B_6.

ridine (for review see 193, 475, 539). As proposed by György and Eckhardt, it was called "pyridoxine" (194).

Until 1942 vitamin B_6 and pyridoxine were considered to be identical. In this year Snell *et al.* (477) found that the activity of "B_6 preparations" from animal material as assayed in microbiological tests varied up to a thousand times. What Snell then called "pseudopyridoxine" was later recognized to be a mixture of pyridoxal and pyridoxamine (27, 52, 164, 199, 215, 414, 415, 473, 475, 478).

Like most vitamins, the compounds of the B_6 group—pyridoxine (I), pyridoxal (II) and pyridoxamine (III) (see fig. 5)—exert their physiological effects after being phosphorylated to form coenzymes. Bound to specific proteins, they act as enzymatic catalysts in certain metabolic reactions. The most important are concerned with the metabolism of amino acids and amines (74, 76, 192, 475).

Although it is not yet possible to explain all physiological actions of vitamin B_6 in biochemical terms, it would be useful to begin with a review of the enzymatic reactions in which vitamin B_6 or its active form pyridoxal-5′-phosphate (Plp) (fig. 5, V) is involved as a coenzyme. The "pharmacological aspects" we will consider should be understood not primarily in terms of pharmacodynamic actions exerted by vitamin B_6 *per se*, but rather in terms of pharmacologically induced alterations of B_6-dependent physiological reactions, which result from a *functional* B_6 deficiency. A large number of drugs with well-defined pharmacological actions is known to promote "functional" B_6 deficiency by a variety of different mechanisms, as can be seen from figure 1: for instance by inhibition of coenzyme synthesis, by chemical inactivation of the coenzyme itself, or by competitive displacement of the coenzyme by structural analogues. A short review on B_6-dependent enzymatic reactions will therefore be confined to those, the disturbance of which could possibly explain the pharmacological action of the respective drug.

I. B_6 ENZYMES

A. Decarboxylation of aromatic amino acids

$$\mathrm{R{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH} \xrightarrow{-CO_2} \mathrm{R{-}\underset{\displaystyle NH_2}{\underset{|}{CH_2}}}$$

Vitamin B_6 is necessary for all nonoxidative metabolic reactions of amino acids. It was first recognized as a coenzyme of bacterial amino acid decarboxylases. Strains of *Streptococcus faecalis*, grown in B_6-free medium, lose most of their decarboxylase activity against tyrosine. The enzymatic activity is restored to normal by the addition of pyridoxal and ATP, from which Plp can be formed (52, 164, 192).

1. 3,4-Dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP). In animals and man tyrosine is decarboxylated to tyramine not only by the intestinal bacterial flora but also by a tissue enzyme, detected first in kidney and liver (219). However, in the biosynthesis of the hormonal catecholamines tyrosine is not decarboxylated to tyramine, but hydroxylated to 3,4-dihydroxyphenyl-

alanine (dopa), which then is decarboxylated to dopamine by the Plp-dependent L-dopa decarboxylase. Dopamine is the immediate precursor of norepinephrine and epinephrine (for review see Holtz, 220).

It appears that L-dopa decarboxylase is identical with the enzyme that decarboxylates 5-hydroxytryptophan (5-HTP) to 5-hydroxytryptamine (serotonin) (230, 536). The suprarenal medulla and sympathetic nerves, which contain no serotonin, decarboxylate 5-HTP as well as L-dopa, while the serotonin-rich carcinoids of the intestine, which contain no catecholamines, decarboxylate L-dopa as well as 5-HTP. The decarboxylation of both amino acids is inhibited by α-methyldopa (536). There is, therefore, no direct correlation between the localization of the decarboxylating enzyme and the distribution of the biogenic amines.

Dopa and 5-HTP are not constituents of proteins but must be formed in the organism from the precursor amino acids tyrosine and tryptophan through the action of hydroxylases. The localization of the specific hydroxylases, in contrast to that of the nonspecific decarboxylase, then should be responsible for the specific distribution of the amines, dopamine, epinephrine, and norepinephrine in the one case, serotonin in the other. In brain, dopa decarboxylase activity and amine content run to a certain degree parallel; for example, both are higher in the brain stem than in the cortex (229, 511). However, the cerebral localization of the single amines is different (10, 511).

Dopamine seems to be not only an intermediary product in the synthesis of the catecholamines, but in certain parts of the body the final product of the biosynthesis and an active substance on its own. In the lung, as well as in the intestine and liver of dogs and cattle, dopamine is practically the only catecholamine to be found (147, 456). According to Sano *et al.* (446) as well as Carlsson (87) nearly all of the brain dopamine is found in the putamen and caudate nucleus, structures which are of importance for the functions of the extrapyramidal system (see section VI).

It is of interest that the injection of dopa as well as 5-HTP produces actions which may be potentiated by simultaneous injection of pyridoxine and which can be even more strongly enhanced by pretreatment of the animals with monoamine oxidase (MAO) inhibitors, which protect the resulting amines from inactivation. Intravenous injection of L-dopa in cats induced a rise of blood pressure and in rabbits hyperglycemia (222, 224); the injection of 5-HTP in guinea pigs caused bronchoconstriction (536). These actions were due to the amines arising from decarboxylation of the injected amino acids *in vivo*. They were potentiated by pyridoxine (31). After injection of dopa and 5-HTP central effects also are to be expected since these amino acids easily pass the blood-brain barrier and are then decarboxylated in the brain to the corresponding amines, dopamine and serotonin. After inhibition of brain MAO, *e.g.*, by previous injection of iproniazid, the injection of dopa or 5-HTP provoked central excitation and shortening of barbiturate and avertin (tribromethanol) sleeping time (221); the threshold for convulsions (electroshock and Metrazol, pentylenetetrazol) was raised (277) and the analgesic action of morphine increased (449, 454). Con-

comitantly the amine content of the brain was elevated. In contrast, reserpine causes a decrease in the amine content of the brain and at the same time sedation (*cf.* 80) and prolongation of barbiturate sleeping time (221); the seizure threshold is lowered (96) and the analgesic effect of morphine is abolished (449, 454).

2. Histidine. Histidine is decarboxylated to histamine not only by bacteria (for review see Gale, 163) but also by tissue extracts. In contrast to the bacterial enzyme, which decarboxylates L-histidine as well as D-histidine, the tissue enzyme decarboxylates only the L- form. Plp is the coenzyme. Histidine decarboxylase was first detected in kidney and liver (226, 530), and later in nervous tissue, with the highest activity in sympathetic nerves (229, 533). It is of interest that the noradrenergic nerves contain more histamine than norepinephrine, the actual transmitter substance (146, 533). A histidine decarboxylating enzyme, different from the histidine decarboxylase of the organs (rabbit kidney, guinea pig kidney and stomach, and fetal rat liver), is found in mast cells (166, 527). Both decarboxylases are Plp-dependent, but in contrast to tissue decarboxylase, the mast cell decarboxylase is not inhibited by α-methyldopa (418, 527, 529).

Since dopa and 5-HTP are decarboxylated by the same enzyme it was suggested by Udenfriend *et al.* (312, 501) that all aromatic amino acids, *e.g.*, dopa, 5-HTP, histidine, tyrosine (*o*-, *m*-, *p*-), tryptophan, phenylalanine, and α-methyldopa are substrates of a nonspecific "aromatic amino acid decarboxylase." However, Awapara *et al.* (25), working with a highly purified enzyme preparation from rat liver, found that only dopa, 5-HTP, and *o*- and *m*-tyrosine (but not *p*-tyrosine, histidine, and tryptophan) were decarboxylated. In agreement with this are results obtained by Hagen (195) with enzyme preparations from pheochromocytoma and by Holtz *et al.* (225) with kidney extracts of different species. Also Blaschko had found that *o*- and *m*-tyrosine in contrast to *p*-tyrosine were easily decarboxylated by the enzyme (67, 68b).

B. Decarboxylation of L-glutamic acid

The enzyme L-glutamic acid decarboxylase, which also has Plp as coenzyme, is found only in the central nervous system and catalyzes the decarboxylation of glutamic acid to γ-aminobutyric acid (GABA) (fig. 2). GABA was first detected by Ackermann and Kutscher (3) in 1910 in putrefying pancreas, and later by Roberts and Frankel (429) and Awapara *et al.* (24) in the CNS of various animal species. It is not found in cerebrospinal fluid, blood, or other organs (506) but it occurs in retina (283). The carbon skeleton of GABA is derived from glucose, as shown in experiments with brain slices and C^{14}-glucose as substrate (53).

Roberts *et al.* (427, 428, 429, 431) found that the GABA concentration in brain is 4 to 5 times lower than that of glutamic acid. Highest concentrations of GABA and highest activity of glutamic acid decarboxylase (GAD) are found in the gray matter of brain (431), but none in peripheral sympathetic nerves or suprarenal medulla, which have particularly high dopa or 5-HTP decarboxylase activity (229, 230). Brain homogenates of small laboratory animals, such as

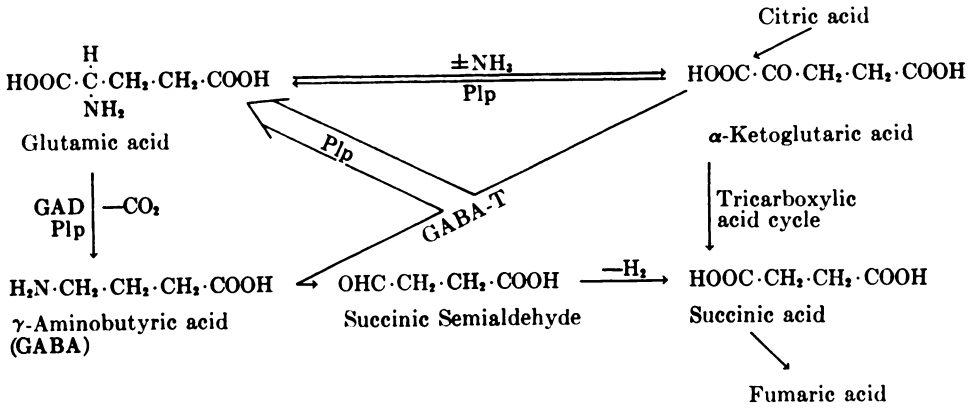


FIG. 2. Metabolism of glutamic acid and GABA in brain: GAD, glutamic acid decarboxylase; GABA-T, GABA-α-ketoglutaric acid transaminase.

mice, rats and guinea pigs, have higher GAD activities than homogenates from brains of larger species, *e.g.*, pig and beef (313). The relative activities found by Lowe *et al.* (313) were: in monkeys 100, in rabbits 187, in rats 251, and in mice 340.

GABA is easily taken up by brain slices, but not by slices of liver, kidney, or diaphragm (140, 447). In mouse brain the GABA content increases with age. GABA content and GAD activity parallel each other until the fifth postnatal day, while during further development GAD activity increases much more strongly than GABA content (430). This indicates that the disappearance of GABA by transamination (see fig. 2) keeps pace with its increased formation. That means an acceleration of turnover with increasing development, which maintains the GABA content at a constant level. Similar results were obtained with chick embryo and rabbit brain (428, 468).

GABA is a metabolite participating in the α-ketoglutaric acid–glutamic acid–succinic acid cycle, accessory to the tricarboxylic acid cycle and thereby involved in the cerebral carbohydrate metabolism (see fig. 2). GABA is also of pharmacological interest in that it exerts inhibitory actions on synaptic transmission in the CNS (for review see 139, 263, 428, 458, 499).

1. GABA and GABA derivatives as central inhibitors. Factor I, isolated by Florey (153) from brain and spinal cord of cattle, which proved to be an inhibitor of the stretch receptor of crustaceans, appeared to be identical with GABA (47). According to Kuffler and Edwards (293) GABA has an action on the stretch receptor of crayfish and lobster like that of the inhibitory neuron. It is unknown whether GABA itself is the transmitter substance. Therefore it is of interest that in mammalian brain GABA has inhibitory actions. From electrophysiological investigations in cats, Purpura *et al.* (410) have concluded that topical application of GABA to the cerebral cortex induces a blockade of excitatory synapses. According to Iwama and Jasper (245) GABA blocks selectively the superficial dendrites, either directly or through a blockade of synaptic

transmission. Grundfest (191) came to the conclusion that GABA does not activate inhibitory synapses, but inhibits excitatory synapses (139, 263).

It was pointed out that probably not γ-aminobutyric acid (GABA) itself but β-hydroxy-γ-aminobutyric acid (206) is the actual central inhibitor. This hydroxyamino acid, administered intracisternally in doses a tenth of those of GABA, suppressed convulsions induced electrically or by injection of Metrazol. It was detected by Ohara *et al.* (381) and by Hayashi (204, 205) as a brain constituent. On the other hand, Mitoma (360) found no transformation of C^{14}-labeled GABA to β-hydroxy-γ-aminobutyric acid in brain homogenates.

In addition to acetylcholine, other choline esters such as butyrylcholine (212, 227), whose action is chiefly nicotinic, and γ-aminobutyrylcholine occur in mammalian brain (259, 295). The latter substance is said to have an inhibitory action 1000 times greater than that of GABA (493). GABA seems to be also the precursor of γ-guanidobutyric acid, which occurs in brain (243, 397) and probably originates from a transamidination between arginine and GABA, as demonstrated by Pisano and Udenfriend (398). In contrast to GABA, which inhibits the superficial excitatory synapses of the brain, the guanidino derivative is supposed to activate the inhibitory axodendritic synapses (411). On the stretch receptor the activity of this compound is one-third to one-seventh that of GABA (293).

The activity of GABA as an inhibitory modulator of neuronal activity can be shown only when it is topically applied to the brain cortex. It shows no detectable central action when injected intravenously because, under physiological conditions, it is unable to penetrate the blood-brain barrier in full-grown animals (171, 468, 500). The intravenous and intraperitoneal injection in mice and rats of as much as 3 g/kg did not increase the GABA content of the brain, and after the injection of 200 mg/kg intravenously in monkeys it was impossible to detect GABA in the cerebrospinal fluid (171). On the other hand, in younger animals, where the blood-brain barrier is not yet fully developed, an increase in the GABA content of the brain after intravenous injection can be found (468) as well as the typical reversal in the EEG of the surface-negative waves to surface-positive waves (409). Also, after local damage to the blood-brain barrier by craniotomy of long duration or application of ethyl chloride on a free circumscribed area of the cortex, the intravenous injection of GABA increased the GABA concentration within the damaged area (56) (see section VII).

2. GABA as an intermediate in brain metabolism. The GABA level in brain is the result of a dynamic equilibrium between formation and utilization. The most important enzyme for the utilization of GABA is the B_6-dependent GABA-α-ketoglutaric acid transaminase (GABA-T), which catalyzes the transamination of α-ketoglutarate to glutamate with the concomitant formation of succinic semialdehyde from GABA (see fig. 2) (for review see 6, 43, 139, 427, 428, 498, 499). It has been calculated from experiments with brain slices that this glutamate-GABA shunt contributes approximately 40% of the oxidative brain metabolism (344). Recently this figure was questioned by Haslam and Krebs (202a). Working with rat brain slices and homogenates they found that the glutamate-

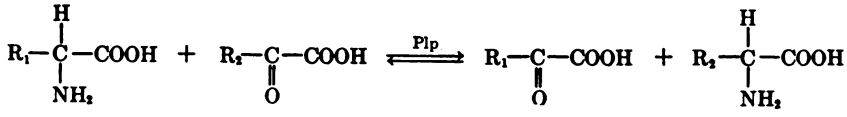
GABA shunt contributes only 10% to the cerebral oxidative metabolism. The oxygen consumption of cat brain slices can be maintained by GABA or succinic semialdehyde as well as by glucose or glutamate as substrates. Under these conditions the P:O ratio is between 2 and 3 (346).

It is of particular interest that the enzymes regulating the glutamate-GABA shunt, *i.e.*, GAD and GABA-T, are B_6-dependent. In contrast to GAD, GABA-T is found in subcortical brain areas in a much higher activity than in cortex. In all areas of the brain the ratio GABA-T/GAD activity is greater than 1 and it reaches values up to 50 in the inferior olivary nucleus and in the superior colliculus (6, 7, 444). The GABA content of the brain is more closely related to GAD than to GABA-T-activity. This can be altered by pharmacological agents which influence the two Plp-dependent enzymes to a different degree, because Plp has different affinities for the two apoenzymes (40, 43) (for details see V D,1).

The pH optimum for the decarboxylase (GAD) is about pH 6.5, while that of the transaminase (GABA-T) is pH 8.2 (432). At pH 7.5 both enzymes have the same percentage of their maximal activity. Therefore, a metabolically induced intracellular shift in pH to the acid side would enhance GABA formation with a concomitant increase in brain GABA, while a shift towards a higher pH would enhance utilization, thus lowering the GABA content.

The internal neuronal pH is mainly regulated by the CO_2-bicarbonate-buffer system. In the hippocampus with its particularly low seizure threshold, CO_2 can reduce spontaneous as well as experimentally augmented activity (135). This perhaps explains why inhibition of the carbonic anhydrase, *e.g.*, by acetazolamide, resulting in an increase in the cerebral CO_2 tension and thus reducing the intracellular pH, has an anticonvulsive action against electroshock in mice (185, 358). It could explain, too, why in cases of epilepsy acetazolamide suppresses the slow EEG waves provoked by hyperventilation (443).

C. Transaminases



These B_6-dependent enzymes are of basic importance for the formation of amino acids from nitrogen-free keto acids and thus for protein synthesis. Therefore, they are necessary for growth and cell replacement. The most important transaminases are those concerned with the transamination of glutamic acid, since they are capable of catalyzing the transfer of the NH_2 group of glutamic acid to almost any keto acid (for review see 348).

Formation of the transaminases mainly takes place in liver and muscle. Even under physiological conditions, small amounts of the intracellularly formed enzymes are released into the blood (422). An increased transaminase titer in serum is a criterion of cell injury, for example, after myocardial infarct or after liver injury produced by alcohol or carbon tetrachloride. An increased serum

titer can occur also with other enzymes, *e.g.*, lactic acid dehydrogenase, aldolase, and muscle creatine phosphokinase (for review see 422). Inhibitors of oxidative metabolism, such as CO, iodoacetate, fluoroacetate, and DNP, increase the release of intracellular enzymes into the blood (83).

The pattern of abnormal serum enzyme activity depends upon the enzymatic equipment of the affected organ (422). The most active transaminases found in human serum are glutamic-oxalacetate transaminase (GOT) and glutamic-pyruvate transaminase (GPT). GOT occurs in highest concentration in the heart, GPT in the liver. In cases of myocardial infarct, the serum GOT activity, almost exclusively, increases in proportion to the extent of necrosis. On the other hand, in acute toxic hepatosis (phosphorus, carbon tetrachloride) both transaminases are increased; the increase in serum GOT is even more pronounced than that of GPT. At the same time enzyme activities in the liver decrease. The determination of transaminase activity in serum is therefore of importance for clinical diagnosis (422).

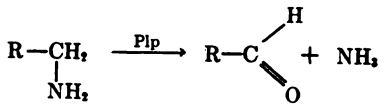
Activation of the anterior pituitary by stress causes an increase in the transaminase activity of various organs, chiefly in the liver (for review see 274), resulting from an augmented enzyme synthesis or an activation of enzyme precursors brought about by corticosteroids (274). Administration of corticoids (*e.g.*, prednisone, cortisone, or hydrocortisone) for several days induced hepatic GPT activity up to 13 times the initial values. Although the induced GPT activity could not be further stimulated by Plp, in B_6 deficiency hydrocortisone led to an increase of only about 50%, while, together with Plp, it was capable of increasing the activity by 600% (438). Since transaminases, especially GPT, are of importance also for hepatic gluconeogenesis, it is understandable that cortisone, which normally stimulates gluconeogenesis very strongly, produced only an insignificant increase in liver glycogen in B_6-deficient rats (138).

Racemization. It has been known for a long time that growth in the rat is promoted by D-amino acids as well as by L-amino acids. For example, L- and D-tryptophan are of equal value in the "growth test" (55). In experimentally produced anemia in the dog D-histidine and D-tyrosine were utilized to the same extent as the L- derivatives in the regeneration of hemoglobin (537). Since only the naturally occurring L-amino acids can be used for protein synthesis and growth, the conclusion was drawn that the unnatural D- forms were transformed by "racemization" to the natural L- forms. The experiments of Rose (435) have thrown light on the mechanism underlying "racemization." He found that keto acids were able to replace the corresponding L-amino acids in the growth test. For example, phenylpyruvic acid was equivalent to L-phenylalanine, and α-keto-γ-methyl-valeric acid to L-leucine or L-isoleucine. D-Amino acids are deaminated by D-amino acid oxidase to form optically inactive keto acids, which then are reaminated by optically specific transaminases to form L-amino acids. In agreement with this were results obtained after the administration of DL-dopa: in guinea pigs the amount of dopamine formed *in vivo* by L-dopa decarboxylase and excreted into the urine corresponded to the injected amount of the L- form, while in rats the amount of the urinary dopamine exceeded that which

could possibly have been derived only from the L- form (223, 366). This species difference was explained by the fact that rats have a much higher D-amino acid oxidase activity in liver and kidney than guinea pigs (287). Thus, in the rat D-dopa could be quantitatively deaminated to dihydroxyphenylpyruvic acid; the latter then was transaminated to L-dopa, the substrate of L-dopa decarboxylase.

Thus, also in this way, vitamin B_6 is indirectly involved in the racemization of amino acids in animals. True racemases dependent on vitamin B_6 have so far been detected only in bacteria, not in animal tissues.

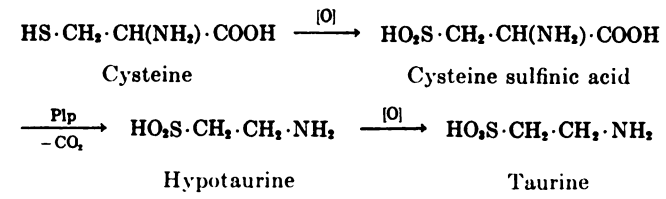
D. Amine oxidases



These enzymes are of particular interest because they inactivate and thereby detoxify some pharmacologically active amines by oxidative deamination. First indications that Plp may be a coenzyme were given by observations that histaminase was inhibited by carbonyl reagents (120, 534, 567). Electrophoretically pure histaminase preparations from hog kidney, which deaminated histamine, but not cadaverine and putrescine, contained Plp and flavin (254, 255). Thus, B_6-dependent enzymes—histidine decarboxylase (see section I A, 2) and histaminase—catalyze synthesis as well as breakdown of histamine.

Similarly, the monoamine oxidase of plasma was presumed to have Plp as coenzyme because of the inhibitory action of carbonyl reagents (492). Yamada *et al.* have recently shown that the crystalline enzyme which acts only on aliphatic amines such as spermine, butylamine, amylamine, and heptylamine, but not on aromatic amines like tyramine, norepinephrine, tryptamine, and serotonin, contains a $Cu^{++}{}_4(Plp)_2$-complex (557, 558). The monoamine oxidase of liver, kidney, intestine and brain, which deaminates the aromatic monoamines, is not B_6-dependent (for review see 400).

E. Enzymes of the metabolism of sulfur-containing amino acids

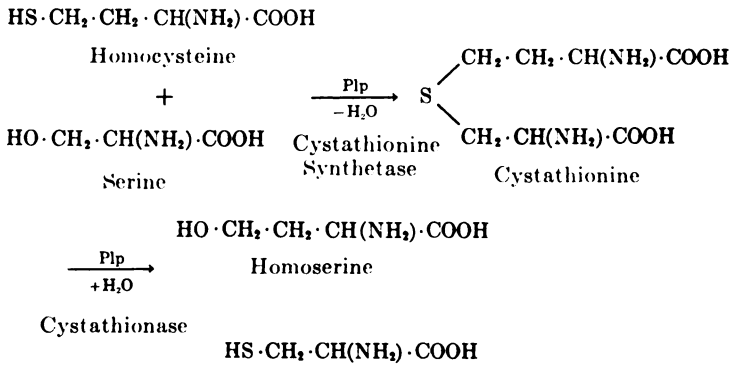


As first shown by Blaschko *et al.* (66, 68) cysteic acid is decarboxylated by a B_6-dependent enzyme in the mammalian liver. Because cysteine sulfinic acid is more rapidly decarboxylated by the same enzyme, this is the most likely main pathway of taurine formation (25a, 55a, 68c, 230a).

The enzyme in brain and liver is very sensitive to a dietary B_6 deficiency. This

explains why taurine excretion in urine ceases in a B_6-deficient state and reappears when pyridoxine again is added to the diet (55a, 68, 68a). However, in spite of a diminished excretion into the urine, the taurine content in liver (4) and in brain (230b) remains normal, while it is even increased in spleen and muscle (4).

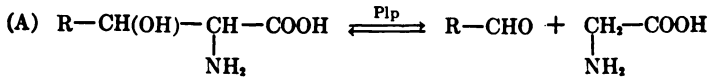
Biosynthesis of cystathionine from homocysteine and serine (cystathionine synthetase) is also B_6-dependent, as is its breakdown by cystathionase to homoserine and cysteine (61a; for review see 76):



In B_6 deficiency the urinary excretion of cystathionine is increased (230c). This could be explained by a higher sensitivity of cystathionase toward B_6 deficiency than of cystathionine synthetase. Cystathioninuria in man has been reported in two adult patients with mental aberrations (160a, 197a). Although no signs of B_6 deficiency could be observed, one of these patients responded to administration of pyridoxine with a reduction of cystathionine excretion and a concomitantly enhanced sulfate excretion. Other vitamins were ineffective (160a). However, there seems to be no causal relationship between this presumably "inborn error of metabolism" and mental disorders, since other members of the two families who were mentally normal displayed the same metabolic disorder (160a, 197a).

Concerning the metabolism of other sulfur-containing amino acids it is of interest that not only transsulfuration from homocysteine to cysteine (as seen above), but also desulfuration of methionine, cysteine, and cysteine sulfinic acid is catalyzed by B_6-containing enzymes (for review see 74, 75, 76).

F. Serine and threonine aldolase

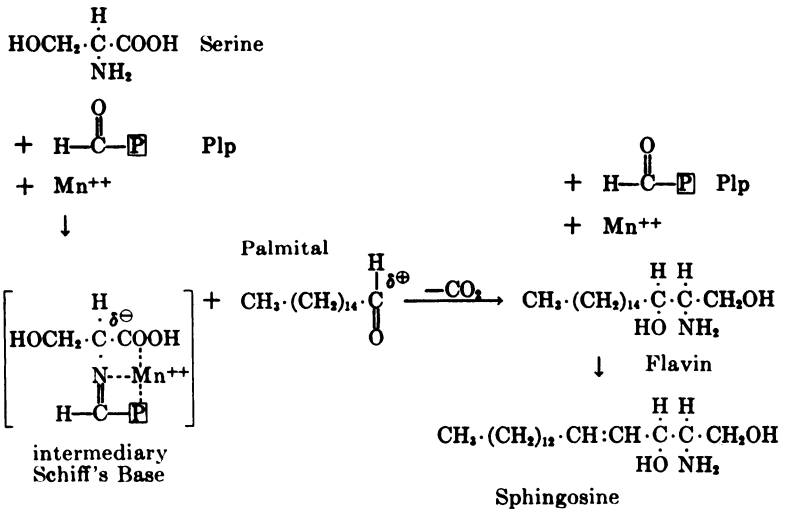


α-Amino-β-hydroxypropionic acid (serine) and α-amino-γ-hydroxybutyric acid (threonine) are metabolized to glycine and the corresponding aldehyde by B_6-dependent aldolases. The formaldehyde formed from serine, in contrast to the acetaldehyde formed from threonine, is coupled to tetrahydrofolic acid

(THFA). The resulting hydroxymethyl-tetrahydrofolic acid is then oxidized by NADP (formerly called TPN) to N_{10}-formyltetrahydrofolic acid. This compound is the biological carrier for one-carbon moieties and is of importance for the synthesis of purines and pyrimidines (8, 203, 249; for review see 75).

(B) Serine + tetrahydrofolic acid → serine-THFA $\xrightarrow[Mn^{++}]{Plp}$ glycine +
hydroxymethyl-THFA $\xrightarrow{NADP}$ N_{10}-formyl-THFA + NADPH

The reaction in (A) from right to left is of importance for the synthesis of 1) δ-aminolevulinic acid, the precursor of heme (see section IX), and 2) the unsaturated amino alcohol sphingosine, a constituent of sphingomyelin. This synthesis most likely results from the reaction between serine and palmitaldehyde in which first serine forms a Schiff's base with Plp and Mn^{++} as chelator. This complex undergoes an aldol condensation with palmital with concomitant decarboxylation. The aldol is then reduced to sphingosine by a flavin-containing enzyme (72, 73, 486).



G. Enzymes of tryptophan metabolism

As can be seen from figure 3, several of the enzymes of the tryptophan metabolism require Plp as coenzyme. The B_6-dependent tryptophan synthetase, which forms tryptophan (I) from indole (II) and serine, as well as tryptophanase, which metabolizes tryptophan to indole (II), ammonia, and pyruvic acid, are found only in bacteria (504, 555). All other metabolic pathways are found in animals and man as well as in bacteria. The B_6-dependent decarboxylation of tryptophan to tryptamine (III) (532) as well as the decarboxylation of 5-hydroxytryptophan (IV) to serotonin (V) (102) is quantitatively negligible. The chief metabolic pathway is the cleavage of the indole ring by tryptophan pyrrolase to an unstable N-formylkynurenine intermediate (276, 284), which in turn is converted to kynurenine (VI) and 3-hydroxykynurenine (VII). These two compounds can be

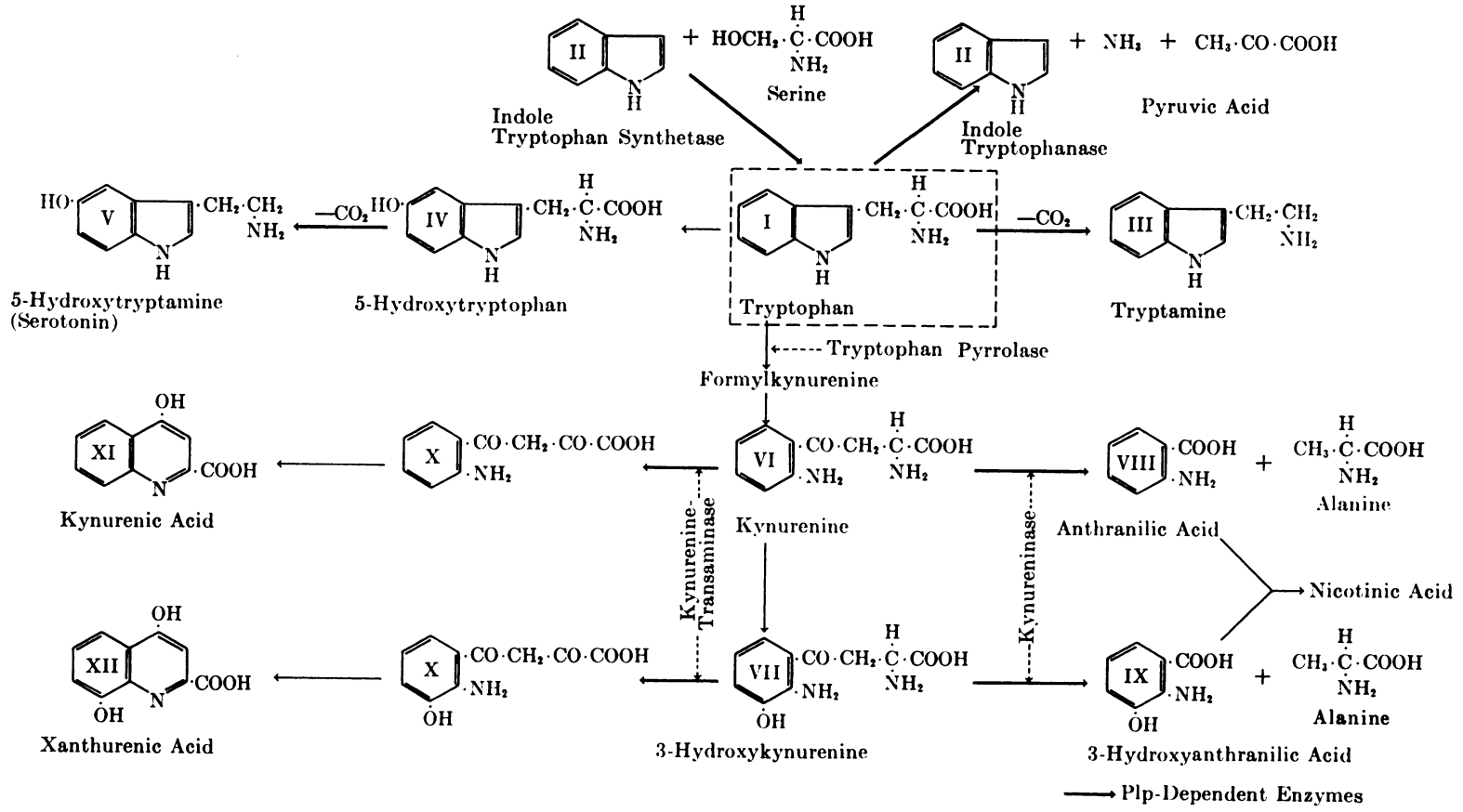


FIG. 3. Metabolism of tryptophan.

further metabolized by two B_6-dependent enzymes: 1) by kynureninase to anthranilic acid (VIII) or to 3-hydroxyanthranilic acid (IX), respectively; and 2) by kynurenine transaminase *via* the corresponding keto acids (X) to form kynurenic acid (XI) or xanthurenic acid (XII), respectively (75, 76, 81, 174).

In vitamin B_6 deficiency there is an inhibition of these two enzymes, and therefore an augmented excretion of kynurenine (VI) and 3-hydroxykynurenine (VII) as well as their acetylated derivatives (114). Kynurenic acid (XI) and especially xanthurenic acid (XII), which normally appear only in trace amounts in the human urine (405), are excreted in large amounts (305, 402). The "xanthurenic acid excretion test," *i.e.*, the abnormal increase of urinary xanthurenic acid after loading with L- or DL-tryptophan, is clinically used to detect vitamin B_6 deficiency (271, 272, 273, 304, 324, 512, 513). This paradoxical phenomenon, that a metabolite, xanthurenic acid, is formed and excreted in larger amounts than normal in spite of a lack of the coenzyme necessary for its formation, can be explained in the following manner.

Under physiological conditions the formation of formylkynurenine by tryptophan pyrrolase is the rate-limiting step in the degradation of tryptophan. Thus, even in B_6 deficiency, xanthurenic acid excretion is not necessarily increased. However, when a tryptophan load is imposed, this means not only an increased amount of substrate to be metabolized, but also a substrate-induced stimulation of tryptophan pyrrolase activity. Tryptophan pyrrolase activity is no longer rate limiting, and the conversion of kynurenine and 3-hydroxykynurenine to anthranilic acid and 3-hydroxyanthranilic acid, respectively, by the B_6-dependent kynureninase becomes the rate-limiting reaction. The formation of kynurenic acid and xanthurenic acid by transamination does not become rate-limiting because of the different affinities of the two apoenzymes for Plp: kynureninase is more strongly affected by B_6 deficiency than transaminase; therefore, the overflow of tryptophan metabolites occurs *via* the transamination pathway, leading to an increased formation and excretion of xanthurenic acid. Not yet explained is the fact that kynurenic acid, though formed by the same transaminase as xanthurenic acid, is not necessarily excreted in larger amounts, as is true for xanthurenic acid (for review see 13, 274).

However, the "xanthurenic acid excretion test" is not always positive in B_6 deficiency. Thus, hydrazides (*e.g.*, isonicotinic acid hydrazide, hydroxylamine, hydrazine, and isopropylhydrazine) which form hydrazones with Plp, thereby blocking its coenzyme function, even in convulsive doses did not increase xanthurenic acid excretion in rats after a tryptophan load (32, 436). On the other hand, subconvulsive doses of thiosemicarbazide and of thiocarbohydrazide as well as of the antimetabolite deoxypyridoxine caused an abnormally high xanthurenic acid excretion (32, 543). In the dog there was an increased excretion of xanthurenic acid after the administration of INH, but not after deoxypyridoxine (545).

H. Pyridoxal-5'-phosphate as a cofactor of phosphorylase

This enzyme, which contains Plp (33, 257), catalyzes the breakdown of glycogen by splitting off glucose-1-phosphate. Baranowski *et al.* (33), working with a

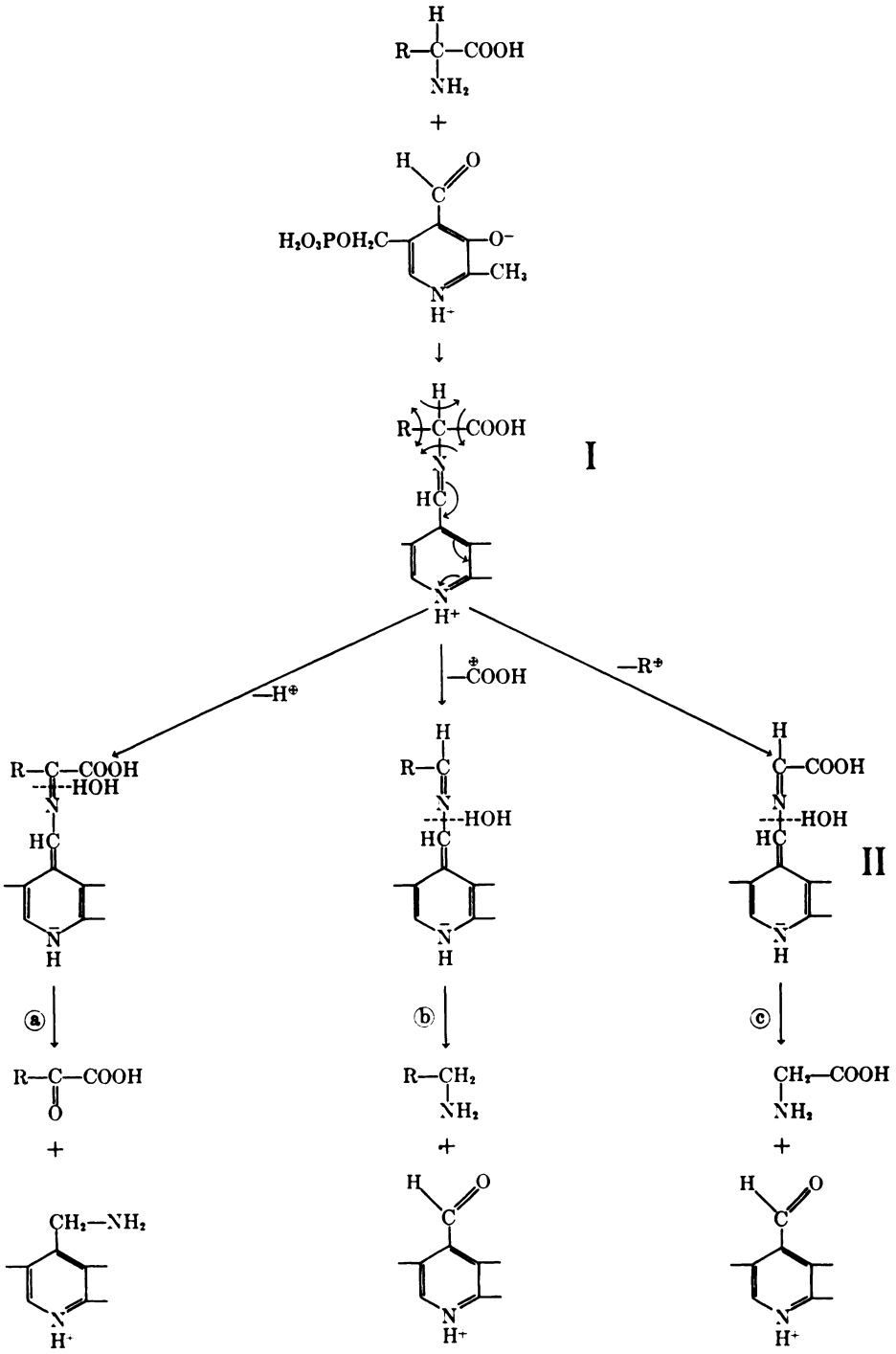


FIG. 4. The general mechanism for reactions catalyzed by pyridoxal-5′-phosphate.

crystalline muscle phosphorylase "a" preparation were able to isolate Plp as a barium salt. It is of interest that this was the first time that Plp was isolated from a natural source. The phosphorylase is the only B_6-containing enzyme not involved in the metabolism of the amino acids. Although the chemically induced dissociation of Plp from the purified enzyme results in an inactivation of the latter, B_6 presumably has not the function of a coenzyme (241). It seems to be necessary for the stabilization of the enzyme structure. In B_6 deficiency the total activity of muscle phosphorylase is diminished but the glycogen content of the muscle is unaffected, presumably because the activity of phosphorylase "a," the active form of the glycogen-metabolizing enzyme, remains unaltered (242, 315).

I. On the mechanism of reactions catalyzed by pyridoxal-5'-phosphate

In order to understand certain pharmacological actions which can be referred to the influence of a drug on B_6-catalyzed enzymatic reactions, it is necessary to have some knowledge of the biochemical mechanisms by which Plp participates in these reactions. Braunstein (74) and Snell *et al.* (352, 475) have shown that almost all of these reactions proceed by the same mechanism, whereby pyridoxal, phosphorylated in the 5' position, is the most important catalytic form of vitamin B_6. Even in the absence of the apoenzyme, decarboxylation, transamination, and racemization of amino acids can be catalyzed by Plp or pyridoxal itself, if heavy metals are added (352). The phosphorylated 5-hydroxymethyl group, together with the pyridine nitrogen, is important for the binding of the coenzyme on the apoenzyme, but does not take part in the actual chemical or enzymatic reaction. The free aldehyde group in the position *para* to the pyridine-nitrogen as well as the free phenolic group in the position *ortho* to the aldehyde group are essential and participate in these reactions. Amino acids require a free amino group in order to react with Plp. N-Alkylated amino acids for instance are not decarboxylated.

As can be seen from figure 4, the first step in every Plp-catalyzed reaction is the formation of a Schiff's base (I) or of transitional Schiff's bases (II) (214, 352, 396, 452, 531). This results in a labilization of all four bonds around the α-C-atom of the amino acid (352, 396). Depending upon the substrate affinity and the apoenzyme specificity there then follows: a) transamination, which consists in the formation of the corresponding keto acid with the splitting off of H^+ while the NH_2-group becomes coupled to pyridoxal to form pyridoxamine; b) decarboxylation, *i.e.*, cleavage of the carboxyl-carbon—α-carbon bond and the formation of the corresponding amine plus CO_2; or c) splitting off of the alkyl residue R with the formation of a lower homologue of the reacting amino acid (*cf.* section I F above).

II. METABOLISM OF VITAMIN B_6

All forms in which vitamin B_6 occurs in the organism (see fig. 5)—pyridoxine (I), pyridoxal (II) and pyridoxamine (III)—are practically equivalent because they can be enzymically converted into one another (74, 475, 476). Pyridoxine is

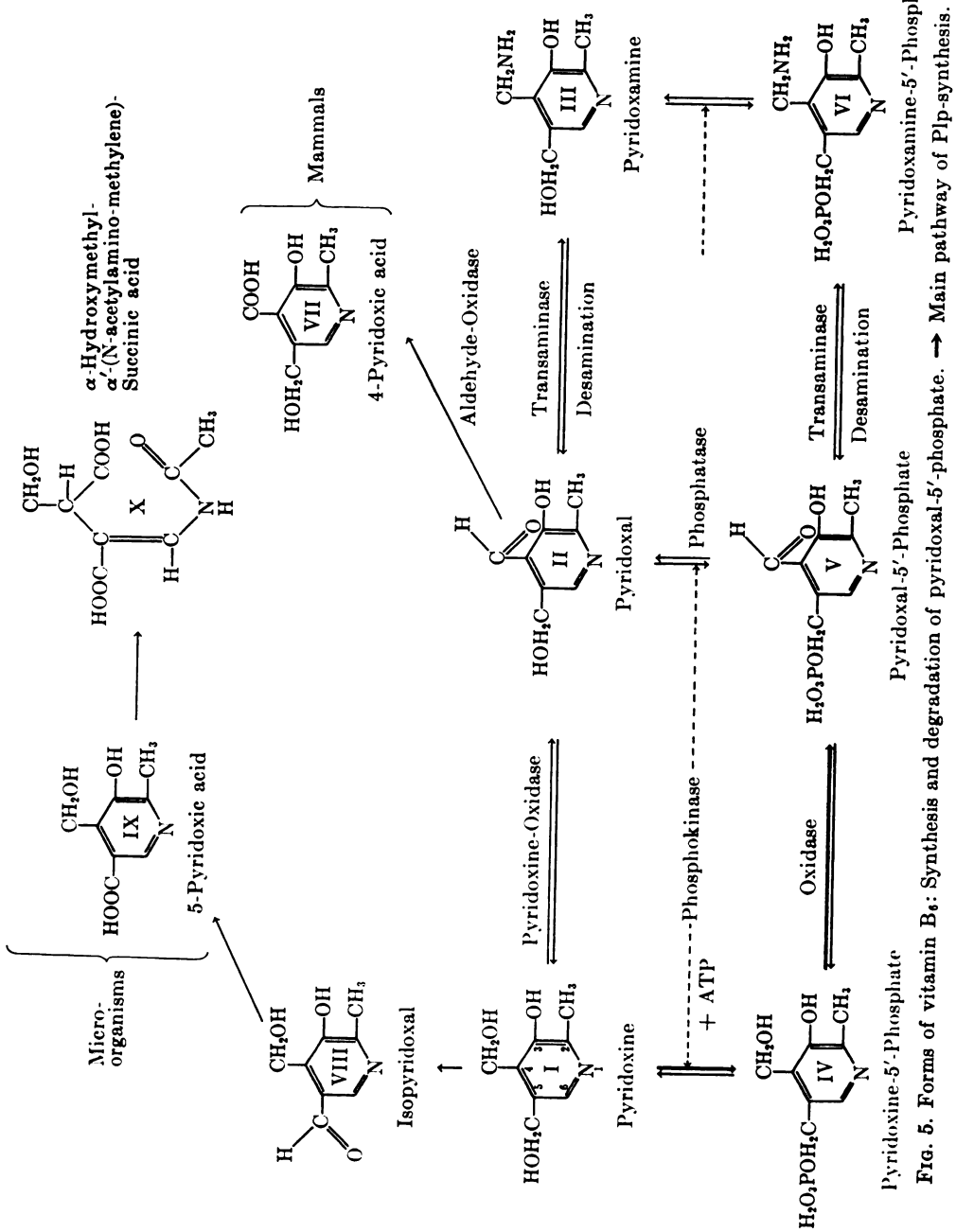


FIG. 5. Forms of vitamin B_6: Synthesis and degradation of pyridoxal-5'-phosphate. → Main pathway of Plp-synthesis.

oxidized by an oxidase to pyridoxal (519) which can be further transaminated to form pyridoxamine. All three forms can be phosphorylated in position 5′ by an ATP-dependent phosphokinase (335). The oxidation of pyridoxine to pyridoxal presumably is preceded by phosphorylation (335, 519). Vitamin B_6 occurs in the tissues, *e.g.*, brain and liver, predominantly in the phosphorylated forms, Plp (V) and pyridoxamine-5′-phosphate (VI) (28, 314). The degradation of the phosphorylated forms is initiated by a phosphatase. In the case of pyridoxine-5′-phosphate (IV), the resulting pyridoxine (I) is oxidized to pyridoxal (II); in the case of pyridoxamine-5′-phosphate (VI) the resulting pyridoxamine (III) is deaminated to pyridoxal (II). Pyridoxal is then oxidized by a nonspecific aldehyde oxidase to pyridoxic acid (VII). This enzyme is found in liver, kidney, and other tissues. Pyridoxic acid is the only excretion product of metabolized vitamin B_6 (304, 476).

In bacteria the hydroxymethyl group of pyridoxine in position 5, but not that in position 4, is oxidized to form isopyridoxal (VIII), which is subsequently further oxidized to 5-pyridoxic acid (IX). Further degradation results in the cleavage of the ring to form α-hydroxymethyl-α'-(N-acetylamino-methylene)-succinate (X) (240, 434).

III. ABSOLUTE AND RELATIVE B_6 DEFICIENCY

While the rat is able to meet a considerable part of its B_6 requirement through bacterial synthesis in the intestine (141, 353), it appears that man is completely dependent upon the oral intake of the vitamin (111, 304, 488, 509). In view of the ubiquitous distribution of B_6 in all foodstuffs a mixed diet is generally sufficient to fulfill this requirement. The human B_6 requirement is reported to be 2 to 3 mg per day (304, 509). The increasing use of chemical and physical methods for the preparation and preservation of foodstuffs can, however, endanger the fulfillment of the vitamin B_6 requirement. The storage as well as the sterilization of meat and milk products by heat or radiation can lead to a significant loss of B_6 activity, particularly of pyridoxal and pyridoxamine (123, 417, 421). On the other hand, pasteurization or drying of milk causes only slight loss of vitamin activity (237, 556).

In 1952 and 1953 "epidemic B_6-deficiency symptoms" occurred in the USA caused by the exclusive use of a vitamin B_6-poor milk preparation (Liquid SMA, liquid synthetic milk adapted) in the nutrition of babies (59, 109, 332, 362). The most marked signs were epileptiform convulsions, and these could be promptly relieved by the administration of pyridoxine. Because of the relatively low B_6 content of human milk, 130 μg/l (488, 556), the occurrence of convulsions is possible also in breastfed babies. Indeed, Bessey *et al.* (59) have reported two such cases, in which the convulsions disappeared upon daily administration of 0.26 mg of pyridoxine.

A *relative* B_6 deficiency, in spite of a normal intake, may result from a variety of causes, among which those discussed below are prominent.

A. High protein diet

The "requirement" for vitamin B_6 is, like that of the vitamins B_1 and B_2, not constant, but depends on other nutritional and metabolic factors (304). Since vitamin B_6 is primarily concerned with amino acid and protein metabolism, it is conceivable that on a protein-rich diet the B_6 requirement is raised and the appearance of the syndrome of deficiency is accelerated and accentuated (49, 91, 378).

B. Thyroxine, 2,4-dinitrophenol, insulin

Abnormal metabolic conditions, such as fever, neoplasms and hyperthyroidism, may also induce a relative B_6 deficiency (271, 324, 517, 554). The treatment of rats with 0.1 to 0.2 mg/kg DL-*thyroxine* for 10 days resulted in a significant fall in the pyridoxine and Plp content of the liver and heart (322). Apparently, there must have been also an ATP deficiency, since the lowered myocardial and hepatic Plp content could not be raised by pyridoxine administration, but could be raised by ATP. There is a corresponding decrease in the activity of B_6-dependent enzymes, for example cysteic acid decarboxylase, dopa decarboxylase, glutamic-pyruvic transaminase, threonine dehydrase, *etc.* (95, 232). It appears that the diminished enzyme activity (*e.g.*, dopa decarboxylase activity in the liver) induced by thyroxine results from an impairment of the apoenzyme, too (85, 228).

2,4-Dinitrophenol (*DNP*), which does not inhibit pyridoxal-phosphokinase *in vitro* (338), is the prototype of an agent which indirectly inhibits the phosphorylation of pyridoxal through uncoupling oxidative phosphorylation with a resultant fall in the synthesis of energy-rich phosphates (ATP). DNP also inhibits the active transport of amino acids (*e.g.*, histidine and methionine) across the intestinal wall. This transport is activated by B_6 derivatives (see section VII). Plp is able to abolish the inhibitory action of DNP. On the other hand, the non-phosphorylated B_6 derivatives—pyridoxal and pyridoxine—are able to prevent inhibition only if they are injected before DPN (246, 247, 502).

Insulin coma is associated with a fall of ATP and creatine phosphate in brain. The diminished decarboxylation of 5-HTP found in brain under conditions of severe insulin hypoglycemia can be attributed to an ATP deficiency which may result in the reduced phosphorylation of pyridoxal (108).

C. Pregnancy

From the third month of pregnancy there is an increased xanthurenic acid excretion, which is particularly large when symptoms of toxemia are present (485, 512, 514, 515). A single dose of 25 mg of pyridoxine can restore the xanthurenic acid excretion to normal for 3 to 5 days. Thiamine, riboflavin, and nicotinic acid are ineffective (82, 512). The chief metabolic product of vitamin B_6, pyridoxic acid, is excreted in normal amounts by pregnant women, but after loading with 25 mg of pyridoxine—"pyridoxine load test" (448)—they excrete significantly less pyridoxic acid than the controls (515). This indicates a retention of pyridoxine as a result of an increased requirement in pregnancy.

Whereas the B_6 content (taken as total activity) of blood, skin, and urine is

normal in pregnancy (160), the Plp content of the plasma and leukocytes is significantly lower than normal (516, 518). Administration of 100 mg of pyridoxine results in a smaller increase in the Plp content of plasma and leukocytes in pregnant women than in nonpregnant women (516).

In this connection it is of interest that estrogens (estradiol disulfate, stilbestrol) are extremely potent inhibitors of vitamin B_6-dependent enzymes. In concentrations as low as 10^{-7} M they inhibit dopa decarboxylase, cysteine sulfinic acid decarboxylase, kynurenine transaminase, and phosphorylase "a." Estrone sulfate and the glucuronide of pregnandiol were less potent. According to Mason *et al.* the steroid hormones and Plp compete for the apoenzyme. That the steroid hormones have an affinity for the apoenzyme is also indicated by the fact that they are able to protect the enzyme from proteolytic inactivation by chymotrypsin (325, 326, 327).

D. Vitamin B_6 dependence

Hereditary disturbances in vitamin B_6 metabolism can also lead to a relative B_6 deficiency and its signs. Among these are the cases of epileptiform convulsions which appear as soon as the first hour after birth in certain babies (235, 236). These convulsions are refractory to barbiturates and other antiepileptic therapy but are completely relieved in a few minutes by pyridoxine. A maintenance dose of 2 to 15 mg/day is required to prevent their recurrence (59, 69a, 105, 110, 169, 321, 377, 460, 461). During the convulsions, as in insulin seizures, the cerebral blood flow, oxygen consumption, and respiratory quotient fall, and they return to normal as soon as the convulsions cease after the injection of pyridoxine (480). Tower (499) concluded from these results that the convulsions have their origin in a deficiency of specific substrate. On the other hand, during convulsions due to electroshock or Metrazol brain metabolism is augmented and falls to values below normal only in the postconvulsive phase.

In "absolute" B_6 deficiency (for instance, of dietary origin) associated with convulsions, usually one finds a low blood B_6 content and a decreased urinary vitamin B_6 activity and pyridoxic acid. In the hereditary cases convulsions occur despite a normal B_6 intake and are due to an endogenous B_6 deficiency. After a pyridoxine load Coursin (110) observed, concomitant with a low B_6 blood level, an abnormally high urinary excretion of pyridoxic acid in some cases and of pyridoxal and pyridoxamine in another case. This indicates that the metabolism or excretion of the vitamin is increased above normal in this syndrome, and that this produces an endogenous B_6 deficiency.

These cases probably belong to the group of "inborn errors of metabolism" of which classic examples are phenylketonuria, argininosuccinuria, maple sirup disease, *etc.* (273). This interpretation is supported by observations of the occurrence of several such metabolic anomalies within one and the same family (236, 321, 377, 460).

IV. B_6 DEFICIENCY PRODUCED BY ANTIMETABOLITES

According to Snell and co-workers (335, 519) an ATP-dependent phosphokinase phosphorylates pyridoxine to pyridoxine-5′-phosphate. A flavin-containing enzyme which occurs largely in liver and to a lesser extent in kidney and

brain then oxidizes pyridoxine-5′-phosphate to Plp (519). This enzyme is inhibited *in vitro* by heavy metals (Cu^{++}, Hg^{++}, *p*-chloromercuribenzoate). Plp itself is a competitive inhibitor. Moreover, 4-deoxypyridoxine-5′-phosphate and pyridoxaloxime-5′-phosphate are inhibitors because they possess a greater affinity for the oxidase than pyridoxine-5′-phosphate (519). More details on the inhibition of phosphokinase are given in section IV A.

A. Structural analogues of vitamin B_6

Without realizing it, Abderhalden (2) was presumably the first to use a B_6 antimetabolite, in 1938. In experiments designed to accomplish the synthesis of vitamin B_1 (thiamine) from its pyrimidine and thiazole moieties *in vivo*, he observed that the injection of the pyrimidine component (2-methyl-4-amino-5-hydroxymethyl-pyrimidine), later named toxopyrimidine (fig. 6, III), produced a typical running fit in mice and rats, *i.e.*, an uncontrollable running movement followed by convulsions. It was only much later that toxopyrimidine was recognized as an antivitamin on account of its structural similarity to the B_6 molecule (317, 318). The toxopyrimidine convulsions can be suppressed by pyridoxine, pyridoxal, or pyridoxamine.

Experimentally, antimetabolites of vitamin B_6 have the advantage that one can produce acute B_6 deficiency syndromes in animals without time-consuming feeding of B_6-deficient diets. Probably the most thoroughly investigated antimetabolite is 4-deoxypyridoxine (fig. 6, I). In this compound the 4-hydroxymethyl group essential for vitamin function (352) is chemically altered. ω-

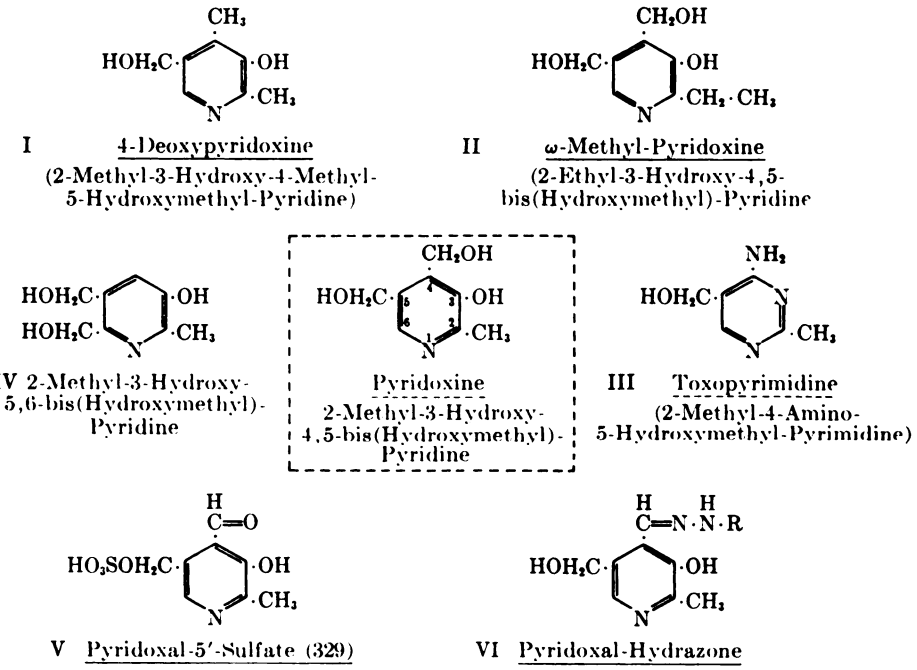


FIG. 6. Vitamin B_6 antimetabolites.

Methyl-pyridoxine (fig. 6, II), however, appears to act as an antivitamin in the rat, causing convulsions, in spite of a normal chemical structure at position 4 (445). On the other hand, this compound can act as a coenzyme of bacterial tyrosine decarboxylase (382).

In animals 4-deoxypyridoxine is highly toxic, especially under conditions of dietary B_6 deficiency (386). Thus the acrodynia in rats is accentuated (143). The lethal action of the antimetabolite on chick embryos can be suppressed by vitamin B_6 (113). Deficiency symptoms after 4-deoxypyridoxine in man reported by Vilter *et al.* (510), consist of dermatitis, glossitis, cheilosis, and neuritis.

The bacterial tyrosine decarboxylase (in *Streptococcus faecalis*) is inhibited *in vitro* only by 4-deoxypyridoxine-5′-phosphate and not by 4-deoxypyridoxine (51, 503). The nonphosphorylated analogue is inactive in concentrations 50,000 times greater than an effective dose of the phosphorylated compound (239). The same is true for toxopyrimidine (318). It is therefore reasonable to assume that 4-deoxypyridoxine acts as an antimetabolite in the animal and human body only after it has been phosphorylated and can compete with the physiologically occurring Plp for the binding sites on the apoenzyme (503). The extent to which a B_6 enzyme is inhibited by an antimetabolite depends upon the relative affinities of the phosphorylated antimetabolite and of Plp for the apoenzyme (474, 475). Therefore, the administration of an antimetabolite *in vivo* does not necessarily inhibit all B_6-dependent enzymes to the same degree, but particularly those whose coenzyme will easily dissociate from the apoenzyme (for review see 116, 238, 475).

Deoxypyridoxine also inhibits the phosphorylation of pyridoxine and pyridoxal by phosphokinase, an enzyme found in high concentration in the cerebral cortex. Yeast, bacterial, and animal phosphokinase can be inhibited by B_6 analogues which themselves undergo phosphorylation (*e.g.*, 4-deoxypyridoxine) and by B_6 analogues that have a high affinity for the phosphokinase although they are not substrates of the enzyme (*e.g.*, 5-deoxypyridoxal) (238, 239, 475). Other analogues (*e.g.*, toxopyrimidine) can be phosphorylated, but are not inhibitors of the phosphokinase (239, 338, 475). Although carbonyl reagents (*e.g.*, hydrazine, hydroxylamine, and semicarbazide) do not inhibit phosphokinase, their oximes or hydrazones (fig. 6, VI), formed in the reaction with pyridoxal *in vitro* (presumably also *in vivo*), are among the strongest inhibitors of the enzyme (336, 337, 338) (see section V D).

B. Substrate analogues

Substrate analogues that have a high affinity to the apoenzyme and are poorly metabolized by the corresponding enzyme may be strong inhibitors by competition with the usual substrate.

In order to elucidate the role of catecholamines in hypertension, many investigations have been performed to block the enzymatic biosynthesis of these amines by inhibition of L-dopa decarboxylase. Thus Clark *et al.* (103, 104, 201) found some compounds chemically related to dopa, *e.g.*, 5-(3,4-dihydroxycinnamoyl)-salicylic acid, caffeic acid, and others, to be rather potent inhibitors of the decarboxylase *in vitro*.

1. α-Methyldopa (3,4-dihydroxyphenyl-α-methylalanine). In 1954, Sourkes (481) investigated dopa analogues which contain a methyl group on the α-C-atom of the side chain. Most active inhibitors are α-methyl-metatyrosine and α-methyldopa. The inhibitory action is due to a competition of the inhibitor with the substrate for the apoenzyme. Inactivation of the coenzyme Plp, by formation of Schiff's bases with subsequent transformation to tetrahydroisoquinoline derivatives (see section V A) plays only a minor role. These inhibitors are active also *in vivo*. *Alpha*-methyldopa suppresses the increase in blood pressure in response to an intravenous injection of L-dopa (126). It also inhibits the decarboxylation of 5-HTP to serotonin, *in vivo* and *in vitro* (536). The inhibitory action of α-methyldopa *in vivo* lasts only a few hours (536). In agreement with this, the decreased serotonin content in brain brought about by α-methyldopa returns to normal 6 to 8 hours after injection (471, 472). In contrast, the simultaneously occurring depletion of norepinephrine in brain and heart lasts for days (213, 294, 365, 482).

Although the corresponding amines, α-methyldopamine and α-methyl-metatyramine (Aramine), are not inhibitors of dopa decarboxylase, they have the same action as α-methyldopa in depleting tissue norepinephrine (403). Therefore the depleting action probably is not due to α-methyldopa itself, but to α-methyldopamine, produced slowly by decarboxylation of the amino acid (526). Evidence was given by Carlsson and Lindquist (88) that the depletion of the physiological catecholamines as produced by α-methyldopa and α-methyl-metatyrosine is brought about by replacement of the catecholamines by the corresponding α-methylated amines, which obviously can also be hydroxylated in the side chain.

Thus, the pharmacological action of α-methyldopa, particularly its hypotensive action (178), of which clinical use is made in the treatment of hypertension (21, 86, 177, 302, 469), may be explained by the replacement of the physiological transmitter, norepinephrine, by less vasoactive amines. Controversial results were obtained with α-methyldopa in the treatment of patients with carcinoid (69, 132, 470).

In the initial stages of treatment with α-methyldopa, fatigue and sedation were encountered (21, 132, 379). Other side-effects can be Parkinsonian symptoms (190). Obviously, also in these cases the methylated amines that originate from α-methyldopa cannot substitute functionally for the normal endogenous amines (see section VI).

In hypertensive rats the blood-pressure-lowering effect of α-methyldopa was completely prevented by a hydrazine derivative (NSD 1039; N_1-2-hydroxybenzyl-N_1-methyl-hydrazine) (119) which is one of the most potent inhibitors of dopa decarboxylase, presumably by inactivation of Plp (418). This also indicates that α-methyldopa itself is not the hypotensive agent. Apparently it has to be decarboxylated to the hypotensive methylated amines. From this it follows that it is not the inhibition of dopa decarboxylase by α-methyldopa, but the decarboxylation of α-methyldopa by the B_6-dependent dopa decarboxylase which is *de facto* the cause of its hypotensive action.

2. Phenylketonuria and B_6 enzymes. Oligophrenia phenylpyruvica, an inborn error in metabolism, is probably due primarily to a lack of the liver enzyme which

hydroxylates phenylalanine to tyrosine. This leads to an increase in the phenylalanine and phenylpyruvic acid concentrations in blood and urine. Clinically this is followed by cerebral manifestations such as oligophrenia and epileptiform convulsions.

In patients with phenylketonuria the values for serotonin in plasma and for 5-hydroxyindoleacetic acid in urine are lowered (392, 393, 394). Also the plasma epinephrine and norepinephrine concentrations (523) as well as the urinary excretion of these amines and of dopamine are decreased (369). The values are restored to normal when these patients are maintained on a diet poor in phenylalanine (60, 275). Feeding high doses of phenylalanine to young rats reduced the serotonin content of brain and blood (23, 522, 563). The explanation of these disturbances in amine metabolism could be an inhibition of amine synthesis. Phenylpyruvate and phenylacetate (but not phenylalanine) inhibit the B_6-dependent decarboxylation of dopa and 5-HTP (121, 149, 197, 201). In agreement with this, epinephrine synthesis from C^{14}-*p*-tyrosine in adrenal slices is inhibited by phenylpyruvate, while at the same time the accumulation of C^{14}-dopa in the medium indicates dopa decarboxylase inhibition (71). On the other hand, phenylalanine competitively inhibits the uptake of the precursor amino acids for amine synthesis into the cells. Thus, the uptake of tyrosine by brain slices is strongly inhibited by phenylalanine, while that of histidine, arginine, ornithine, and proline is much less affected (343, 372). A single intraperitoneal injection of 1 g of L-phenylalanine per kg inhibited the uptake of 5-HTP and thereby the formation of serotonin in the brain to about 60%; D-phenylalanine was less potent and phenylpyruvate had no effect (343).

A decrease in cerebral amine content itself appears not to be responsible for the cerebral effects of phenylpyruvic oligophrenia, since rats with an increased serotonin content of the brain induced by treatment with a monoamine oxidase inhibitor (isocarboxazide) showed a reduction of intelligence (high incidence of mistakes in the Hebb-Williams maze test) similar to that of rats kept on a diet rich in phenylalanine and having a low serotonin level in brain (23, 311, 563).

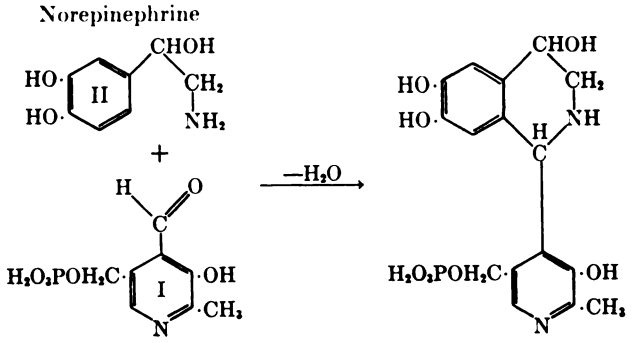
A disturbance in amine metabolism, convulsions, and dermatitis, as observed in patients with phenylketonuria, may indicate a B_6 deficiency. However, long-lasting treatment with vitamin B_6 did not have any therapeutic effect (340).

V. B_6 DEFICIENCY BY INACTIVATION OF PYRIDOXAL-5′-PHOSPHATE (PLP)

The most important functional group of Plp is the aldehyde group in position 4. Blockade of this group inactivates coenzyme function.

A. Amino acids and amines

The amount of CO_2 released under anaerobic conditions by brain homogenates is increased if L-glutamic acid is added to the medium (see section I B). However, if the homogenate is preincubated for a few minutes with L-dopa, dopamine, or norepinephrine, the formation of CO_2 is depressed and is even lower than that seen in the controls to which no glutamic acid has been added (230). This inhibition, which can be reduced by the addition of Plp, is explained by a stoichiometric reaction of catecholamines having a primary NH_2-group with the



Pyridoxine-5′-Phosphate

Tetrahydroisoquinoline Derivative

coenzyme, to form Schiff's bases. These rearrange themselves irreversibly to tetrahydroisoquinoline derivatives. Thus the catecholamines as well as the coenzyme lose their pharmacological and biochemical activities (216, 230, 455).

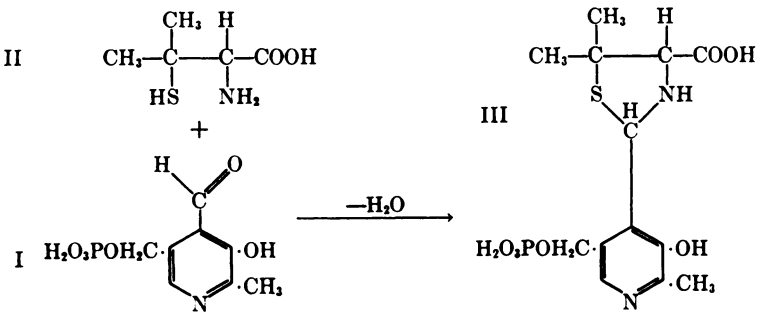
The reaction may be of importance for the *in vitro* estimation of Plp-dependent enzyme activities. In the manometric estimation of dopa decarboxylase, for example, the substrate (dopa) as well as the reaction product (dopamine) is equally able to react with Plp, causing a rapid inactivation of the coenzyme, and thereby to stop the enzymic decarboxylation.

Epinephrine, which is not an inhibitor of dopa decarboxylase or glutamic acid decarboxylase, becomes inhibitory when it is allowed to take up 4 or 6 moles of oxygen per mole. The inhibitory action of oxidized catecholamines cannot be reduced by the addition of pyridoxal-5′-phosphate. Their site of attack is, therefore, probably the apoenzyme (230). This is presumably why Werle and Koch (531) found that epinephrine, catechol, resorcinol, or pyrogallol inhibits histidine decarboxylase and dopa decarboxylase after incubation for 30 minutes in an oxygen atmosphere with tissue enzyme preparations.

B. Penicillamine

D-Penicillamine (α-amino-β-thioisovaleric acid) results from chemical degradation of penicillin and is also a metabolic product of the antibiotic in man (106). It finds clinical application in heavy metal poisoning and Wilson's disease. It accelerates the elimination not only of Cu, but also of Pb, Fe and Hg through chelate formation (18, 154, 451). In rats the L- isomer is significantly more toxic. The oral LD50 for the DL- form is 365 mg/kg, whereas that of the D- isomer is greater than 2500 mg/kg (18, 19). Chronic administration of the L- isomer to rats leads to inhibition of growth, anorexia, and acrodynia as well as to tonic-clonic convulsions (running fit) (19, 549, 550). These effects as well as the accompanying increased xanthurenic acid excretion in the urine can be suppressed by pyridoxine (290, 291).

L-Penicillamine inhibits the absorption of amino acids from the intestine (5, 502) (see section VII). *In vitro* it is an inhibitor of numerous B_6-dependent enzymes, *e.g.*, of transaminases including GOT and GPT (207, 290), of cysteine desulfhydrase (20), and of α-aminolevulinic acid synthetase (303). If the co-

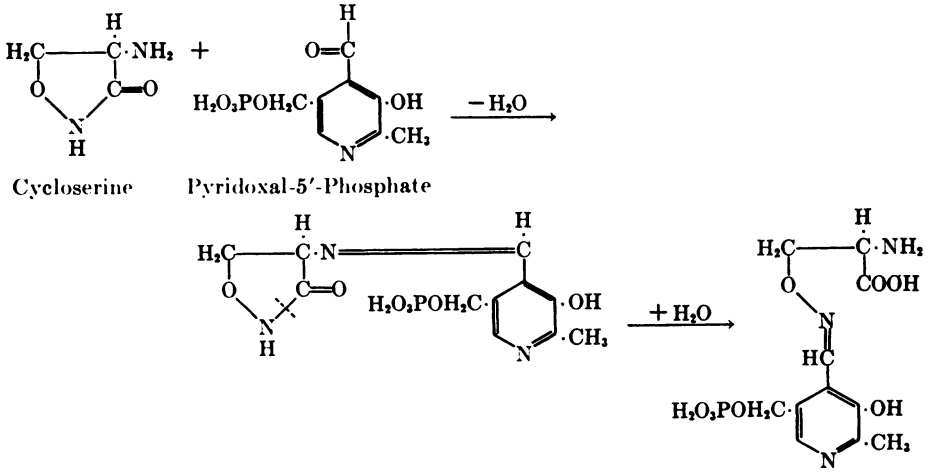


enzyme of these enzymes, Plp, is incubated with penicillamine prior to its addition to the apoenzyme, the enzyme activity is inhibited by both L- and D-isomers. However, if Plp and the apoenzyme are preincubated first, then only L-penicillamine has an inhibitory effect (502). Both isomers (II) are capable of blocking free Plp (I) by forming a thiazolidine derivative (III) nonenzymatically, as is true also for other SH-containing amino acids (316, 320, 551). However, L-penicillamine obviously also inhibits the holoenzyme (502).

Because of the toxicity of L-penicillamine, only the D- isomer should be employed in the therapy of Wilson's disease and heavy metal poisoning (18).

C. Cycloserine

The toxic action of this tuberculostatic drug can be explained at least in part by its inhibition of B_6-catalyzed enzymatic reactions (144). Among the most important side-effects seen frequently with therapeutic dosage are sedation, psychic disturbances, pareses, and above all epileptic convulsions (157, 161, 308, 407, 568). Cycloserine brings forth seizures and ataxia also in the mouse and rabbit (161). Pyridoxine reduces cycloserine toxicity (144, 433). While Dengler



Cycloserine

Pyridoxal-5'-Phosphate

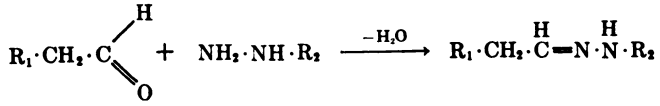
Intermediary Schiff's Base

Oxime of Plp and β-Amino-oxyalanine

(125) could not establish an increased xanthurenic acid excretion in patients treated with cycloserine, in rats with high doses there is an enhanced excretion of xanthurenic acid, kynurenine, and 3-hydroxykynurenine due to inhibition of B_6-dependent kynureninase (17). Dopa decarboxylase and glutamic acid decarboxylase are also inhibited *in vitro* by cycloserine (125); the same is true for other decarboxylases and transaminases of animal (380) and bacterial origin (15, 16, 562). Plp abolishes the inhibition. It is probable that, after formation of an unstable Schiff's base and splitting of the isoxazolidinone ring, a stable pyridoxal-oxime of β-amino-oxy-α-alanine results (260, 440a).

D. Hydrazides

Braunstein (76) wrote in 1953: "In our investigations, we made it a rule first to examine the sensitivity towards carbonyl reagents of all enzymatic reactions in which participation of pyridoxal phosphate could be assumed." Hydrazine and hydrazine derivatives of the general structure R—NH—NH_2 are typical carbonyl reagents in that they react with the carbonyl oxygen of aldehydes and ketones to form hydrazones.



However, the hydrazones resulting from the reaction between Plp and hydrazides are still active as coenzymes *in vitro*, as shown by Palm for dopa decarboxylase (391). Whereas on addition of free coenzyme decarboxylation soon comes to an end because of inactivation of Plp by the substrate, the addition of the hydrazone, formed for instance by incubation of isonicotinylhydrazide (INH) with Plp, promotes a continuous reaction, which results in quantitative decarboxylation of the substrate. This is presumably due to a slow hydrolysis of the hydrazone, splitting off small amounts of free coenzyme (391). This reaction could be useful in the quantitative manometric estimation of dopa decarboxylase activity. The results were confirmed by Gonnard (182). His assumption that the hydrazone itself is the activating factor (181), however, would be difficult to reconcile with the enzymatic mechanism of decarboxylation (see section I, 1).

Hydrazine itself is of toxicological importance because of its widespread industrial application (131). Mono- and di-substituted hydrazines are of pharmacological and toxicological interest in that a whole series of them have found therapeutic application in the last few years. Among these are the tuberculostatic isonicotinylhydrazide (isoniazid, INH) and the monoamine oxidase inhibitor isonicotinyl isopropylhydrazide (iproniazid, Marsilid) (for review see 400). Compounds such as semicarbazide, thiosemicarbazide, carbohydrazide, and thiocarbohydrazide served for a long time to detect Plp-dependent reactions *in vitro* and *in vivo*. Since most of them penetrate into the central nervous system, they often have been used to examine the role of vitamin B_6 in the CNS. The most characteristic pharmacological action of those hydrazides that penetrate the blood-brain barrier consists in the induction of convulsions (for review see

544). Dieke (129) as well as Parks *et al.* (395) detected the convulsive action of hydrazides such as semicarbazide and thiosemicarbazide, McGrath *et al.* (342) that of hydrazine. Convulsions occurring in tuberculous patients after treatment with INH were reported by numerous authors (94, 112, 286, 292, 440). The hydrazide-induced convulsions have been described by Jenney and Pfeiffer (251) as follows: "These compounds produce maximal, tonic-extensor convulsions which closely resemble the grand mal seizures of epilepsy or supramaximal electroshock and intravenous Metrazol, and differ from most drug-induced seizures, in that an animal may have as many as five maximal seizures before recovery or death." In 1890, Loew (310) had observed in guinea pigs and rabbits the appearance of opisthotonos, intermittent clonic convulsions, and pareses starting in the hind limbs after the injection of hydrazine. He wrote: "The diamide, thus, is like hydroxylamine in numerous organisms a strong poison. Both compounds are chemically characterized by their affinity to aldehydes and ketones."

The convulsive hydrazide doses are very different from species to species. The dog appears to be particularly sensitive, while small rodents are strikingly insensitive. Thus the convulsive doses (mg/kg) of semicarbazide are: dog 10, cat 40, man 40, monkey 60, guinea pig 75. On the other hand, they are 116, 150, and 175 mg/kg for the mouse, rat, and rabbit, respectively (544).

The death of a tuberculous patient who was under treatment with INH, after a single injection of Irgapyrine (phenylbutazone + amidopyrine) led Dienemann and Simon (130) to investigate the influence of INH on the convulsion threshold of amidopyrine, Metrazol, and procaine. In all three cases, INH lowered the seizure threshold. Epileptics appear to be particularly sensitive to the convulsive action of INH (151). Alcohol is supposed to favor the onset of convulsions (189). In animals semicarbazide lowers the threshold for Metrazol but not that for strychnine (251, 419). Also the threshold for electroshock convulsions is lowered after administration of hydrazides (265). Hydrazide convulsions are particularly easy to provoke by acoustical and optical stimuli (32, 265, 413, 419). In combination with flicker light, semicarbazide has been used clinically to produce grand mal seizures in schizophrenic patients resistant to therapy (420).

Like the convulsions in B_6 deficiency, either dietary or hereditary, those brought about experimentally by hydrazides are largely refractory to antiepileptic drugs (barbiturates, hydantoins). Dilantin (diphenylhydantoin) prevented neither semicarbazide nor INH convulsions in rats (251, 419); it only prolonged the survival time. NaBr and Mesantoin (N-methylphenylethylhydantoin) were somewhat active, as was the oxazolidine derivative trimethadione, which has shown itself to be of value in therapy of petit mal epilepsy, and which is one of the most specific antagonists of Metrazol-induced convulsions (148, 183, 251). Hydrazide-induced convulsions have in common with those of B_6 deficiency that they can be suppressed immediately by pyridoxine or its congeners (32, 252). Parks *et al.* (395) were the first to show that pyridoxamine acts as an antidote against poisoning with thiosemicarbazide.

The various hydrazides have very different convulsive potencies (32, 251). The sulfur-containing derivatives, *e.g.*, thiosemicarbazide and thiocarbohydrazide, are much more potent than semicarbazide or carbohydrazide. In mice

after intramuscular injection the ED100's of carbohydrazide (1.66 mmol/kg) and semicarbazide (1.80 mmol/kg) are 10 to 50 times higher than those of the corresponding thio compounds (0.038 and 0.121 mmol/kg) (32). The ED100 of the B_6 antimetabolite 4-deoxypyridoxine is 1 mmol/kg. The convulsive action of hydrazides can be prevented by simultaneous injection of pyridoxine. On a molar basis, a dose of pyridoxine approximately one-half that of semicarbazide, carbohydrazide, thiocarbohydrazide, cyanoacetic hydrazide, or hydrazine is necessary to reduce the ED100 to the ED50. On the other hand for thiosemicarbazide, deoxypyridoxine, and isopropylhydrazine, the ED100 can be correspondingly reduced by pyridoxine in 1/8th, 1/22nd and 1/42nd of the molar dose of the hydrazine (32).

INH occupies an exceptional position in that even a molar dose of pyridoxine two times that of INH does not prevent INH convulsions in the rat and mouse (32, 134, 319, 413). On the other hand some authors showed that pyridoxine antagonized the convulsant effect of INH in the dog (545) and decreased its toxicity in mice and man (419). Plp or pyridoxal actually accelerates the appearance of INH seizures and produces convulsions in animals given subconvulsive doses of INH (see section V D, 2) (32, 107, 375).

1. Hydrazides and GABA metabolism. Since Plp is also the coenzyme of glutamic acid decarboxylase, it could be expected that hydrazides would affect cerebral glutamic acid metabolism, particularly the formation of GABA. According to Killam (262, 264), convulsive doses of hydrazide lower GABA content of the brain through inhibition of glutamic acid decarboxylase. This has been confirmed for a variety of hydrazides (32, 43, 139, 150, 263, 333, 375, 427, 428, 544). Roberts *et al.* (432) could show an inhibition of the decarboxylation of intracisternally injected C^{14}-L-glutamic acid to GABA after thiosemicarbazide in rats. This suggested that hydrazide-induced seizures were caused by a diminished cerebral GABA content. However, other investigators showed that the causal relationship between glutamate-GABA metabolism in brain and the occurrence of convulsions is more complicated. In mice, 1) the GABA content of the brain remains unchanged after convulsive doses of thiosemicarbazide; 2) the simultaneous injection of thiosemicarbazide and a convulsion-preventing dose of pyridoxine significantly lowers the GABA content of the brain; and 3) the GABA content of the brain, lowered by convulsive doses of other hydrazides, is still more lowered by the simultaneous injection of a convulsion-preventing dose of pyridoxine (32). Obviously, pyridoxine in protective doses activates not only the decarboxylation of glutamic acid, with a formation of GABA and an increase in the GABA content of the brain, but also, and to a greater extent, the transamination between GABA and α-ketoglutaric acid, leading to the disappearance of GABA and thus to a decrease in the GABA content of the brain (see fig. 2). Thus, it appears that it is not the cerebral GABA content as such that is of decisive importance for the susceptibility of cerebral motor centers to convulsive stimuli but the rate of the turnover in the glutamate-GABA shunt (32).

Confirmation of these findings is given by experiments of Rindi and Ferrari (425), in which pyridoxamine could prevent toxopyrimidine-induced convulsions but not the accompanying fall in the GABA content. Moreover, experiments of

Baxter and Roberts (45), as well as Maynert and Kaji (333), showed that pyridoxine or pyridoxal prevented the convulsions induced by semicarbazide and thiosemicarbazide, but not the fall in cerebral GABA.

The lack of a causal relationship between the cerebral GABA content and seizure threshold is also supported by the fact that mouse strains susceptible to spontaneous seizures had approximately a 50% higher cerebral GABA content than other nonsusceptible strains (371). On the other hand, in susceptible strains the Plp content decreased more rapidly in dietary B_6 deficiency (314). Also, in cats there has been found no correlation between GABA content and electroshock thresholds in various cortical areas. The hippocampus, which has a particularly low seizure threshold (convulsions can be induced purely through mechanical excitation), has an especially high GABA content (46).

That the activity of GAD alone does not determine the appearance of convulsions, is shown in that various hydrazide-induced convulsions appear at completely different levels of GAD activity, that is, at different degrees of inhibition. After equipotent convulsive doses, the enzyme activity of mouse brain was inhibited only by 23 and 43% with thiocarbohydrazide or thiosemicarbazide, respectively, while after carbohydrazide, semicarbazide, or INH, it was inhibited by 65 to 70% (32). From experiments by Killam (262) with thiosemicarbazide in the cat, on the other hand, it may be concluded that there exists a parallel between the degree of GAD inhibition and the appearance of electroencephalographic seizure potentials. Incubation of the hydrazides with Plp showed, in agreement with the affinity constants reported by Wiegand (538), that semicarbazide, for instance, has a greater affinity to the coenzyme than the sulfur-containing compound, although the latter possessed a much higher convulsive potency (32). This is perhaps explained by the greater lipid solubility of the thio- derivatives.

The crucial experiment to support the hypothesis that a depression of the GABA turnover rather than a lowering in the GABA content of the brain is the cause of hydrazide convulsions, would be to inhibit GABA-T specifically or at least to a higher degree than GAD, thereby increasing the cerebral GABA content, and still produce convulsions. Hydroxylamine *in vivo* inhibits the transaminase more strongly than the decarboxylase. GABA-T inhibited *in vitro* by hydroxylamine cannot be reactivated as readily as GAD. This probably results from the higher affinity of Plp to the transaminase apoenzyme; therefore the dissociation of inactivated coenzyme (pyridoxal-phosphate-oxime) from the apoenzyme, and its replacement by Plp would be slower than in the case of GAD (44, 45). In rats, the administration of hydroxylamine results in convulsions shortly after the injection, while 1½ hours later the cerebral GABA content is raised to 180% of normal (41, 42, 137). This action is extraordinarily species- and strain-dependent. In Wistar rats, the rise in GABA amounts to only 38%, in Sprague-Dawley rats to 9%; in Royal-Hart rats and mice there is no increase at all (162, 494). The increased GABA content and the inhibition of GABA-T caused by hydroxylamine is associated with an increase in the seizure threshold for electroshock and Metrazol, but not for thiosemicarbazide (42, 137). This hydrazide led to convulsions in rats, even if the GABA content remained normal

or was raised by the simultaneous injection of hydroxylamine (42). Similar results were obtained in mice with diacetylmonoxime; thiosemicarbazide convulsions could not be prevented despite a normal or even augmented GABA content of the brain (162). A dose of 100 mg of hydrazine hydrochloride per kg raised the GABA content of mouse brain by 300% after 12 hours, but did not protect the animals against Metrazol- and semicarbazide-provoked convulsions (333).

A very active inhibitor of GABA-T is β-amino-oxyacetic acid (NH_2OCH_2—COOH), which causes vomiting, sedation, and muscular paralysis in dogs and cats at low doses, while higher doses result in convulsions which are correlated with a strong elevation of cerebral GABA levels (44, 521). DaVanzo *et al.* (118) found that in mice, rats, and cats, amino-oxyacetic acid exerts a certain protective action against methionine-sulfoximine and semicarbazide convulsions, which is maximal 1 hour after the administration of amino-oxyacetic acid. After 8 hours, however (when the GABA levels were maximally increased), this protective effect had disappeared.

According to Massieu *et al.* (328), another hydrazide, L-glutamic acid γ-hydrazide, inhibits GAD more strongly than GABA-T, *in vitro*. However, it elicits convulsions, although the cerebral GABA content increases to 6 to 10 times the normal. Also α,γ-diaminobutyric acid, which inhibits GAD but not GABA-T *in vitro*, led to fatal convulsions with an increase of brain GABA by 200 to 300% after a latency of 24 hours. It has been suggested that diaminobutyric acid is transformed in the body to an inhibitor of GABA-T (258, 344).

It appears that there is a relationship between hydrazide-induced seizures and a lack of cerebral Plp, the coenzyme of GAD and GABA-T. Hydrazides inactivate the coenzyme by hydrazone formation and impair its synthesis by inhibition of phosphokinase, the enzyme necessary for the phosphorylation of pyridoxine and pyridoxal. As found by Snell and co-workers (336, 337, 338), hydrazones as well as oximes of pyridoxal are potent inhibitors of phosphokinase. Indeed, after the administration of hydrazides the corresponding hydrazones of pyridoxal and Plp have been detected in brain and urine (28, 543, 544). In agreement with this, Bain and Williams (28, 544) found that after INH, deoxypyridoxine, or toxopyrimidine the cerebral Plp content was diminished whereas the pyridoxal and pyridoxine concentrations were increased, possibly due to an inhibition of phosphokinase. Decreased coenzyme levels in brain were found also if seizures were suppressed by high doses of phenobarbital (28). Also the convulsant action of structural analogues of vitamin B_6, such as 4-deoxypyridoxine, toxopyrimidine, and others is due to a competitive displacement of Plp from the apoenzyme as well as to an inhibition of phosphokinase and possibly of pyridoxine oxidase (338, 519).

It seems reasonably safe to conclude from what has been stated that B_6 deficiency can be the cause of convulsions. However, the causal connection between the occurrence of seizures and alterations in the metabolism of GABA remains obscure. Although a decrease in brain GABA may result in a lack of a cerebral inhibitor, thereby lowering the seizure threshold, the GABA content of the brain *per se* is not the decisive factor, because convulsions have been found to occur with decreased as well as with increased concentration of brain GABA.

Therefore, it appears doubtful that the role of GABA as a central synaptic inhibitor is of importance for the excitability of motor centers.

The main point could be that a decrease of the GABA content by inhibition of GAD as well as an increase of the GABA concentration by inhibition of GABA-T reduces the turnover of the brain specific glutamate-GABA shunt, which participates to about 40% in the oxidative metabolism of the brain. From this it follows that under all circumstances B_6 deficiency will lead to a diminished oxygen consumption in the brain. This has indeed been observed in kittens with a dietary B_6 deficiency (345) as well as in brain slices of rats pretreated with hydrazides. In both instances, the GABA content was decreased. On the other hand, the administration of α,γ-diaminobutyric acid to rats, causing an increase of the cerebral GABA concentration, nevertheless produced a diminished oxygen consumption (344). It has previously been mentioned that a decreased oxygen consumption of the brain was observed in a "B_6-dependent" patient (480). Seizures due to B_6 deficiency can be blocked only by vitamin B_6, but not by the administration of GABA (258, 408) or glucose (251). This again proves that the decreased oxygen consumption and the seizures are not caused by a lack of substrate but rather by a deficiency of the enzymatic catalyst of the brain turnover in the glutamate-GABA metabolism.

2. INH convulsions. The convulsive action of INH in animals was first described by Benson *et al.* (54) and by Rubin *et al.* (441). INH is frequently used in suicide attempts: 200 mg/kg are said to be the absolute convulsive dose which leads eventually to death by respiratory paralysis (288, 292). While INH convulsions, as already mentioned, cannot be suppressed by pyridoxine, compounds with a carbonyl group, *e.g.*, pyruvic acid, are reported to exert a protective action, just as α-ketoglutarate and pyruvate antagonize semicarbazide convulsions (39, 251, 465). This protection is probably due to the formation of hydrazones, occurring already outside the brain. The hydrazone formed from INH and pyruvate has been detected as a urinary excretion product (244).

The failure of pyridoxine or pyridoxal to protect against INH convulsions in the experiments of Quadbeck and Sartori (413) could be caused by a too low dosage of Plp, since pyridoxal was given only in one-fourth of the convulsive INH dose. A part of the circulating Plp may be inactivated by INH with hydrazone formation already in the peripheral blood, so that too little free coenzyme reaches the brain to prevent the convulsive action of INH. However, even when a dose (750 mg/kg) is injected which is twice the convulsive INH dose (200 mg/kg) on a molar base, convulsions occur in mice. Indeed, they occur earlier than after INH administration alone. Subconvulsive doses of INH together with Plp cause seizures in all animals (32). Plp appears to accelerate the penetration of INH into the brain. The convulsion-promoting action of pyridoxal phosphate occurs only with INH—not, for example, with semicarbazide (32). On the other hand Dixon and Williams (130a) have shown that the hydrazones of pyridoxal and Plp with INH and other hydrazides are 10 to 100 times more potent as convulsants than the parent hydrazides upon intracerebral injection. However, pyridoxal and Plp are by themselves convulsant upon intracerebral injection (32, 285).

INH, acting as a hydrazine, can inhibit B_6-dependent reactions, and, as a pyridine can also inhibit those catalyzed by NAD (diphosphopyridine nucleotide) (256). Thus, its convulsive action could be caused by an interference with the energy-delivering reactions of glycolysis and of the Krebs cycle, which are necessary for the synthesis of energy-rich phosphate compounds. It is known that anoxemia and glucose deprivation can lead to convulsions. In favor of this assumption is the fact that the simultaneous injection of 400 mg/kg nicotinamide (two times the molar convulsive dose of INH) protects 50% of the animals from the convulsive action of INH (ED100 = 200 mg/kg) (32). Indeed, the INH analogue of NAD has been isolated from liver and brain homogenates after incubation with INH (256). It is of interest that C^{14}-labeled INH accumulated in the hippocampus of cat brains (38), and that another analogue of nicotinamide, 3-acetylpyridine, which also produces convulsions and can be incorporated by nucleotidase into the NAD molecule, produced histological lesions in the region of the hippocampus and hypothalamus (82a, 256).

3. INH neuritis. Although B_6 cannot protect against the central effects of INH intoxication (convulsions, psychoses, ataxia, and somnolence) it has a protective action against INH-induced peripheral neuropathies (440). The toxic neuropathy resulting from the therapeutic application of INH in tuberculosis is characterized by sensory disturbances and pareses (for review see 292). According to Biehl and Vilter (61) the frequency with which the neuritic symptoms appear is dose-dependent: 10% with 6 to 10 mg/kg daily; 20% with 15 mg/kg; 40% with more than 20 mg/kg daily. Histopathologically, the neurotoxic action of INH manifests itself in a primary degeneration of the myelin sheath with myelin fragmentation and formation of droplets of neutral fat. Inflammatory reactions are not seen. The distal portions of the spinal nerves are most strongly affected, especially in the region of the sciatic plexus. Later the spinal ganglia and cranial nerves are also damaged (268, 269, 270, 289, 564, 565, 566). Similar injury can be produced by the local application of INH to the exposed sciatic plexus of the rat (268).

While some authors believe that high doses of nicotinamide have a curative effect in INH neuritis, others deny any therapeutic effect (for review see 26). In experimentally induced INH neuritis in the rat nicotinamide had only a slight protective action (566). On the other hand, there exist numerous clinical and experimental observations which indicate a causal relationship between INH neuritis and B_6 deficiency. Vilter *et al.* (61, 510) concluded from the similarity between the neuritic effects resulting from high doses of deoxypyridoxine (hot feet, electric foot syndrome, hyporeflexia, weakened vibration perception, *etc.*) and those produced by INH that both were caused by B_6 deficiency. In monkeys dietary B_6 deficiency or deoxypyridoxine leads to swelling of the Betz cells of the motor cortex, and to a lesser degree of the small pyramidal cells, and to eccentric displacement of the nucleus and loss of Nissl substance (508). In peripheral nerves, there is degeneration of the myelin sheath. After deoxypyridoxine, the axon cylinder is also affected, but basal ganglia, cerebellum, and spinal cord are not. Klinghardt observed marked loss of medullary substance in

the exposed sciatic plexus of the rat after local application of a deoxypyridoxine depot (268).

INH-treated patients excreted more B_6 than normal, probably in the form of the hydrazone. Pyridoxic acid excretion was unaltered. Following tryptophan loading, more xanthurenic acid was sometimes excreted. Medication with pyridoxine resulted in the disappearance of the neuritis and of the increased xanthurenic acid excretion (61, 158, 292, 404). B_6 derivatives had a protective effect against INH neuritis also in rats (564, 565, 566).

In many tuberculous patients, the urinary output of xanthurenic acid was already increased before the INH treatment (26). An increased protein catabolism in tuberculosis appears to be the basic disturbance which in turn leads to a latent B_6 deficiency and in this way becomes the pacemaker of the toxic action of INH (26). Although numerous investigations indicate a B_6 deficiency as the cause of INH neuritis, the absence of skin lesions (acrodynia, *etc.*) speaks against this hypothesis. Klinghardt (268, 269) has discussed the possibility of a disturbance in lipid metabolism through coupling of the hydrazine group of INH with fatty acid aldehydes, particularly those of plasmalogens. Although it is known that Plp is the coenzyme for the synthesis of sphingosine, a constituent of sphingomyelin, it has to our knowledge not been considered that a disturbance of this enzymatic reaction through INH could be a pathogenic factor in INH neuritis (see section I, F).

4. Isopropylhydrazine convulsions. As already mentioned, the convulsive dose (ED100) of isopropylhydrazine (IPH) is particularly high (3.64 mmol/kg intramuscularly). The reason could be that this hydrazine—probably the active group of the monoamine oxidase (MAO) inhibitor iproniazid—is by itself a potent inhibitor of this enzyme (399). Blockade of brain MAO, however, increases brain amines (dopamine, norepinephrine, serotonin) and hence presumably elevates seizure threshold. Thus, by elevating the amine content in brain, IPH might antagonize to a certain degree its own convulsive action. In favor of this assumption is the fact that after pretreatment of mice with iproniazid, which is a MAO inhibitor only, L-dopa suppressed the tonic phase of hydrazide-induced seizures (32).

5. Hydrazides and amine metabolism. Whereas hydrazides, potent inhibitors of amino acid decarboxylases *in vitro*, lower the cerebral GABA content by inhibition of GAD *in vivo*, it appears that decarboxylation of aromatic amino acids, *e.g.*, dopa and 5-HTP, is little influenced as indicated by a normal dopamine and serotonin content of the brain even after convulsive doses of hydrazides. Thus, in mice the serotonin content of the brain remains nearly unaltered after administration of 250 mg of semicarbazide per kg (525) a dose which definitely lowers the cerebral GABA content. The same is true for convulsive doses of INH (399). Also dietary B_6 deficiency which diminishes GAD activity and hence the GABA content of brain (345, 428) leaves decarboxylation of aromatic amino acids (L-dopa, 5-HTP) unimpaired so that normal dopamine and norepinephrine levels in brain are maintained (483, 535). In B_6-deficient chickens with a normal cerebral dopamine content, however, the brain serotonin is decreased, a finding that indicates a higher turnover of this amine (525).

Even the most potent decarboxylase inhibitors, such as the recently developed hydrazine and hydroxylamine derivates, *e.g.*, NSD 1024, 1034, and 1035, even in high doses, lowered the endogenous amine content of the brain only a little (79, 84). Obviously the remaining decarboxylase activity is sufficient to maintain the very low normal level of amines.

After phenelzine (phenylethylhydrazine), which inhibits the decarboxylation of injected 5-HTP in mice to 50%, the serotonin content of the brain is even increased, since this hydrazine inhibits MAO more strongly than 5-HTP decarboxylation (133). In hydrazides with the hydrazine group substituted, *e.g.*, iproniazid or nialamide, the inhibitory activity on B_6-dependent enzymes is still more reduced as compared to their strong MAO-inhibiting action (for review see Pletscher, 400).

VI. VITAMIN B_6 AND THE PARKINSON SYNDROME

Relationships between vitamin B_6 and the Parkinson syndrome in man result from the fact that practically the total cerebral dopamine content, formed by B_6-dependent decarboxylation of dopa, is localized in the caudate nucleus, putamen and globus pallidum (see section IA, 1) (58, 87, 446). The suspicion that dopamine is of importance for the function of the extrapyramidal system is supported by the finding that the dopamine content of the caudate nucleus and putamen was strongly reduced in Parkinsonian patients at autopsy (57, 136, 233). Parkinsonians also excrete less dopamine and 5-hydroxyindoleacetic acid in the urine than do normal controls (34, 35, 36). Thus, it is understandable that all measures which tend to increase the dopamine content of the brain have been shown to be therapeutically active, in that they relieve, at least temporarily, the cardinal symptoms of the disease: rigor, tremor, and akinesis. Particularly useful is the administration of dopa, which is decarboxylated in brain and causes an increase in cerebral dopamine. Simultaneous treatment with B_6 and monoamine oxidase inhibitors may even produce a stronger elevation of the endogenous dopamine levels in the brain (37, 50, 57, 64, 200). Thus the observation in 1929 by Behringer and Wilmanns (50) of a therapeutic effect of harmine can perhaps be explained by the fact, as we know today, that harmine is a reversible inhibitor of monoamine oxidase (for review see 400).

Vitamin B_6 (50 to 100 mg/day) was first applied to the treatment of Parkinsonism with success in the 1940's (253). It appears that better remissions have been obtained with higher doses (600 to 1400 mg/day) (29, 152, 188, 200, 442). Recently, Hartmann-von Monakow (200) has come to the conclusion that vitamin B_6 is indicated in all cases of Parkinsonism not resulting from arteriosclerosis. This therapy is particularly useful in the postoperative treatment of patients in whom the globus pallidum has been stereotaxically electrocoagulated. In these cases, the B_6 medication acts to resolve the walking and speech disturbances and the loss of motivation and memory that occur after the operation. The fact that high doses of reserpine (cerebral amine depletion) may produce Parkinsonian symptoms (*e.g.*, akinesis, rigor, and catalepsy) which are relieved by the administration of dopa and monoamine oxidase inhibitors in animals

and man (89, 124, 341), also points to a causal relationship between cerebral dopamine and extrapyramidal function.

Against Parkinsonian symptoms brought about by administration of phenothiazine derivatives, dopa plus B_6 was only slightly active or almost inactive (341). Unlike reserpine, the phenothiazines do not produce amine depletion, but are assumed to block not only peripheral but also central adrenergic effects. Phenothiazine derivatives which contain a piperazine moiety (*e.g.*, trifluperazine, prochlorperazine, perphenazine, thiopropazate and thioproperazine) and (unlike chlorpromazine, promethazine, and others) have only a weak antihistaminic action, most frequently cause such extrapyramidal side effects. In this connection, it is of interest that bulbocapnine, which induces a catatonic state in man and animals, raises the histamine content of the CNS 4-fold in the albino rat (93), presumably by inhibition of histaminase through interaction with its coenzyme Plp (92). The catatonic state as well as the extrapyramidal effects of phenothiazines with weak antihistaminic action, as mentioned above, can be hindered or abolished by strong antihistaminic agents such as diphenhydramine (341).

These results could indicate that the antagonism between adrenergic and histaminergic actions, long recognized in the periphery, as for example on smooth muscle, is perhaps of importance in definite functions of the CNS. In agreement with this would be the fact that anticholinergic drugs such as atropine, scopolamine, Parpanite (caramiphen) and Artane (trihexphenidyl·HCl), have been widely used with success in the treatment of human Parkinsonism for a long time. An absolute dopamine deficiency in the key areas of the extrapyramidal system (reserpine, human Parkinsonism) or a "functional" dopamine deficiency (phenothiazine derivatives) appears to result in an preponderance of cholinergic and histaminergic mechanisms over adrenergic ones leading to the appearance of Parkinsonian symptoms. Recent investigations indicate a further possibility. Like bulbocapnine, papaverine and other opium alkaloids of the isoquinoline type (laudanine, laudanosine), as well as mescaline, can produce a hypokinetic rigidity (145). These alkaloids have in common one, two or—in the case of mescaline—three methoxy groups. According to Ernst (145), the subcutaneous injection of 4-methoxytyramine or 3,4-dimethoxydopamine provokes a striking syndrome of hypokinetic rigidity and a catatonic state in cats, sometimes preceded by tremors. Therefore a disturbance of the methyltransferase system, discovered by Axelrod, resulting in an O-methylation of dopamine in the *para* position with or without O-methylation in the *meta* position, might create such a hypokinetic rigidity also in human Parkinsonism.

VII. VITAMIN B_6 AND ACTIVE TRANSPORT OF AMINO ACIDS

A great number of experiments indicates that vitamin B_6 not only is of importance as a coenzyme for the intermediary metabolism of amino acids, their synthesis and degradation, but also is necessary for the intracellular uptake and accumulation of amino acids by an active, energy-requiring transport (99, 210, 540). The well-known fact that tissue extracts can have a 5- to 10-fold higher

amino acid concentration than the blood plasma indicates the possibility of amino acid uptake against a concentration gradient (505a).

Vitamin B_6 also appears to be necessary for a normal absorption of amino acids by the intestine (99). Thus, less L-alanine was absorbed from the isolated, perfused intestine of guinea pigs pretreated with deoxypyridoxine, as compared with the intestine of control animals (159). Jacobs *et al.* (246, 247, 248) found that pyridoxine increased the absorption of methionine in normal rats (intestine perfused *in situ*), and restored to normal the diminished absorption in B_6-deficient animals (deoxypyridoxine or dietary). L-Penicillamine inhibited the absorption of histidine; again pyridoxine was able to abolish this inhibition (5, 502). In B_6 deficiency also the incorporation of amino acids into protein is inhibited. For instance, enrichment of radioactive phenylalanine in the tissues after injection of C^{14}-phenylalanine into B_6-deficient rats was substantially less than in controls (361). That the underlying mechanism was an inhibition of the incorporation of the amino acid into tissue protein is indicated by the fact that after longer experimental periods the specific activity incorporated into protein remained far below normal, despite a high radioactivity in the protein-free supernatant (361).

A "model amino acid"—α-aminoisobutyric acid—which does not naturally occur in the organism and is not metabolized, but is governed by the same laws of active transport as the natural amino acids, is particularly suitable for such experiments. Thus, if one injects C^{14}-α-aminoisobutyric acid (AIB) into B_6-deficient rats, the serum level of AIB is about 40% higher than in the controls over a long period of time, while the concentration in heart, skeletal muscle, intestine, and kidney is significantly lower (376, 424).

A "membrane" (cell barrier) of particular pharmacological interest is the blood-brain barrier. B_6 appears to be of importance for its permeability too. *Gamma*-aminobutyric acid (GABA) does not penetrate from the blood into the brain in normal adult animals (171, 468, 500). The GABA content of the brain remained unchanged after i.v. injection of 200 mg/kg GABA to rats, but increased almost 200% if the animals were pretreated with the B_6 antimetabolite 4-methoxymethyl-pyridoxine (165). It is an open question whether this increase in the permeability of the blood-brain barrier is due to a damage to the cell structures or to the convulsions induced by the antimetabolite (408).

Experiments on Ehrlich ascites tumor cells *in vitro* have elucidated the mechanism by which vitamin B_6 influences the active transport of amino acids. One of the most important observations was that pyridoxal or Plp, but not pyridoxine or pyridoxamine, stimulates the uptake of glycine and α,γ-diaminobutyric acid by ascites tumor cells (423). Such cells from B_6-deficient animals were clearly less able to accumulate glycine. The uptake could be restored to normal by the addition of pyridoxal. Deoxypyridoxine inhibited the uptake (101).

Oxender and Christensen (387) found a stimulating action of pyridoxal on amino acid transport across a model of an epithelial membrane, a layer of ascites cells, 4 to 5 cells thick, sucked onto a millipore filter. If pyridoxal was added to one side of the membrane after the equilibration of glycine on both sides, there occurred an enrichment of the glycine concentration on the other side. The reac-

tion underlying this stimulatory action of pyridoxal is possibly the same by which it catalyzes enzymatic reactions, namely the formation of Schiff's bases between amino acids and pyridoxal. It is known that pyridoxal can form chelates with cations like Na^+, K^+, Li^+, Ca^{++}, Mg^{++}, or Mn^{++}. Since the stimulated uptake of amino acids produced by pyridoxal is accompanied by an enhanced potassium efflux, the hypothesis was put forward that there is a combined transport of all three components in the form of a chelate (98, 100, 389).

The fact that carbonyl reagents (hydroxylamine, semicarbazide) do not influence the stimulating action of pyridoxal on the cellular uptake of amino acids appears to be an argument against the formation of a Schiff's base as the primary reaction. Difficult to reconcile with a carrier function of pyridoxal is the fact that the uptake of pyridoxal into cells occurs rapidly, but is soon arrested, while its stimulatory action on amino acid uptake continues for a long time thereafter (387, 388, 390). The amino acid influx is therefore independent of the pyridoxal influx. Plp stimulates amino acid transport but weakly. It is taken up by the cell only in very small amounts, because it is probably fixed on the cell surface (390). Hence, it has been discussed recently whether the action of pyridoxal in promoting cellular accumulation of amino acids could be the result of an inhibition of amino acid efflux (210, 211, 217, 218, 388).

VIII. VITAMIN B_6 AND TUMOR GROWTH

Since B_6 in its phosphorylated form is the coenzyme in numerous enzymatic reactions concerned with the synthesis and degradation of amino acids and proteins, the prerequisite for growth, it was to be expected that this vitamin would be of importance for tissue growth and that a deficiency would lead to inhibition of growth, benign as well as malignant.

Dietary B_6 deficiency inhibits the growth of chemically induced as well as implanted tumors. Treatment with *p*-dimethylaminoazobenzene leads to the formation of hepatomas in normal rats but not in animals with a B_6 deficiency (359). With normal intake of B_6, the expansion of the liver tumors was maximal (559). The carcinogenic action of methylcholanthrene in rabbits was also diminished in B_6 deficiency (267). Implants of sarcoma 180 (65), as well as Flexner-Jobling carcinoma, Yale adenocarcinoma, or mouse fibrosarcoma did not take in B_6-deficient rats (267). Growth of "sarcoma 180 solid" in mice was inhibited only when the animals were placed on a B_6-deficient diet 2 weeks before implantation of the tumor (355). In the controls, there was rapid tumor growth which led to death in 5 weeks. In the B_6-deficient animals, there was an initial growth of the implant which ceased after 2 weeks. The tumor was completely reabsorbed and disappeared. All B_6-deficient animals survived. If they were then placed on a B_6-containing diet the tumors did not reappear.

That B_6-deficient diets inhibit the growth of "sarcoma 180 solid" is of particular interest because this tumor is resistant to most antimetabolites such as antivitamins, and folic acid, pyrimidine, or amino acid antimetabolites, with the exception of 6-mercaptopurine (357). On the other hand, B_6-deficient diets had no effect on the survival time of experimental animals with various ascites tumors, adenocarcinoma 755, and Ehrlich carcinoma (solid), although a clear inhibition

of growth did occur. In Ridgway osteogenic sarcoma and Walker carcinosarcoma, B_6-deficient diets had no detectable effect (357). It is possible to develop a subline of sarcoma 180 solid by passing the tumor 20 times through B_6-deficient animals; it then shows unrestrained growth in B_6-deficient animals. When both the normal and subline tumors are implanted in B_6-deficient animals, the subline tumor takes, but the normal tumor does not (356).

B_6 antimetabolites are also able to inhibit tumor growth. Weir and Morningstar (524) reported marked remission of lymphatic leukemia in a patient after deoxypyridoxine treatment. However, Gellhorn and Jones (172) observed no therapeutic effect with deoxypyridoxine and B_6-deficient diets in patients with lymphosarcoma or acute leukemia. In animal experiments the therapeutic action of B_6 deficiency can be shown (357).

In rats the Murphy lymphosarcoma was completely reabsorbed after deoxypyridoxine or methoxypyridoxine treatment (489). Deoxypyridoxine suppressed the ability of fibrosarcoma transplants to take without producing evidences of B_6 deficiency (309). Hydrazides, such as INH (155), can also inhibit tumor growth. Deoxypyridoxine potentiates the INH action (167). Mouse ascites tumor cells are damaged as soon as 9 hours after a single intraperitoneal injection of INH (466). Also hydrazones, such as pyridoxal-methyl- and pyridoxal-dimethylhydrazone, act as cytostatic agents against sarcoma 180. The isonicotinylhydrazone of pyridoxal was also bacteriostatic against various anaerobes. The hydrazones of pyridoxal and semicarbazide, as well as those of Plp with INH and thiosemicarbazide, proved to be inactive. The same was true of the dimethylhydrazone of Plp (541, 542). Combined therapy (dietary deficiency, B_6 antimetabolites, hydrazides) can have stronger therapeutic effects as compared with the action of the single agents. This was shown by Brockman *et al.* (78) with sarcoma 180. The experiment involved the simultaneous administration of deoxypyridoxine and a hydrazide, *e.g.*, benzoylhydrazide, *p*-aminosalicylic acid hydrazide or cyanoaceto-hydrazide, together with a B_6-deficient diet. The strongest inhibition was seen with the combination of B_6-deficient diet, hydrazide and deoxypyridoxine-5′-phosphate. The phosphorylated deoxypyridoxine plus hydrazide was more active in animals on a normal diet than the nonphosphorylated compound.

In the adenocarcinoma of the mouse the B_6 content of the tumor was lower (0.66 μg/g) than that of the organ with the lowest value, *i.e.*, lung (0.73), spleen (0.88), liver (5.2 μg/g) (323, 463). Similar results were obtained in human tumors regardless of their site of origin (30). The B_6 content of liver mitochondria was reduced to one-fifth of normal after feeding 4-dimethylaminoazobenzene (406). However, the NAD and NADP content of tumor tissue was also abnormally low (250). Thus, various enzyme activities are diminished in the tumor tissue. The activity of the cystine desulfurase system, consisting of the NADH-dependent cystine reductase (374) and the Plp-dependent cysteine desulfhydrase (75), is extraordinarily depressed or completely absent because of a deficiency in the two coenzymes (306, 487). Cysteine desulfhydrase is one of the enzymes most sensitive to B_6 deficiency (497). The activity of 5-HTP decarboxylase was also reduced in rat liver tumors. It decreased as soon as 2 weeks after feeding of the

cancerogenic compound 3,4-dimethylamino azobenzene. The normal liver tissue on the borders of the tumors had a normal enzymatic activity (266).

Just as the decrease in the B_6 content of tumors is not specific, but rather includes other vitamins, particularly those of the B group (for review see 187), the activity of various enzymes whose coenzyme is not Plp, *e.g.*, monoamine oxidase, is decreased in tumor tissue (266). The cause of tumor growth inhibition in B_6 deficiency, however, is not, as one might assume, due to an inhibition of transaminases. Against such a relationship would speak, for instance, the fact that cortisone causes marked growth inhibition of Walker carcinosarcoma and of other tumors in rats, but at the same time activates the glutamate-pyruvate transaminase to 14 times normal values (437). On the other hand, DOCA promotes tumor growth and inhibits transaminases (437).

The inhibition of tumor growth in B_6 deficiency is not necessarily caused by a decrease in the activity of vitamin B_6-dependent enzymes. It can also result from a deficit in growth factors for whose formation B_6 is necessary. Thus, the liver content of pantothenic acid, nicotinamide, and riboflavin (495), as well as NAD (546) and coenzyme A (547), falls in B_6 deficiency. This can be partially explained by an inhibition of synthesis. Indeed, coenzyme A synthesis in liver homogenates from B_6-deficient animals is inhibited and can be restored by the addition of Plp (561). Also, NAD synthesis is impaired since the injection of nicotinamide or nicotinic acid leads to significantly lower increases in NAD values in B_6-deficient animals than in normal animals (546).

Likewise the synthesis of purines, pyrimidines, flavines, and pterines depends on Plp-catalyzed reactions. The carbon and nitrogen atoms of these compounds are chiefly supplied by aspartic acid and glycine (for review see Braunstein, 75). Finally, it may be mentioned that the intestinal absorption of vitamin B_{12} is diminished in B_6 deficiency so that serum and liver concentrations of B_{12} are lowered (234, 416).

IX. VITAMIN B_6 AND HEMATOPOIESIS

Anemia can be a result of B_6 deficiency (117, 156, 334, 347, 401, 490, 552) in animals. In B_6-deficient rats blood regeneration following hemorrhage is retarded (464). Snyderman *et al.* (479) reported two children with micro- and hydrocephaly, in whom convulsions and anemia appeared during a B_6-deficient dietary regime.

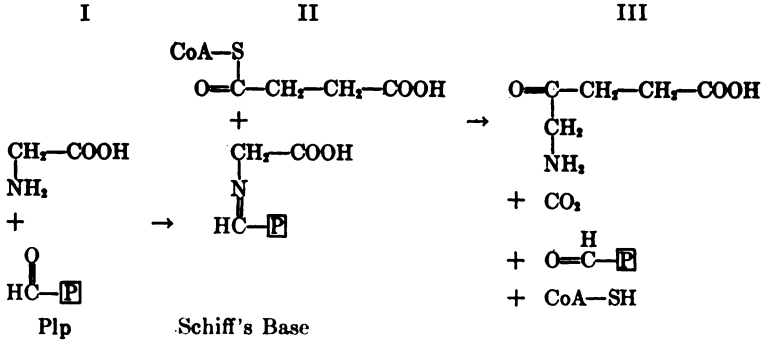
Genuine B_6-deficiency anemia in man was first observed by Harris *et al.* in 1956 (198). This rare clinical syndrome—only 17 cases have been described (122, 168)—agrees hematologically with the experimentally induced anemia in B_6-deficient animals: a microcytic, hypochromic anemia with an accompanying, strongly elevated serum iron level. The hematological picture in the human cases is similar to that of sidero-achrestic anemia (209), a disease of the erythropoietic system, sometimes hereditary, but usually acquired, appearing in older people, associated with a defect in the incorporation of iron into the hemoglobin molecule. The B_6 anemia is characterized by the appearance of sideroblasts and erythroblasts in bone marrow, high serum iron level, and liver hemosiderosis.

Many cases respond specifically to pyridoxine. Fe, Co, vitamin B_{12}, folic acid,

liver extract, ACTH, and corticoids, as well as androgens, are usually ineffective (122, 168, 170, 282). Blood transfusions have only a short-lasting therapeutic effect. Pyridoxine sometimes causes a drastic reticulocytosis as well as a progressive increase in the erythrocyte count and hemoglobin content. At the same time there is a disappearance of pathological cell forms and a fall in the serum iron concentration (170, 282). Pyridoxine alone is not always sufficient for a complete restitution of the hematological disturbance. Liver extracts, yeast extracts, and testosterone can act as adjuvants (122, 168, 231). There seems to be a familial frequency in this B_6-dependent anemia which occurs chiefly in mature individuals (65a). A relationship to Blackfan-Diamond syndrome has been suggested (9, 168).

Recently Kohn *et al.* (282) observed a fulminating anemia after INH treatment superimposed upon an already present mild hypochromic anemia. The serum transaminase activity, taken as a criterion of B_6 deficiency, was reduced to zero. Withdrawal of INH and the institution of B_6 therapy led to a reticulocytosis, increase in hemoglobin, a fall in the serum iron to subnormal values and an increase in the serum transaminase titers. The frequency of the occurrence of anemia during INH treatment deviates extraordinarily between 4 and 34% (354).

Essentially, a defect in heme formation is responsible for the appearance of the hypochromic anemia seen in B_6 deficiency, since B_6 is necessary for the synthesis of protoporphyrins on the one hand and probably for the incorporation of iron on the other. Plp is necessary as a coenzyme for δ-aminolevulinic acid synthetase (see section I F), which condenses glycine (I) and succinate (succinyl-Co A, II) to form δ-aminolevulinic acid (ALA, III). Two molecules of ALA further react by way of porphobilinogen and coproporphyrin to form the iron-free protoporphyrin.



Cartwright and Wintrobe (90), and numerous later authors (175, 176, 261, 373) assumed that the cause of B_6-deficiency anemia in pigs was the result of a disturbed protoporphyrin synthesis because they found that the protoporphyrin content of the erythrocytes was lowered. One can indeed show that heme formation from glycine and succinate in erythrocytes from B_6-deficient animals is inhibited and can be reactivated *in vitro* by the addition of Plp (457). Carbonyl reagents such as D-penicillamine, cysteine, and cyanide also inhibit the ALA-synthesis from glycine and succinate (175). However, a reduced protoporphyrin

content in the erythrocytes of the human anemia is not regularly found (90); the concentration of protoporphyrin was usually normal or even raised (168, 282). The same was true for the excretion of ALA in the urine: it could be normal (168) or even elevated (507).

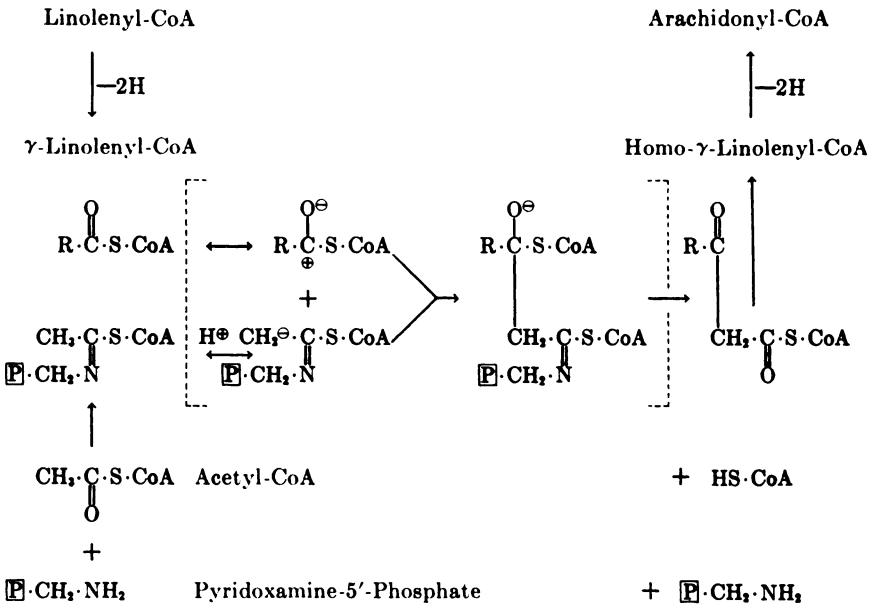
There are other reasons why it is unlikely that the only cause of human B_6-deficiency anemia is a disturbance of protoporphyrin synthesis. Heilmeyer and Clotten (208) could not restore the reduced heme synthesis in erythrocytes by the addition of ALA. In ducks the anemia in B_6 deficiency was not prevented by the injection of ALA (457). The explanation may be that B_6, in addition to its coenzyme function in ALA synthesis, is necessary for the incorporation of iron into the protoporphyrin molecule. Gardner and Nathan (168) found that in spite of normal blood concentrations of B_{12} and folic acid, there was a strong inhibition of Fe^{59} incorporation into the heme of erythrocytes in a patient with a pyridoxine-sensitive anemia. Yamada and Ogawa (560) have reported decreased iron incorporation in bone marrow cultures from B_6-deficient animals. This can also be produced by cycloserine, deoxypyridoxine, or INH and is improved by Plp.

Vitamin B_6, which is necessary for growth in animals and microorganisms, appears to be of importance also for the new formation of blood cells. This is indicated by its therapeutic action in toxic leucopenia (for review see 11, 363, 439).

X. VITAMIN B_6 AND METABOLISM OF FATTY ACIDS

In 1936, Birch (62) and György (193) reported that lipids with a high content of polyunsaturated fatty acids—designated as "vitamin F" at that time—were able to reduce acrodynia in vitamin B_6-deficient rats. Later in 1942, Quackenbush *et al.* (412) obtained similar results: linoleic acid and vitamin B_6 had an additive effect on acrodynia in rats. Further light on the mechanism of this action is shed by the finding of Witten and Holman (553) that vitamin B_6 is essential for the conversion of linoleate into the more unsaturated tetraenoic arachidonate. This agrees with the fact that in B_6-deficient rats the serum content of tetraenoic acids is decreased (184), and that supplementation of the diet with peanut oil in B_6-deficient chickens leads to a much more pronounced increase in linoleate than in arachidonate, as compared with control animals (115). The importance of vitamin B_6 for the utilization of linoleate is further documented by the finding that in B_6 deficiency, with or without supplementation with linoleic acid, the content of linoleate particularly in the phospholipid fraction of the liver is increased while concomitantly arachidonate is decreased (12, 450, 491, 548).

The mechanism of the action of vitamin B_6 might possibly be explained by *in vitro* experiments of Wakil (520) using liver mitochondria; pyridoxal- and pyridoxamine-phosphate appeared to be cofactors in the elongation of the fatty acid chain by incorporation of acetyl CoA. Pyridoxamine phosphate has been suggested to form a Schiff's base with acetyl CoA, which may activate the methyl group for the incorporation into the fatty acid molecule as shown in the following scheme adapted from Wakil (520).



It must be emphasized, however, that there are distinct differences in the histological manifestation of changes in the skin due to essential fatty acid deficiency and B_6 deficiency. The edema associated with essential fatty acid deficiency ("Burr-Burr syndrome") is predominantly localized in the ventral trunk skin, but edema caused by B_6 deficiency is confined to the dorsal skin of the hind paws. The tissue content of vitamin B_6 is normal in fatty acid deficiency (for review see Aaes-Jørgensen, 1).

The dermatitis seborrheica sicca often seen in B_6-deficient human beings responded favorably to oral or local therapy with vitamin B_6 as well as to high doses of linoleic acid (510), but the concomitant glossitis and neuritis did not respond to linoleic acid.

The weight loss in B_6 deficiency is essentially due to the mobilization of fat depots (48). In spite of a dietary supplementation with corn oil, B_6-deficient animals were unable to deposit fat, although they could still utilize carbohydrates (in contrast to protein) for the synthesis of fat (48, 127, 464).

The relationship between B_6 deficiency and atherosclerosis is still obscure. Rinehart and Greenberg (426) found in B_6-deficient monkeys vast atherosclerotic lesions in the abdominal aorta, and the iliac, femoral, renal, and coronary arteries, manifested by swelling of the intima with deposition of mucoids, and in more advanced stages by fibrous plaques without deposition of lipids in the media. While these findings were confirmed by Mushett and Emerson (367, 368) in monkeys and dogs, Olson (384) observed in B_6-deficient rats hypertension but no atherosclerotic changes of the vessels. Several authors reported hypercholesterolemia in rats, chickens, and monkeys, if B_6 deficiency was accompanied by atheromatous changes and deposition of cholesterol in the aorta (115, 184, 186). Since, according to Shah *et al.* (462), cholesterol synthesis is not

accelerated in B_6 deficiency, hypercholesterolemia rather seems to be due to an increased mobilization of cholesterol in the liver (184).

The relationship between human atherosclerosis and B_6 deficiency is controversial (for review see 439, 528). A therapeutic effect of vitamin B_6 appears doubtful (439), and only occasionally can an increased urinary excretion of xanthurenic acid be observed in atherosclerotic patients (528), whereas according to Boxer *et al.* (70), the Plp content of the leukocytes is sometimes significantly decreased.

XI. RADIOPROTECTIVE ACTION OF VITAMIN B_6

In 1948, Goldfeder *et al.* (180) reported that pretreatment with pyridoxine prolonged the survival time of mice exposed to 350 r radiation, from 14 days to 100 days. Bridges and Koch and others (77, 280, 297) could not confirm these results with pyridoxine, but found that Plp, injected 15 minutes before the radiation, did protect. On the other hand, radiation damage is not more severe in the B_6-deficient rat, and the B_6 content of the liver is not reduced by radiation (339). Also, radiation sensitivity of mice is not increased by pretreatment with INH or deoxypyridoxine; it is increased by penicillamine (280, 296). In the monkey, radiation caused only a brief increase in the B_6 excretion (383). In the rat after a 525 r radiation dose, there was a strong inhibition of the absorption of pyridoxine from the intestine, which became maximal after 3 days and disappeared after 7 to 17 days (128).

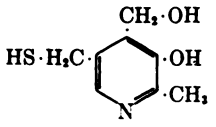
The increased excretion of xanthurenic acid in the rat and mouse could also be indicative of a radiation-induced B_6 deficiency (202, 301, 349). Following radiation, the xanthurenic acid excretion is first diminished for a short time and then increases sharply during the following 7 to 10 days, while the taurine excretion is first increased and later falls to subnormal values. At the same time the content of cysteine sulfinic acid in the spleen is increased, probably due to an inhibition of the B_6-dependent cysteine sulfinic acid decarboxylase (4, 301, 351). Other intermediary products of tryptophan metabolism, such as kynurenine, kynurenic acid, and anthranilic acid, are also excreted in increased amounts. The cause of this is probably a radiation-induced activation of hepatic tryptophan pyrrolase which results in overloading of the capacity of the B_6-dependent kynureninase by the increased amounts of metabolites (496). *In vitro*, the different forms of vitamin B_6 are rapidly destroyed by irradiation (330, 370).

The fact that serotonin and to a lesser extent 5-HTP as well as ATP exert a radioprotective action also could speak for a relationship between B_6 enzymes and radiation damage (297, 298, 300). The relatively weak action of 5-HTP is potentiated to approximately that of serotonin when it is injected along with Plp and ATP 15 minutes before irradiation. It appears that ionizing radiation inhibits decarboxylation of 5-HTP, possibly because of an ATP deficiency (297, 298). Reserpine also has a protective effect when administered 12 hours before irradiation (299, 350).

Vitamin B_6 not only hinders the lethal action of high doses of radiation in animals, but also diminishes the toxic action of sublethal doses in man. Most

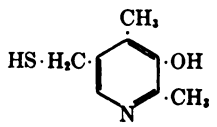
radiologists believe that the clinical symptoms of radiation sickness, *e.g.*, nausea and vomiting, can be favorably influenced by B_6. Maxfield *et al.* (331) could bring about the disappearance of the radiation-induced vomiting and nausea within 30 minutes by the administration of 25 mg of pyridoxine hydrochloride. Similar results have been reported by other authors (173, 196, 385). The usual appearance of nausea during 600 r radiation therapy 5 times a week could be suppressed by the intravenous injection of 25 mg of pyridoxine. Withdrawal of pyridoxine immediately led to the recurrence of nausea and vomiting. The accompanying diarrhea was not influenced by B_6 (459). However, Silverman *et al.* (467) denied any curative effect against deep radiation in a comparison of pyridoxine, Dramamine (dimenhydrinate), and placebo.

The radioprotective action of sulfur-containing B_6 analogues (without antimetabolic effects) reported in the last few years by Langendorff *et al.* (278, 281, 296) must be placed beside the well-known protection exerted by sulfur-containing compounds of the cysteine-cysteinamine group. 2-Methyl-3-hydroxy-4-hydroxymethyl-5-mercaptomethyl-pyridine was active, even when administered as late as 2 hours after irradiation. The protective action is probably less the result of the B_6 skeleton, than of the SH-group found in position 5, as shown by the following formulae:



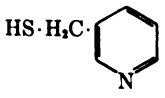
5-Mercaptopyridoxine

active



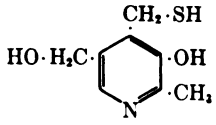
4-Deoxy-5-Mercaptopyridoxine

active



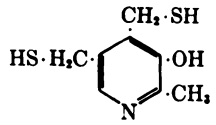
5-Mercaptomethyl-Pyridine

inactive



4-Mercaptopyridoxine

inactive



4,5-Dimercaptopyridoxine

inactive

Although nothing is known about the mechanism of the radioprotective effect of vitamin B_6, particularly of its antiemetic action, a comparison with its therapeutic effect in hyperemesis gravidarum appears feasible (for review see 11, 439). In both cases there is an increase in protein breakdown and transformation with an augmented influx of protein degradation products, such as peptides and amino acids, into the circulation, and on the other hand a relative B_6 deficiency. This may explain why vitamin B_6 has an antiemetic action only in radiation sickness and in pregnancy. Although its use is often recommended in cases of motion sickness, Chinn *et al.* (97) were unable to demonstrate any beneficial effect

of vitamin B_6 in extensive investigations on a great number of persons. Pyridoxine proved to be ineffective also in experimental vomiting provoked in dogs by application of apomorphine (453).

XII. PHARMACOLOGY AND TOXICOLOGY OF VITAMIN B_6 AND ITS DERIVATIVES

As a vitamin, B_6 is active even in small doses in B_6 deficiency. In B_6-saturated organisms, however, effects can be demonstrated only with high doses. It seems doubtful whether these effects are actually due to its vitamin character, *i.e.*, to an alteration of B_6-dependent biochemical reactions (285).

The toxicity (1/LD50) of the three naturally occurring forms of vitamin B_6 (pyridoxine, pyridoxamine, pyridoxal), as well as of pyridoxic acid, is much higher after intravenous than after subcutaneous or oral application. Pyridoxal is 2 to 5 times more toxic than pyridoxine or pyridoxamine (279, 285, 505). Replacement of the hydroxyl groups by sulfhydryl groups increases the toxicity (279, 285), while a still more pronounced increase in toxicity can be achieved by methylating the hydroxymethyl group in position 4 ($R—CH_2—OH \rightarrow R—CH_2—OCH_3$): the product, 4′-methyl-pyridoxine, is an antimetabolite to vitamin B_6 (505).

The toxic effects of high doses of vitamin B_6 are mainly of central origin: impaired postural reflexes, tachypnea, convulsions, and paralyses; in addition, salivation and vomiting occur in cats and pigeons (279, 285, 505). Degeneration of the dorsal columns and the dorsal roots of the spinal cord, of ganglia and sensory nerves were observed postmortem in dogs and cats which had received 2 to 6 g of pyridoxine per day for a long period of time (14). Following the administration of 200 mg of pyridoxine to cats for 20 days Astruc (22) found changes of the adrenals resembling those after nonspecific stress.

It is of interest to note that a number of signs of B_6 hypervitaminosis are similar to those found in B_6 deficiency. This might possibly be explained by the observation that following the administration of large amounts of pyridoxine, pyridoxamine, or pyridoxal in mice, the pyridoxamine- and pyridoxal-phosphate content in the central nervous system was significantly lowered (28).

In cats, 10 mg of pyridoxine had a slight sedative effect if given intracisternally, while 20 mg caused excitation after a transient period of sedation; 3 mg of pyridoxal or 0.3 mg of pyridoxal-phosphate were followed by marked excitation, salivation and short-lasting convulsive seizures. Pyridoxamine caused sedation, whereas pyridoxamine-phosphate had no effect (32, 285). In human beings pyridoxine is said to exhibit sedative actions (484).

In contrast to the centrally excitatory 5-sulfhydryl derivatives of pyridoxine, 5-chloro-pyridoxine causes sleep-like sedation (279). Dipyridoxolyl-5′-disulfide and dipyridoxolyl-4′-disulfide, in contrast to pyridoxine, produced sedation and a prolongation of the spontaneous sleeping time, and caused blepharospasm and miosis (285, 364). Long-term administration of the disulfides and of pyridoxine to rhesus monkeys resulted in a reduction of the spontaneous motor activity and aggressiveness (364).

In Regnier's test with pyridoxine and several of its derivatives (*e.g.*, 4-pyri-

doxic acid, 4-deoxypyridoxine-5′-disulfide, and pyridoxamine-5′-disulfide) a centrally induced analgesia is demonstrable. This seems to be a direct action not related to any coenzyme function, since these compounds, with the exception of pyridoxine itself, have little or no anticonvulsive properties against seizures induced by toxopyrimidine (285).

In isolated organs (ileum, uterus), pyridoxine and its derivatives had no characteristic effects (for review see Unna, 505). Since the chronotropic and inotropic effects on isolated rabbit atria as described by Levine *et al.* (307) are associated with an increased uptake of sodium and a decreased release of potassium, they could perhaps be explained by an alteration of the active transport of ions (see section VII).

Pyridoxine itself has no effect on the blood pressure if given intravenously. However, it potentiated the blood-pressure-raising effect of L-dopa, as shown in cats (31) and in man (124). This effect is due to its function as a coenzyme for dopa decarboxylase.

CONCLUDING REMARKS

In the present contribution we have attempted to demonstrate that vitamin B_6, particularly its catalytically most important form pyridoxal-5′-phosphate (Plp), is a many-sided biocatalyst by virtue of its high chemical reactivity. For the same reason it is particularly susceptible to chemical inactivation. As with other vitamins, a *chronically* insufficient intake of vitamin B_6 results in typical signs of an avitaminosis in man and animals. Moreover, many drugs exert acute effects by inactivation of Plp, thus interfering with B_6-catalyzed biochemical reactions. Therefore, vitamin B_6 has more "pharmacological aspects" than other vitamins.

1. Even a short-lasting functional inactivation of Plp by carbonyl reagents, *e.g.*, semicarbazide, thiocarbohydrazide, and others, leading to the formation of hydrazones by reacting with the aldehyde group of Plp, promptly induces convulsions within minutes after their application, this result indicating a functional avitaminosis of the brain. Convulsive carbonyl reagents, such as hydrazine and hydroxylamine, are widely used industrially, and they may also be found among drugs used in man. Thus, the tuberculostatic agent isonicotinylhydrazide (INH) directly reacts with Plp, whereas cycloserine can do so only after biotransformation to a hydroxylamine derivative. While single application of a high dose of hydrazides provokes acute cerebral convulsions, chronic administration, even in "therapeutic" doses, can lead to a widespread neuritis in the peripheral nervous system. It must be admitted, however, that the intimate mechanism of these actions has not been established clearly despite many efforts. The reasons may be found in the extraordinarily large number of reactions in which Plp serves as coenzyme. In addition, all these reactions are susceptible to inhibition to a highly varying degree, depending upon the differing affinities of the coenzyme to the apoenzymes. Thus, convulsive doses of hydrazides inhibit the brain-specific, Plp-dependent glutamic acid-decarboxylase, thereby leading to a decrease of the γ-aminobutyric acid (GABA) in the brain; at the

same time, however, the activity of the equally Plp-dependent aromatic amino acid decarboxylase is unchanged and the amine content of brain remains normal. On the other hand, there are carbonyl reagents, *e.g.*, hydroxylamine and hydrazine, which induce convulsions, although they cause an elevation of brain GABA. These compounds apparently inhibit the GABA-α-ketoglutaric acid transaminase to a higher degree than the glutamic acid decarboxylase. From this it follows that there is no causal relationship between the GABA content of the brain and the susceptibility of cerebral motor centers to convulsive stimuli; more likely the lowered turnover in the glutamic acid-GABA pathway is the decisive factor because of its importance for the oxidative metabolism of the brain. However, it still remains to be established whether an impairment of the GABA pathway is of importance at all for the induction of convulsions, or whether the detectable biochemical alterations are secondary to an unknown process. In this connection it must be emphasized that vitamin B_6 has a protective action against convulsions due to B_6 deficiency only, but is devoid of any therapeutic effect in the treatment of human epilepsy.

As to the neuritic symptoms caused by long-term administration even of therapeutic doses of hydrazides, *e.g.*, INH, it should be realized that Plp is the coenzyme of the biosynthesis of sphingosine and hence of sphingomyelin, an essential constituent of nervous tissue.

2. Some of the most important B_6-catalyzed reactions are involved in the biosynthesis of amino acids from nitrogen-free precursors, and therefore in the synthesis of protein. B_6 is also involved in the intestinal absorption and in the active transport of amino acids. From this results its importance for growth and cell replacement. Tumor growth can be inhibited by dietary as well as by chemically induced B_6 deficiency. The function of vitamin B_6 in amino acid and protein metabolism might also be the base for its antiemetic action in cases of hyperemesis gravidarum and radiation sickness, since in both instances we are dealing with alterations in the protein metabolism with an abnormally high B_6 requirement.

3. It is of interest that impairment of vitamin B_6-catalyzed metabolic reactions can be the mechanism underlying the toxic action of drugs, *e.g.*, the convulsive hydrazides. On the other hand, therapeutic drug effects, *e.g.*, the antihypertensive action of α-methyldopa, may be mediated by vitamin B_6 through its coenzyme function.

4. B_6 is also necessary for the synthesis of other biocatalysts in the organism. We have pointed out that the synthesis of heme is specifically B_6-dependent, since Plp is necessary for the formation of δ-aminolevulinic acid, the precursor of pyrrols and porphyrins. Therefore, anemia is a specific symptom of B_6 deficiency, while rather nonspecific signs can arise from the fact that B_6 is also essential for the synthesis of numerous enzymic cellular heme derivatives. Other rather nonspecific signs of B_6 deficiency could be the result of a decreased synthesis of NAD and coenzyme A, or of an impaired intestinal absorption of vitamin B_{12}. For these reasons it is difficult or impossible satisfactorily to interpret manifestations resulting from a B_6 deficiency in biochemical terms.

5. Since vitamin B_6, like other vitamins, is not a pharmacon but a physiological biocatalyst, it follows that specific therapeutic effects from its administration can be expected only in cases of a lack of this compound, as for instance in babies fed with B_6-poor milk preparations or in cases of vitamin B_6-dependence, where B_6 is the specific anticonvulsant. Also the administration of vitamin B_6 in arteriosclerosis could be to a certain degree a treatment directed against the etiology of the disease since this vitamin is involved in the enzymatic biosynthesis of unsaturated fatty acids. The same could be true for the treatment of Parkinsonians with vitamin B_6 because of its coenzyme function in the synthesis of dopamine. The clinical aspects of vitamin B_6 discussed in our contribution were confined to diseases in which causal relationships between pathogenesis and a disturbance of vitamin B_6-catalyzed reactions could be assumed.

REFERENCES

1. Aaes-Jørgensen, E.: Essential fatty acids. Physiol. Rev. **41**: 1–15, 1961.
2. Abderhalden, E.: Beitrag zum Problem der für die B_1 (Aneurin bzw. Thiamin) Avitaminose charakteristischen Erscheinungen. Pflüg. Arch. ges. Physiol. **240**: 647–652, 1938.
3. Ackermann, D. and Kutscher, Fr.: Über die Aporrhegmen. Hoppe-Seyl. Z. **69**: 265–272, 1910.
4. Aebi, H., Lauber, K., Schmidli, B. and Zuppinger, A.: Die Wirkung ionisierender Strahlen auf die Taurinausscheidung der Ratte. Biochem. Z. **328**: 391–404, 1957.
5. Akedo, H., Sugawa, T., Yoshikawa, S. and Suda, M.: Intestinal absorption of amino acids: The effect of vitamin B_6 on the absorption of *l*-amino acids through the intestine. J. Biochem., Tokyo **47**: 124–130, 1960.
6. Albers, R. W.: Gamma-aminobutyric acid. In: The Neurochemistry of Nucleotides and Amino Acids, ed. by R. O. Brady and D. B. Tower, pp. 146–158. John Wiley & Sons, Inc., New York, 1960.
7. Albers, R. W. and Brady, R. O.: The distribution of glutamic decarboxylase in the nervous system of the rhesus monkey. J. biol. Chem. **234**: 926–928, 1959.
8. Alexander, N. and Greenberg, D. M.: Studies on the purification and properties of the serine-forming enzyme system. J. biol. Chem. **220**: 775–785, 1956.
9. Altman, K. I.: Unpublished. (Cit. from 170.)
10. Amin, A. H., Crawford, T. B. B. and Gaddum, J. H.: The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. J. Physiol. **126**: 596–618, 1954.
11. Anonymous: Die Vitamin-B_6-Gruppe, ed. by Deutsche Hoffmann-La Roche AG, 1960.
12. Anonymous: The effect of pyridoxine on the conversion of linoleic acid to arachidonic acid. Nutr. Rev. **20**: 109–110, 1962.
13. Anonymous: Vitamin B_6-deficiency and tryptophan metabolism. Nutr. Rev. **21**: 89–91, 1963.
14. Antopol, W. and Tarlov, I. M.: Experimental study of the effects produced by large doses of Vitamin B_6. J. Neuropath. **1**: 330–336, 1942.
15. Aoki, T.: Experimental studies on the mode of action of cycloserine. I. Effects on glutamic acid decarboxylase. Kekkaku **32**: 418, 1957. (Cit. from 125.)
16. Aoki, T.: Experimental studies on the mode of action of cycloserine. II. The influence on glutamic acid decarboxylase. Kekkaku **32**: 544, 1957. (Cit. from 125.)
17. Aoki, T.: Experimental studies on the mode of action of cycloserine. III. Influence on indol formation. Kekkaku **32**: 605, 1957. (Cit. from 125.)
18. Aposhian, H. V.: Biochemical and pharmacological properties of the metal binding agent penicillamine. Fed. Proc. **20**: 185–188, 1961.
19. Aposhian, H. V. and Aposhian, M. M.: N-Acetyl-DL-penicillamine, a new oral protective agent against the lethal effects of mercuric chloride. J. Pharmacol. **126**: 131–135, 1959.
20. Aposhian, H. V. and Bradham, L. S.: Metabolism in vitro of the sulphydryl amino acids, L- and D-penicillamine. Biochem. Pharmacol. **3**: 38–41, 1959.
21. Arnold, O. H.: α-Methyldopa. Wirkungen und Nebenwirkungen bei der Therapie des arteriellen Hochdrucks. Dtsch. med. Wschr. **87**: 2288–2290, 1962.
22. Astruc, J.: Der Einfluss des Pyridoxols auf den histologischen Bau der Nebennierenrinde. Arzneim.-Forsch. **12**: 440–443, 1962.
23. Auerbach, V. H., Waisman, H. A. and Wykoff, L.: Phenylketonuria in the rat associated with decreased temporal discrimination learning. Nature, Lond. **182**: 871–872, 1958.
24. Awapara, J., Landua, A. J., Fuerst, R. and Seale, B.: Free γ-aminobutyric acid in brain. J. biol. Chem. **187**: 35–39, 1950.
25. Awapara, J., Sandman, R. P. and Hanly, C.: Activation of dopa decarboxylase by pyridoxalphosphate. Arch. Biochem. Biophys. **98**: 520–525, 1962.

25a. Awapara, J. and Wingo, W. J.: On the mechanism of taurine formation from cysteine in the rat. J. biol. Chem. **203**: 189–194, 1953.

26. AXT, F., BÜNGER, P. AND LASS, A.: Vitamin B-Stoffwechsel und Polyneuritis bei Isoniazidbehandlung. Fortschr. Neurol. Psychiat. **24:** 369–391, 1956.
27. BADDILEY, J. AND MATHIAS, A. P.: An ambiguous synthesis of decarboxylase. J. chem. Soc. **1952:** 2583–2591.
28. BAIN, J. A. AND WILLIAMS, H. L.: Concentrations of B_6 vitamers in tissues and tissue fluids. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 275–293. Pergamon Press, New York, 1960.
29. BAKER, A. B.: Treatment of paralysis agitans with vitamin B_6. J. Amer. med. Ass. **116:** 2484–2487, 1941.
30. BALLANTYNE, R. M. AND MCHENRY, E. W.: Vitamin B_6 and biotin in human cancer tissue. Cancer Res. **9:** 689–691, 1949.
31. BALZER, H. AND HOLTZ, P.: Beeinflussung der Wirkung biogener Amine durch Hemmung der Aminoxydase. Arch. exp. Path. Pharmak. **227:** 547–558, 1956.
32. BALZER, H., HOLTZ, P. AND PALM, D.: Untersuchungen über die biochemischen Grundlagen der konvulsiven Wirkung von Hydraziden. Arch. exp. Path. Pharmak. **239:** 520–552, 1960.
33. BARANOWSKI, T., ILLINGWORTH, B., BROWN, D. H. AND CORI, C. F.: The isolation of pyridoxal-5-phosphate from crystalline muscle phosphorylase. Biochim. biophys. Acta **25:** 16–21, 1957.
34. BARBEAU, A.: Preliminary observations on abnormal catecholamine metabolism in basal ganglia diseases. Neurology **10:** 446–451, 1960.
35. BARBEAU, A., JASMIN, G. AND DUCHASTEL, Y.: Biochemistry of Parkinson's disease. Neurology **13:** 56–58, 1963.
36. BARBEAU, A., MURPHY, G. F. AND SOURKES, T. L.: Excretion of dopamine in diseases of basal ganglia. Science **133:** 1706–1707, 1961.
37. BARBEAU, A., MURPHY, G. F. AND SOURKES, T. L.: Les catécholamines dans la maladie de Parkinson. In: Monoamines et Système Nerveux Central; Symposium Bel-Air, Genève, 1961, pp. 247–262. Georg et Cie., S.A. Genève, 1962.
38. BARLOW, C. F., SCHOOLAR, J. C., AND ROTH, L. J.: Distribution of carbon-14-labeled isoniazid in brain. Neurology **7:** 820–824, 1957.
39. BARRETO, R. C. R. AND MANO, D. B.: Prevention of the convulsant and lethal effects of isonicotinic hydrazide by pyruvic acid. Biochem. Pharmacol. **8:** 409–412, 1961.
40. BAXTER, C. F. AND ROBERTS, E.: The γ-aminobutyric acid–α-ketoglutaric acid transaminase of beef brain. J. biol. Chem. **233:** 1135–1139, 1958.
41. BAXTER, C. F. AND ROBERTS, E.: Elevation of γ-aminobutyric acid in rat brain with hydroxylamine. Proc. Soc. exp. Biol., N. Y. **101:** 811–815, 1959.
42. BAXTER, C. F. AND ROBERTS, E.: Demonstration of thiosemicarbazide-induced convulsions in rats with elevated brain levels of γ-aminobutyric acid. Proc. Soc. exp. Biol., N. Y. **104:** 426–427, 1960.
43. BAXTER, C. F. AND ROBERTS, E.: Gamma-aminobutyric acid and cerebral metabolism. In: The Neurochemistry of Nucleotides and Amino Acids, ed. by R. O. Brady and D. B. Tower, pp. 127–145. John Wiley & Sons, Inc., New York, 1960.
44. BAXTER, C. F. AND ROBERTS, E.: Elevation of γ-aminobutyric acid in brain by selective inhibition of γ-aminobutyric-α-ketoglutaric-acid transaminase. J. biol. Chem. **236:** 3287–3294, 1961.
45. BAXTER, C. F. AND ROBERTS, E.: Effect of 4-methoxymethylpyridoxine and carbonyl-trapping agents on amino acid content of mammalian brain and other tissues. In: Amino Acid Pools. Distribution, Formation and Function of Free Amino Acids, ed. by J. T. Holden, pp. 499–508. Elsevier Publ. Co., Amsterdam, 1962.
46. BAXTER, C. F., ROBERTS, E. AND EIDELBERG, E.: γ-Aminobutyric acid and seizure susceptibility in areas of normal cat brain cortex. J. Neurochem. **5:** 203–205, 1959–60.
47. BAZEMORE, A. W., ELLIOTT, K. A. C. AND FLOREY, E.: Isolation of factor I. J. Neurochem. **1:** 334–339, 1957.
48. BEARE, J. L., BEATON, J. R. AND MCHENRY, E. W.: Studies on vitamin B_6. III. Carcass composition of vitamin B_6-deficient rat. J. biol. Chem. **202:** 589–595, 1953.
49. BEATON, J. R., BEARE, J. L. AND MCHENRY, E. W.: Factors affecting development of acrodynia in pyridoxine-deficient rats. J. Nutr. **48:** 325–334, 1952.
50. BEHRINGER, K. AND WILMANNS, K.: Zur Harmin-Banisterin-Frage. Dtsch. med. Wschr. **55:** 2081–2086, 1929.
51. BEILER, J. M. AND MARTIN, G. J.: Inhibition of the action of tyrosine decarboxylase by phosphorylated desoxypyridoxine. J. biol. Chem. **169:** 345–347, 1947.
52. BELLAMY, W. D. AND GUNSALUS, I. C.: Tyrosine decarboxylation by streptococci; growth requirements for active cell production. J. Bact. **48:** 191–199, 1944.
53. BELOFF-CHAIN, A., CATANZARO, R., CHAIN, E. B., MASI, I. AND POCCHIARI, F.: Fate of uniformly labeled ^{14}C-glucose in brain slices. Proc. roy. Soc., ser. B **144:** 22–28, 1955–56.
54. BENSON, W. M., STEFKO, P. L. AND ROE, M. D.: Pharmacologic and toxicologic observations on hydrazine derivatives of isonicotinic acid (Rimifon, Marsilid). Amer. Rev. Tuberc. **65:** 376–391, 1952.
55. BERG, C. P. AND POTGIETER, M.: Tryptophane metabolism; growth-promoting ability of *dl*-tryptophane. J. biol. Chem. **94:** 661–673, 1932.
55a. BERGERET, B., CHATAGNER, F. AND FROMAGEOT, C.: Quelques relationes entre le phosphate de pyridoxal et la décarboxylation de l'acide cystéine-sulfinique par divers organs du rat normal ou du rat carence en vitamine B_6. Biochim. biophys. Acta **117:** 128–135, 1955.
56. BERL, S., PURPURA, D. P., GONZALES-MONTEAGUDO, O. AND WAELSCH, H.: Effects of injected amino acids on metabolic changes occurring in epileptogenic and nonepileptogenic lesions of the cerebral cortex. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 445–453. Pergamon Press, New York, 1960.
57. BERNHEIMER, H., BIRKMAYER, W. AND HORNYKIEWICZ, O.: Verteilung des 5-Hydroxytryptamins (Serotonin) im Gehirn des Menschen und sein Verhalten bei Patienten mit Parkinsonsyndrom. Klin. Wschr. **39:** 1056–1059, 1961.

58. Bertler, A. and Rosengren, E.: Occurrence and distribution of dopamine in brain and other tissues. Experientia 15: 10-11, 1959.
59. Bessey, O. A., Adam, D. J. D. and Hansen, A. E.: Intake of vitamin B_6 and infantile convulsions: a first approximation of requirements of pyridoxine in infants. Pediatrics 20: 33-44, 1957.
60. Bickel, H. and Grüter, W.: Prophylaxe und Behandlung der Phenylketonurie. Dtsch. med. Wschr. 86: 39-43, 1961.
61. Biehl, J. P. and Vilter, R. W.: Effects of isoniazid on pyridoxine metabolism. J. Amer. med. Ass. 156: 1549-1552, 1954.
61a. Binkley, F., Christensen, G. M. and Jensen, W. N.: Pyridoxine and transfer of sulfur. J. biol. Chem. 194: 109-113, 1952.
62. Birch, T. W.: The relation between vitamin B_6 and the unsaturated fatty acid factor. J. biol. Chem. 124: 775-793, 1938.
63. Birch, T. W., György, P. and Harris, L. J.: Differentiation of the antiblack tongue and the P-P-factors from lactoflavin and vitamin B_6 (so called "rat pellagra factor"). Biochem. J. 29: 2830-2850, 1935.
64. Birkmayer, W. and Hornykiewicz, O.: Der *l*-3,4-Dioxyphenylalanin (=Dopa)-Effekt bei Parkinson-Akinese. Wien. klin. Wschr. 73: 787-788, 1961.
65. Bischoff, F., Ingraham, L. P. and Rupp, J. J.: Influence of vitamin B_6 and pantothenic acid on growth of sarcoma 180. Arch. Path. 35: 713-716, 1943.
65a. Bishop, R. C. and Bethell, F. H.: Hereditary hypochromic anemia with transfusion hemosiderosis treated with pyridoxine; report of a case. New Engl. J. Med. 261: 486-489, 1959.
66. Blaschko, H.: *l*-(−)-Cysteic acid decarboxylase. Biochem. J. 36: 571-574, 1942.
67. Blaschko, H.: Substrate specificity of amino acid decarboxylases. Biochim. biophys. Acta 4: 130-137, 1950.
68. Blaschko, H., Carter, C. W., O'Brien, J. R. P. and Sloane Stanley, G. H.: Pyridoxine in relation to amine formation in the mammalian liver. J. Physiol. 107: 18-19P, 1948.
68a. Blaschko, H., Datta, S. P. and Harris, H.: Pyridoxine deficiency in rat: liver *l*-cysteic acid decarboxylase activity and urinary amino acids. Brit. J. Nutr. 7: 364-371, 1953.
68b. Blaschko, H., Holton, P. and Sloane Stanley, G. H.: Enzymic formation of pressor amines. J. Physiol. 108: 427-439, 1949.
68c. Blaschko, H. and Hope, D. B.: Enzymic decarboxylation of cysteic and cysteine sulfinic acids. J. Physiol. 126: 52-53P, 1954.
69. Blumberg, A., Dubach, U. C., Kreis, W. and Müller, H. R.: Klinische und biochemische Untersuchungen beim Carcinoidsyndrom. Dtsch. med. Wschr. 87: 921-929, 1962.
69a. Bower, B. D.: The tryptophan load test in the syndrome of infantile spasms with oligophrenia. Proc. R. Soc. Med. 54: 540-544, 1961.
70. Boxer, G. E., Pruss, M. P. and Goodhart, R. S.: Pyridoxal-5-phosphoric acid in whole blood and isolated leukocytes of man and animals. J. Nutr. 63: 623-636, 1957.
71. Boylen, J. B. and Quastel, J. H.: Effects of *l*-phenylalanine and sodium-phenylpyruvate on the formation of adrenaline from *l*-tyrosine in adrenal medulla in vitro. Biochem. J. 80: 644-648, 1961.
72. Brady, R. O., Formica, J. V. and Koval, G. J.: The enzymatic synthesis of sphingosine. II. Further studies on the mechanism of the reaction. J. biol. Chem. 233: 1072-1076, 1958.
73. Brady, R. O. and Koval, G. J.: The enzymatic synthesis of sphingosine. J. biol. Chem. 233: 26-31, 1958.
74. Braunstein, A. E.: Pyridoxal-catalyzed reactions as the basis of nitrogen metabolism. In: Proc. Int. Symposium of Enzyme Chemistry, ed. by K. Ichihara, pp. 135-142. Maruzen, Tokyo, 1957.
75. Braunstein, A. E.: Pyridoxal phosphate. In: The Enzymes, ed. by P. D. Boyer, H. Lardy and K. Myrbäck, 2nd ed., vol. 2, pp. 113-184. Academic Press, New York, 1960.
76. Braunstein, A. E.: Die Funktionen von Vitamin B_6 bei den Prozessen des Aminosäurestoffwechsels. Ergebn. Biol. 35: 27-56, 1953. (In: Zur Biochemie des Eiweisses, pp. 121-161. VEB Dtsch. Verlag der Wissenschaften, Berlin, 1956.)
77. Bridges, B. A. and Koch, R.: Radiation-protection by some sulfhydryl derivatives of pyridoxine and a new BAL preparation. Int. J. Rad. Biol. 3: 49-54, 1961.
78. Brockman, R. W., Thomson, J. R., Schabel, F. M., Jr. and Skipper, H. E.: The inhibition of growth of sarcoma 180 by combination of B_6 antagonists and acid hydrazides. Cancer Res. 16: 788-795, 1956.
79. Brodie, B. B., Kuntzman, R., Hirsch, C. W. and Costa, E.: Effects of decarboxylase inhibition on the biosynthesis of brain monoamines. Life Sci. 3: 81-84, 1962.
80. Brodie, B. B., Spector, S. and Shore, P. A.: Interactions of drugs with norepinephrine in the brain. Pharmacol. Rev. 11: 548-564, 1959.
81. Brown, R. R.: Studies on the metabolism of tryptophan in man. Rec. chem. Progr. 22: 29-37, 1961.
82. Brown, R. R., Thornton, M. J. and Price, J. M.: The effect of vitamin supplementation on the urinary excretion of tryptophan metabolites by pregnant women. J. clin. Invest. 40: 617-623, 1961.
82a. Brunnemann, A., Coper, A. and Herken, H.: Isolierung von 3-Acetylpyridin-Adenindinucleotid (3-APAD) aus dem Gehirn 3-Acetylpyridin-vergifteter Ratten. Arch. exp. Path. Pharmak. 244: 223-236, 1962.
83. Bruns, F. H., Brosswitz, E., Dennemann, H., Horn, H. D. and Noltmann, E.: Studien über die Ursachen des Enzymverlustes der geschädigten Zelle. Klin. Wschr. 39: 342-346, 1961.
84. Burkard, W. P., Gey, K. F. and Pletscher, A.: A new inhibitor of decarboxylase of aromatic amino acids. Experientia 18: 411-412, 1962.
85. Canal, N. and Maffai-Faccioli, A.: Effect of thyroxine treatment on some vitamin B_6 dependent enzymes. Rev. esp. Fisiol. 15: 225-232, 1959.

86. CANNON, P. J., WHITLOCK, R. T., MORRIS, R. C., ANGERS, M. AND LARAGH, J. H.: Effect of α-methyl dopa in severe and malignant hypertension. J. Amer. med. Ass. 179: 673–681, 1961.
87. CARLSSON, A.: The occurrence, distribution and physiological role of catecholamines in the nervous system. Pharmacol. Rev. 11: 490–493, 1959.
88. CARLSSON, A. AND LINDQUIST, M.: In vivo decarboxylation of α-methyl dopa and α-methyl metatyrosine. Acta physiol. scand. 54: 87–94, 1962.
89. CARLSSON, A., LINDQVIST, M. AND MAGNUSSON, T.: 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature, Lond. 180: 1200, 1957.
90. CARTWRIGHT, G. E. AND WINTROBE, M. M.: Studies on free erythrocyte protoporphyrin, plasma copper and plasma iron in normal and in pyridoxine-deficient swine. J. biol. Chem. 172: 557–565, 1948.
91. CERECEDO, L. R., FOY, J. R. AND DERENZO, E. C.: Protein intake and vitamin B_6-deficiency in rat. II. Effect of supplementing low-protein, pyridoxine-deficient diet with cystine or with methionine. Arch. Biochem. 17: 397–402, 1948.
92. CHAPMAN, J. E. AND WALASZEK, E. J.: Potentiation of histamine responses by bulbocapnine. Med. exp. 6: 95–100, 1962.
93. CHAPMAN, J. E. AND WALASZEK, E. J.: Katatone Zustände und Histaminspiegel im Zentralnervensystem. Cit. from Dtsch. med. Wschr. 87: 1277, 1962.
94. CHAPTAL, I., JEAU, R., CRASTES DE PAULET, A., BONNET, H., PETIT, M. AND GUILLAUMOT, R.: Accidents provoqués par l'isoniazide au cours de traitement prolongés de la tuberculose de l'enfant. Pediatrie 15: 623–634, 1960.
95. CHATAGNER, F., BERGERET, B. AND LABOUESSE, J.: Influence d'hormones ovariennes et thyroïdiennes sur deux décarboxylases d'acides aminés dans le foie du rat. Mise en évidence de l'existence de deux régulations indépendantes. Biochim. biophys. Acta 35: 231–237, 1959.
96. CHEN, G., ENSOR, C. R. AND BOHNER, B.: A facilitation action of reserpine on the central nervous system. Proc. Soc. exp. biol., N. Y. 86: 507–510, 1954.
97. CHINN, H. I.: Evaluation of drugs for protection against motion sickness aboard transport ships. J. Amer. med. Ass. 160: 755–760, 1956.
98. CHRISTENSEN, H. N.: Possible role of chelation between alkali metals and pyridoxal in biological transport. Science 122: 1087–1088, 1955.
99. CHRISTENSEN, H. N.: Intestinal absorption with special reference to amino acids. Fed. Proc. 21: 37–42, 1962.
100. CHRISTENSEN, H. N. AND RIGGS, T. R.: Structural evidences for chelation and Schiff's base formation in amino acid transfer into cells. J. biol. Chem. 220: 265–278, 1956.
101. CHRISTENSEN, H. N., RIGGS, T. R. AND COYNE, B. A.: Effects of pyridoxal and indolacetate on cell uptake of amino acids and potassium. J. biol. Chem. 209: 413–427, 1954.
102. CLARK, C. T., WEISSBACH, H. AND UDENFRIEND, S.: 5-Hydroxytryptophan decarboxylase: preparation and properties. J. biol. Chem. 210: 139–148, 1954.
103. CLARK, W. G.: Studies on inhibition of L-dopa decarboxylase *in vitro* and *in vivo*. Pharmacol. Rev. 11: 330–349, 1959.
104. CLARK, W. G. AND POGRUND, R. S.: Inhibition of dopa decarboxylase in vitro and in vivo. Circulation Res. 9: 721–733, 1961.
105. COCHRANE, W. A.: The syndrome of infantile spasms and progressive mental deterioration related to amino acid and pyridoxine metabolism, p. 115. Proc. 9th int. Congr. Pediatrics, Montreal, 1959.
106. COOK, A. H.: Penicillin and its place in science. Fortschritte der Chemie organischer Naturstoffe VII, pp. 193–247. Springer Verlag, Wien, 1950.
107. COSTA, E.: Vitamin B_6 accentuates in the mouse the convulsant action of isonicotinyl hydrazide. Boll. Soc. ital. Biol. sper. 28: 1015–1016, 1952.
108. COSTA, E. AND HIMWICH, H. E.: Brain serotonin metabolism in insulin hypoglycemia. In: Biochemistry of the Central Nervous System, ed. by F. Brücke, vol. 3, pp. 283–290. Pergamon Press, New York, 1960.
109. COURSIN, D. B.: Convulsive seizures in infants with pyridoxine-deficient diet. J. Amer. med. Ass. 154: 406–408, 1954.
110. COURSIN, D. B.: Seizures in vitamin B_6 deficiency. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 294–301. Pergamon Press, New York, 1960.
111. COURSIN, D. B.: Present status of vitamin B_6 metabolism. Amer. J. clin. Nutr. 9: 304–314, 1961.
112. CRAMER, H. AND BINET, J.-L.: B_6-Avitaminose und Nervensystem. Die peripher- und zentralnervösen Komplikationen der Isonikotinsäurehydrazid(INH)-Therapie. Ärztl. Forsch. 16: 53–59, 1962.
113. CRAVENS, W. W. AND SNELL, E. E.: Desoxypyridoxine and development of the chick embryo. Fed. Proc. 8: 380, 1949.
114. DALGLIESH, C. E., KNOX, W. E. AND NEUBERGER, A.: Intermediary metabolism of tryptophan. Nature, Lond. 168: 20–22, 1951.
115. DAM, H., KRISTENSEN, G., NIELSEN, G. AND SØNDERGARD, E.: Effect of pyridoxine deficiency on cholesterol and polyenoic fatty acids in chicks. Acta physiol. scand. 44: 67–76, 1958.
116. DANIEL, L. J.: Inhibitors of vitamins of the B complex. Nutr. Abstr. Rev. 31: 1–13, 1961.
117. DASILVA, A. C., FAJER, A. B., DEANGELIS, R. C., PONTES, M. A., GIESBRECHT, A. M. AND FRIED, R.: The domestic cat as laboratory animal for experimental nutrition studies. VII. Pyridoxine deficiency. J. Nutr. 68: 213–228, 1959.
118. DAVANZO, J. P., GREIG, M. E. AND CRONIN, M. A.: Anticonvulsant properties of amino-oxyacetic acid. Amer. J. Physiol. 201: 833–837, 1961.
119. DAVIS, R. A., DRAIN, D. J., HORLINGTON, M., LAZARE, R. AND URBANSKA, A.: The effect of L-α-methyl-dopa

and N-2-hydroxy-benzyl-N-methyl-hydrazine (NSD 1039) on the blood pressure of renal hypertensive rats. Life Sci. 3: 193–197, 1963.

120. Davison, A. N.: Pyridoxal phosphate as a coenzyme of diaminoxydase. Biochem. J. 64: 546–548, 1956.
121. Davison, A. N. and Sandler, M.: Inhibition of 5-hydroxy-tryptophan-decarboxylase by phenylalanine metabolites. Nature, Lond. 181: 186–187, 1958.
122. Dawson, D. W., Leeming, J. T., Oelbaum, M. H., Pengelly, C. D. and Wilkinson, J. F.: Pyridoxine-responsive hypochromic anemia. A report of two cases. Lancet 2: 10–14, 1961.
123. Day, E. J., Alexander, H. D., Sauberlich, H. E. and Salmon, W. D.: Effects of gamma radiation on certain water-soluble vitamins in raw ground beef. J. Nutr. 62: 27–38, 1957.
124. Degkwitz, R., Frowein, R., Kulenkampff, C. and Mohs, U.: Über die Wirkungen des L-Dopa beim Menschen und deren Beeinflussung durch Reserpin, Chlorpromazin, Iproniazid und Vitamin B_6. Klin. Wschr. 38: 120–123, 1960.
125. Dengler, H.: Zur Hemmung der L-Glutaminsäure- und L-Dopa-decarboxylase durch D-Cycloserin und andere Isoxazolidone. Arch. exp. Path. Pharmak. 243: 366–381, 1962.
126. Dengler, H. and Reichel, G.: Die Beeinflussung der Blutdruckwirkung von Dopa und Dops durch einen Decarboxylaseinhibitor. Arch. exp. Path. Pharmak. 232: 324–326, 1957.
127. Desikachar, H. S. R. and McHenry, E. W.: Some effects of vitamin B_6 deficiency on fat metabolism in rats. Biochem. J. 56: 544–547, 1954.
128. Detrick, L. E., Uphan, H. C., Sprinsteen, R. W. and Haley, T. J.: Pyridoxine absorption by irradiated intestine. Fed. Proc. 21: 425, 1962.
129. Dieke, S. H.: Thiosemicarbazide: A new toxic derivative of thiourea. Proc. Soc. exp. Biol., N. Y. 70: 688–693, 1949.
130. Dienemann, G. and Simon, K.: I. Mitteilung eines Todesfalles nach kombinierter Verabreichung von Irgapyrin und Neoteben (INH). II. Tierexperimentelle Untersuchungen zu der vorstehenden Arbeit. Münch. med. Wschr. 95: 221–222, 1953.
130a. Dixon, R. H. and Williams, H. L.: The toxicity of pyridoxal and pyridoxal phosphate hydrazones in mice. Fed. Proc. 21: 338, 1962.
131. Drews, A., Eversmann, K. and Fritze, E.: Über eine orale Vergiftung mit Hydrazin. Med. Welt 23: 1295–1297, 1960.
132. Dubach, U. C. and Blumberg, A.: Über die therapeutische Verwendung von α-Methyl-dopa beim Carcinoidsyndrom. Klin. Wschr. 39: 973, 1961.
133. Dubnick, B., Leeson, G. A. and Phillips, G. E.: In vivo inhibition of serotonin synthesis in mouse brain by β-phenylethyl-hydrazine derivatives. Biochem. Pharmacol. 11: 45–52, 1962.
134. Dubnick, B., Leeson, G. A. and Scott, C. C.: Effect of forms of vitamin B_6 on acute toxicity of hydrazines. Tox. appl. Pharmacol. 2: 403–409, 1960.
135. Dunlop, C. W.: Effects of carbon dioxide in the rhinencephalon in the marsupial phalanger. Amer. J. Physiol. 191: 200–202, 1957.
136. Ehringer, H. and Hornykiewicz, O.: Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. Klin. Wschr. 38: 1236–1239, 1960.
137. Eidelberg, E., Baxter, C. F., Roberts, E., Saldias, C. A. and French, J. D.: Anticonvulsant properties of hydroxylamine and elevation of cerebral γ-aminobutyric acid in cats. Proc. Soc. exp. Biol., N. Y. 101: 815–817, 1959.
138. Eisenstein, A. B.: Inhibition of gluconeogenic action of cortisol in pyridoxine-deficient rats. Biochim. biophys. Acta 36: 580–581, 1959.
139. Elliott, K. A. C. and Jasper, H.: Gamma aminobutyric acid. Physiol. Rev. 39: 383–406, 1959.
140. Elliott, K. A. C. and van Gelder, N. M.: Occlusion and metabolism of γ-aminobutyric acid by brain tissue. J. Neurochem. 3: 28–40, 1958.
141. Elvehjem, C. A.: Role of intestinal bacteria in nutrition. J. Amer. diet. Ass. 22: 959–963, 1946.
142. Elvehjem, C. A., Madden, R. J., Strong, F. M. and Woolley, D. W.: Relation of nicotinic acid and nicotinic acid amide to canine black-tongue. J. Amer. chem. Soc. 59: 1767–1768, 1937.
143. Emerson, G. A.: The antivitamin B_6 activity of desoxypyridoxine in the rat. Fed. Proc. 6: 406–407, 1947.
144. Epstein, I. G., Nair, K. G., Mulinos, M. G. and Haber, A.: Pyridoxine and its relation to cycloserine neurotoxicity, a pharmacological and clinical study. Antibiot. Annu. 472–481, 1959.
145. Ernst, A. M.: Phenomena of the hypokinetic rigid type caused by O-methylation of dopamine in the para-position. Nature, Lond. 193: 178–179, 1962.
146. Euler, U. S. von and Åström, A.: Liberation of histamine and sympathin by stimulation of isolated splenic nerves from cattle. Acta physiol. scand. 16: 97–104, 1949.
147. Euler, U. S. von and Lishajko, F.: Dopamine in mammalian lung and spleen. Acta physiol. pharm. néerl. 6: 295–303, 1957.
148. Everett, G. M. and Richards, R. K.: Comparative anticonvulsive action of 3,5,5-trimethyloxazolidine-2,4-dione (tridione), dilantin and phenobarbital. J. Pharmacol. 81: 402–407, 1944.
149. Fellman, J. H.: Inhibition of dopa decarboxylase by aromatic acids associated with phenylpyruvic oligophrenia. Proc. Soc. exp. Biol., N. Y. 93: 413–414, 1956.
150. Ferrari, R. A. and Arnold, A.: The effect of central nervous system agents on rat brain γ-aminobutyric acid level. Biochim. biophys. Acta 52: 361–367, 1961.
151. Fetterhof, K., Holmes, C. X. and Martin, G. E.: Hazards of isoniazid therapy in epileptics. Amer. Rev. Tuberc. 66: 501, 1952.

152. FINKE, H.: Über die Behandlung des Parkinsonischen Syndroms mit hohen Dosen von Vitamin B_6. Münch. med. Wschr. **96**: 637–639, 1954.
153. FLOREY, E.: Further evidence for the transmitter-function of factor I. Naturwissenschaften **34**: 424–425, 1957.
154. FOREMAN, H.: Use of chelating agents in treatment of metal poisoning (with special emphasis on lead). Fed. Proc. **20**: 191–196, 1961.
155. FORNI, P. V.: Idrazide dell'acido isonicolinico e neoplasie. Rass. Med. **29**: 245–247, 1952.
156. FOUTS, P. J., HELMER, O. M., LEPKOVSKY, S. AND JUKES, T. H.: Production of microcytic hypochromic anemia in puppies on synthetic diet, deficient in the rat antidermatitis factor (vitamin B_6). J. Nutr. **16**: 197–207, 1938.
157. FREERKSEN, E., KRÜGER-THIEMER, E. AND ROSENFELD, M.: Cycloserine (D-4-amino-isoxazolidin-3-on). Antibiot. Chemother., Basel **6**: 303–396, 1959.
158. FREYTAG, G.: Untersuchungen zur Frage eines Vitamin B_6-Mangels bei Kindern unter INH-Therapie. Inauguraldissertation, Heidelberg, 1960.
159. FRIDHANDLER, L. AND QUASTEL, J. H.: Absorption of amino acids from isolated surviving intestine. Arch. Biochem. Biophys. **56**: 424–440, 1955.
160. FRIEDMAN, B. I., BECKER, L., THOMPSON, C. AND VILTER, R. W.: Vitamin B_6 metabolism in the post-partum period. J. Lab. clin. Med. **46**: 817–821, 1955.
160a. FRIMPTER, G. W., HAYMOVITZ, A. AND HORWITH, M.: Cystathioninuria. New Engl. J. Med. **268**: 333–339, 1963.
161. FUST, B.: D-Cycloserin. Med. Welt **12**: 470–478, 1958.
162. GABOUREL, J. D.: Anticonvulsant properties of diacetylmonoxime (DAM). Biochem. Pharmacol. **5**: 283–286, 1961.
163. GALE, E. F.: The bacterial amino acid decarboxylases. Advanc. Enzymol. **6**: 1–32, 1946.
164. GALE, E. F. AND EPPS, H. M. R.: Studies on bacterial amino-acid decarboxylases. Biochem. J. **38**: 232–242, 1944.
165. GAMMON, G. D., GUMNIT, R., KAMRIN, R. P. AND KAMRIN, A.: The effect of convulsant doses of analeptic agents upon the concentration of amino acids in brain tissue. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 328–330. Pergamon Press, New York, 1960.
166. GANROT, P. O., ROSENGREN, A. M. AND ROSENGREN, E.: On the presence of different histidine decarboxylating enzymes in mammalian tissues. Experientia **17**: 263–264, 1961.
167. GARATTINI, S. AND VEGETO, A.: Carenza in vitamina B_6 e inibita crescita tumorale. Giorn. ital. Chemioterap. **2**: 41–45, 1955.
168. GARDNER, F. H. AND NATHAN, D. G.: Hypochromic anemia and hemochromatosis. Response to combined testosterone, pyridoxine and liver extract. Amer. J. med. Sci. **243**: 447–457, 1962.
169. GARTY, R., YONIS, Z., BRAHAM, J. AND STEINITZ, K.: Pyridoxine dependent convulsions in an infant. Arch. Dis. Childh. **37**: 21–24, 1962.
170. GEHRMANN, G.: Pyridoxinmangelanämie beim Menschen. Folia haemat., Frankf. **2**: 225–235, 1958.
171. VAN GELDER, N. M. AND ELLIOTT, K. A. C.: Disposition of γ-aminobutyric acid administered to mammals. J. Neurochem. **3**: 139–143, 1958.
172. GELLHORN, A. AND JONES, L. O.: Pyridoxine-deficient diet and desoxypyridoxine in the therapy of lymphosarcoma and acute leukemia in man. Blood **4**: 60–65, 1948.
173. GENDRAU, J. E. AND LAFLEUR, L.: La pyridoxine (vitamin B_6) et le "mal des radiations." Un. méd. Can. **73**: 666–670, 1944.
174. GHOLSON, R. K., HENDERSON, L. M., MOURKIDES, G. A., HILL, R. J. AND KOEPPE, R. E.: The metabolism of DL-tryptophan-α-C^{14} by the rat. J. biol. Chem. **234**: 96–98, 1958.
175. GIBSON, K. D., LAVER, W. G. AND NEUBERGER, A.: Initial stages in the biosynthesis of porphyrins. 2. The formation of δ-aminolaevulic acid from glycine and succinylcoenzyme A by particles from chicken erythrocytes. Biochem. J. **70**: 71–80, 1958.
176. GIBSON, K. D., MATTHEW, M., NEUBERGER, A. AND TAIT, G. H.: Biosynthesis of porphyrins and chlorophylls. Nature, Lond. **192**: 204–208, 1961.
177. GILLESPIE, L.: Clinical pharmacology of newer antihypertensive agents, monoamine oxidase and decarboxylase inhibitors, bretylium tosylate and guanethidine. Ann. N. Y. Acad. Sci. **88**: 1011–1020, 1960.
178. GOLDBERG, L. I., DACOSTA, F. M. AND OZAKI, M.: Actions of the decarboxylase inhibitor α-methyl-3,4-dihydroxyphenylalanine in the dog. Nature, Lond. **188**: 502–504, 1960.
179. GOLDBERGER, J. AND LILLIE, R. D.: Experimental pellagra-like condition in the albino rat. Publ. Hlth Rep., Wash. **41**: 1025–1029, 1926.
180. GOLDFEDER, A., COHEN, L., MILLER, C. AND SINGER, M.: Agents influencing experimental radiation injury effects of folic acid and pyridine. Proc. Soc. exp. Biol., N. Y. **67**: 272–278, 1948.
181. GONNARD, P. AND FENARD, S.: Décarboxylase glutamique et hydrazones de phospho-5-pyridoxal. J. Neurochem. **9**: 135–142, 1962.
182. GONNARD, P. AND NGUYEN CHI, J. P.: Action de l'hydrazide isonicotinique et de composés de structure voisine sur la dopadécarboxylase. Bull. Soc. Chim. biol., Paris **40**: 485–495, 1958.
183. GOODMAN, L. S., TOMAN, J. E. P. AND SWINYARD, E. A.: The anticonvulsant properties of tridione; laboratory and clinical investigations. Amer. J. Med. **1**: 213–228, 1946.
184. GOSWAMI, A. AND SADHU, D. P.: Polyenoic acid in hypercholesterolaemia induced by pyridoxine deficiency in rats. Nature, Lond. **187**: 786–787, 1960.
185. GRAY, W. D., MAREN, T. H., SISSON, G. M. AND SMITH, F. H.: Carbonic anhydrase inhibition: VII. Carbonic anhydrase inhibition and anticonvulsant effect. J. Pharmacol. **121**: 160–170, 1957.
186. GREENBERG, L. D. AND RINEHART, J. F.: Plasma cholesterol fed control and pyridoxine deficient monkeys. Proc. Soc. exp. Biol., N. Y. **76**: 580–583, 1951.
187. GREENSTEIN, J. P.: Biochemistry of Cancer, 2nd ed. Academic Press, New York, 1954.

188. GRINSCHGL, G.: Über Vitamin B_6 (Pyridoxin) und seinen therapeutischen Wirkungsmechanismus unter besonderer Berücksichtigung extrapyramidaler Störungen. Wien. klin. Wschr. **63:** 659–663, 1951.
189. GROCHOWSKA, Z. AND KALUZA, J.: Fatal intoxication with isonicotinic acid hydrazide. Gruślica, Polen **28:** 807–813, 1960.
190. GRODEN, B. M.: Parkinsonism occurring with methyldopa treatment. Brit. med. J. **1:** 1001, 1963.
191. GRUNDFEST, H.: Central inhibition and its mechanism. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 47–65. Pergamon Press, New York, 1960.
192. GUNSALUS, I. C., BELLAMY, W. D. AND UMBREIT, W. W.: A phosphorylated derivative of pyridoxal as the coenzyme of tyrosine decarboxylase. J. biol. Chem. **155:** 685–686, 1944.
193. GYÖRGY, P.: The history of vitamin B_6. Amer. J. clin. Nutr. **4:** 313–317, 1956.
194. GYÖRGY, P. AND ECKHARDT, R. E.: Vitamin B_6 and skin lesions in rats. Nature, Lond. **144:** 512, 1939.
195. HAGEN, P.: Observations on the substrate specificity of dopa decarboxylase from ox adrenal medulla, human phaeochromocytoma and human argentaffinoma. Brit. J. Pharmacol. **18:** 175–182, 1962.
196. VAN HALTERN, H. L.: The use of pyridoxine HCl in radiation sickness. Radiology **47:** 377–380, 1946.
197. HANSON, A.: Inhibition of brain glutamic acid decarboxylase by phenylalanine metabolites. Naturwissenschaften **45:** 423, 1958.
197a. HARRIS, H., PENROSE, L. S. AND THOMAS, D. H. H.: Cystathioninuria. Ann. hum. Genet. **23:** 442–453, 1958–59.
198. HARRIS, J. W., WHITTINGTON, R. M., WEISMAN, R. AND HORRIGAN, D. L.: Pyridoxine responsive anemia in human adult. Proc. Soc. exp. Biol., N. Y. **91:** 427–432, 1956.
199. HARRIS, S. A., HEYL, D. AND FOLKERS, K.: The structure and synthesis of pyridoxamine and pyridoxal. J. biol. Chem. **154:** 315–316, 1944.
200. HARTMANN-VON MONAKOW, K.: Die Bedeutung des Vitamin B_6 für die Neurologie. Psychiat. Neurol. **142:** 387–403, 1961.
201. HARTMANN, W. J., AKAWIE, R. I. AND CLARK, W. G.: Competitive inhibition of 3,4-dihydroxyphenyl-alanine (Dopa) decarboxylase in vitro. J. biol. Chem. **216:** 507–529, 1955.
202. HARTWEG, H. AND BÖWING, G.: Zur Wirkung des Vitamin B_6 im strahlengeschädigten Organismus. Strahlentherapie **106:** 289–293, 1958.
202a. HASLAM, R. J. AND KREBS, H. A.: The metabolism of glutamate in homogenates and slices of brain cortex. Biochem. J. **88:** 566–578, 1963.
203. HATEFI, Y., OSBORN, M. J., KAY, L. D. AND HUENNEKENS, F. M.: Hydroxymethyl tetrahydrofolic dehydrogenase. J. biol. Chem. **227:** 637–647, 1957.
204. HAYASHI, T.: Inhibition and excitation due to γ-aminobutyric acid in the central nervous system. Nature, Lond. **182:** 1076–1077, 1958.
205. HAYASHI, T.: Comparison of inhibitory action of γ-aminobutyric acid and β-hydroxy-γ-aminobutyric acid on the motor system of higher animals. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 515–522. Pergamon Press, New York, 1960.
206. HAYASHI, T. AND NAGAI, K.: Action of ω-amino acids on the motor cortex of higher animals, especially γ-amino-oxy-butyric acid as the real inhibitory principle of brain. Abstr. XX Int. Physiol. Congr., p. 410, 1956.
207. HEDDLE, J. G., MCHENRY, E. W. AND BEATON, G. H.: Penicillamine and vitamin B_6 interrelationships in the rat. Canad. J. Biochem. Physiol. **41:** 1215–1222, 1963.
208. HEILMEYER, L. AND CLOTTEN, R.: (Cit. from 282.)
209. HEILMEYER, L., EMMRICH, J., HENNEMANN, H. H., KEIDERLING, W., LEE, M., BILGER, R. AND SCHUBOTHE, H.: Über eine chronische hypochrome Anämie bei zwei Geschwistern auf der Grundlage einer Eisenverwertungsstörung (Anaemia hypochromica sideroachrestia hereditaria). Folia haemat., Frankf. **2:** 61–75, 1958.
210. HEINZ, E.: Aktiver Transport von Aminosäuren. In: Biochemie des aktiven Transports, pp. 167–188. Springer Verlag, Berlin, 1961.
211. HEINZ, E.: Some remarks on active transport and exchange diffusion of amino acids in Ehrlich cells. In: Amino Acid Pools. Distribution, Formation and Function of Free Amino Acids, ed. by J. T. Holden, pp. 539–544. Elsevier Publ. Co., Amsterdam, 1962.
212. HENSCHLER, D.: Zur Frage des Vorkommens von Butyrylcholin im Rindergehirn. Hoppe-Seyl. Z. **305:** 97–104, 1956.
213. HESS, S. M., CONNAMACHER, R. H., OZAKI, M. AND UDENFRIEND, S.: The effects of α-methyl-dopa and α-methyl-meta-tyrosine on the metabolism of norepinephrine and serotonin *in vivo*. J. Pharmacol. **134:** 129–138, 1961.
214. HEYL, D., LUZ, E., HARRIS, S. A. AND FOLKERS, K.: The chemistry of vitamin B_6. Pyridoxylamines. J. Amer. chem. Soc. **70:** 1670–1671, 1948.
215. HEYL, D., LUZ, E., HARRIS, S. A. AND FOLKERS, K.: Phosphates of the vitamin B_6 group. I. The structure of codecarboxylase. J. Amer. chem. Soc. **73:** 3430–3433, 1951.
216. HEYL, D., LUZ, E., HARRIS, S. A. AND FOLKERS, K.: VIII. Additional pyridoxylideneamines and pyridoxylamines. J. Amer. chem. Soc. **74:** 414–416, 1952.
217. HOLDEN, J. T.: The effect of vitamin B_6 deficiency on glutamic acid accumulation by *Lactobacillus arabinosus*. J. biol. Chem. **234:** 872–876, 1959.
218. HOLDEN, J. T.: Transport and accumulation of amino acids by microorganisms. In: Amino Acid Pools. Distribution, Formation and Function of Free Amino Acids, ed. by J. T. Holden, pp. 566–594. Elsevier Publ. Co., Amsterdam, 1962.
219. HOLTZ, P.: Über Tyraminbildung im Organismus. Arch. exp. Path. Pharmak. **186:** 684–693, 1937.
220. HOLTZ, P.: Role of L-dopa decarboxylase in the biosynthesis of catecholamines in nervous tissue and the adrenal medulla. Pharmacol. Rev. **11:** 317–329, 1959.

221. HOLTZ, P., BALZER, H., WESTERMANN, E. AND WEZLER, E.: Beeinflussung der Evipannarkose durch Reserpin, Iproniazid und biogene Amine. Arch. exp. Path. Pharmak. **231:** 333-348, 1957.
222. HOLTZ, P. AND CREDNER, K.: Dopahyperglykämie. Hoppe-Seyl. Z. **280:** 9-15, 1943.
223. HOLTZ, P. AND CREDNER, K.: Über die Konfigurationsänderung des *d*-Dioxyphenylalanins im Tierkörper. Hoppe-Seyl. Z. **280:** 39-48, 1944.
224. HOLTZ, P. AND CREDNER, K.: Dopadecarboxylase, Renin und renale Hypertonie. Arch. exp. Path. Pharmak. **204:** 244-266, 1947.
225. HOLTZ, P., CREDNER, K. AND WALTER, H.: Über die Spezifität der Aminosäure-decarboxylasen. Hoppe-Seyl. Z. **262:** 111-119, 1939.
226. HOLTZ, P. AND HEISE, R.: Über Histaminbildung im Organismus. Arch. exp. Path. Pharmak. **186:** 377-386, 1937.
227. HOLTZ, P. AND SCHÜMANN, H. J.: Butyrylcholin in Gehirnextrakten. Naturwissenschaften **41:** 306, 1954.
228. HOLTZ, P., STOCK, K. AND WESTERMANN, E.: Über den Einfluss des Thyroxins und des Hungerns auf die Aktivität der Monoaminoxydase und Dopadecarboxylase der Leber. Arch. exp. Path. Pharmak. **228:** 322-330, 1956.
229. HOLTZ, P. AND WESTERMANN, E.: Über die Dopadecarboxylase und Histidindecarboxylase des Nervengewebes. Arch. exp. Path. Pharmak. **227:** 538-546, 1956.
230. HOLTZ, P. AND WESTERMANN, E.: Hemmung der Glutaminsäure-decarboxylase des Gehirns durch Brenzkatechinderivate. Arch. exp. Path. Pharmak. **231:** 311-332, 1957.
230a. HOPE, D. B.: Pyridoxal phosphate as the coenzyme of the mammalian decarboxylase for *l*-cysteine sulfinic and *l*-cysteic acid. Biochem. J. **59:** 497-500, 1955.
230b. HOPE, D. B.: The persistence of taurine in the brains of pyridoxine deficient rats. J. Neurochem. **1:** 364-369, 1957.
230c. HOPE, D. B.: *l*-Cystathionine in the urine of pyridoxine-deficient rats. Biochem. J. **66:** 486-489, 1957.
231. HORRIGAN, D. L.: An unidentified erythropoietic substance in liver. Blood **18:** 535-542, 1961.
232. HORVATH, A.: Inhibition by thyroxine of enzymes requiring pyridoxal-5-phosphate. Nature, Lond. **179:** 968, 1957.
233. HORNYKIEWICZ, O.: Dopamin (3-Hydroxytyramin) im Zentralnervensystem und seine Beziehung zum Parkinson-Syndrom des Menschen. Dtsch. med. Wschr. **87:** 1807-1810, 1962.
234. HSU, J. M. AND CHOW, B. F.: Effect of pyridoxine deficiency on the absorption of vitamin B_{12}. Arch. Biochem. **72:** 322-330, 1957.
235. HUNT, A. D., JR.: Abnormally high pyridoxine requirement. Summary of evidence suggesting relation between this finding and clinical pyridoxine "deficiency." Amer. J. clin. Nutr. **5:** 561-565, 1957.
236. HUNT, A. D., JR., STOKES, J., JR., MCCRORY, W. W. AND STROUD, H. H.: Pyridoxine dependency: a report of a case of intractable convulsions in an infant controlled by pyridoxine. Pediatrics **13:** 140-145, 1954.
237. HURLEY, N. A. AND STEWART, R. A.: The vitamin B_6 content of infant foods. Pediatrics **26:** 679-683, 1960.
238. HURWITZ, J.: The enzymic phosphorylation of vitamin B_6-derivatives and their effects on tyrosine decarboxylase. Biochim. biophys. Acta **9:** 496-498, 1952.
239. HURWITZ, J.: Enzymatic phosphorylation of vitamin B_6 analogues and their effect on tyrosine decarboxylase. J. biol. Chem. **217:** 513-525, 1955.
240. IKAWA, M., RODWELL, V. W. AND SNELL, E. E.: Bacterial oxidation of vitamin B_6. II. Structure of "260 compound." J. biol. Chem. **233:** 1555-1559, 1958.
241. ILLINGWORTH, B. I., JANSZ, H. S., BROWN, D. H. AND CORI, C. F.: Observation on the function of pyridoxal-5-phosphate in phosphorylase. Proc. nat. Acad. Sci., Wash. **44:** 1180-1191, 1958.
242. ILLINGWORTH, B. I., KORNFELD, R. AND BROWN, D. H.: Phosphorylase and uridinediphosphoglucose-glycogen transferase in pyridoxine deficiency. Biochim. biophys. Acta **42:** 486-489, 1960.
243. IRREVERRE, F., EVANS, R. L., HAYDEN, A. R. AND SILBER, R.: Occurrence of gamma-guanidinobutyric acid. Nature, Lond. **180:** 704-705, 1957.
244. IWAINSKY, H. AND SIEGEL, D.: Über die Toxizitätsminderung von Isonicotinsäurehydrazid durch Brenztraubensäure. Beitr. Klin. Tuberk. **118:** 271-274, 1958.
245. IWAMA, K. AND JASPER, H. H.: The action of gamma aminobutyric acid upon cortical electrical activity in the cat. J. Physiol. **138:** 365-380, 1957.
246. JACOBS, F. A., COEN, L. J. AND HILLMAN, R. S. L.: Influence of pyridoxine, pyridoxal-phosphate, desoxypyridoxine and 2,4-dinitrophenol on methionine absorption. J. biol. Chem. **235:** 1372-1375, 1960.
247. JACOBS, F. A., FLAA, R. C. AND BELK, W. F.: Pyridoxal phosphate requirement for intestinal absorption of *l*-tyrosine. J. biol. Chem. **235:** 3224-3227, 1960.
248. JACOBS, F. A. AND HILLMAN, R. S. L.: Intestinal absorption of the methionine isomers as affected by deoxypyridoxine. J. biol. Chem. **232:** 445-451, 1958.
249. JAENICKE, E.: Conversion of β-carbon of serine to N^{10}-formyltetrahydrofolic acid. Fed. Proc. **15:** 281, 1956.
250. JEDEIKIN, L. A. AND WEINHOUSE, S.: Metabolism of neoplastic tissue. IV. Assay of oxidized and reduced diphosphopyridine nucleotide in normal and neoplastic tissues. J. biol. Chem. **213:** 271-280, 1955.
251. JENNEY, E. H. AND PFEIFFER, C. C.: The convulsant effect of hydrazides and the antidotal effect of anticonvulsants and metabolites. J. Pharmacol. **122:** 110-123, 1958.
252. JENNEY, E. H., SMITH, R. P. AND PFEIFFER, C. C.: Pyridoxine as an antidote to semicarbazide seizures. Fed. Proc. **12:** 333, 1953.
253. JOLLIFFE, N.: Effects of vitamin B_6 in paralysis agitans. Trans. Amer. neurol. Ass. **66:** 54-59, 1940.
254. KAPELLER-ADLER, R. AND MCFARLANE, H.: Recent studies with histaminase. Biochem. J. **82:** 49-50, 1962.
255. KAPELLER-ADLER, R. AND MCFARLANE, H.: Purification and identification of hog-kidney histaminase. Biochim. biophys. Acta **67:** 542-565, 1963.
256. KAPLAN, N. O.: Coenzyme metabolism of the brain. In: The Neurochemistry of Nucleotides and Amino Acids, ed. by R. O. Brady and D. B. Tower, pp. 70-88. Wiley & Sons, Inc., New York, 1960.

257. KENT, A. B., KREBS, E. G. AND FISCHER, E. H.: Properties of crystalline phosphorylase b. J. biol. Chem. 232: 549–558, 1958.
258. KESSEL, D.: Increased brain γ-aminobutyrate in diaminobutyrate intoxication. Fed. Proc. 18: 258, 1959.
259. KEWITZ, H.: Nachweis von 4-Amino-n-Butyrylcholin im Warmblütergehirn. Arch. exp. Path. Pharmak. 237: 308–318, 1959.
260. KHOMUTOV, R. M., KARPEYSKII, M. J., SEVERIN, E. S. AND GNUCHEV, N. V.: On the mechanism underlying the interaction of cycloserine and pyridoxal enzymes. Dokl. Akad. Nauk SSSR 140: 492–495, 1961.
261. KIKUCHI, G., KUMAR, A., TALMAGE, P. AND SHEMIN, D.: The enzymatic synthesis of δ-aminolevulinic acid. J. biol. Chem. 233: 1214–1219, 1958.
262. KILLAM, K. F.: Convulsant hydrazides. II. Comparison of electrical changes and enzyme inhibition induced by the administration of thiosemicarbazide. J. Pharmacol. 119: 263–271, 1957.
263. KILLAM, K. F.: Possible role of gamma-aminobutyric acid as an inhibitory transmitter. Fed. Proc. 17: 1018–1024, 1958.
264. KILLAM, K. F. AND BAIN, J. A.: Convulsant hydrazides. I. In vitro and in vivo inhibition of vitamin B_6-enzymes by convulsant hydrazides. J. Pharmacol. 119: 255–262, 1957.
265. KILLAM, K. F., DASGUPTA, S. R. AND KILLAM, E. K.: Studies of the action of convulsant hydrazides as vitamin B_6 antagonists in the central nervous system. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 302–316. Pergamon Press, New York, 1960.
266. KIZER, D. E. AND CHAN, S. K.: The effect of hepatocarcinogenesis upon 5-hydroxytryptophan decarboxylase and serotonin deaminase. Cancer Res. 21: 489–495, 1961.
267. KLINE, B. E., RUSCH, H. P., BAUMANN, C. A. AND LAVIK, P. S.: The effect of pyridoxine on tumor growth. Cancer Res. 3: 825–829, 1943.
268. KLINGHARDT, G. W.: Untersuchungen zur Neurotoxizität des Isonicotinsäurehydrazids und ihrer Verhütung. Verh. dtsch. Ges. inn. Med. 64: 352–356, 1958–59.
269. KLINGHARDT, G. W.: Experimentelle degenerative Nervenschädigungen durch Verbindungen des Hydrazins und des Hydroxylamins. Naturwissenschaften 48: 381, 1961.
270. KLINGHARDT, G. W., RADENBACH, K. L. AND MROWKA, F.: Neurologische Komplikationen bei der Tuberkulosebehandlung mit Isonicotinsäurehydrazid. Wien. med. Wschr. 104: 304–306, 1954.
271. KNAPP, A.: Tryptophanbelastung und Vitamin B_6-Mangel. I. Mitteilung. Die Ausscheidung von Xanthurensäure, Kynurenin, Trigonellinamid und 5-Hydroxyindolessigsäure bei verschiedenen Erkrankungen. Dtsch. Gesundheitswes. 16: 941–951, 1961.
272. KNAPP, A.: Tryptophanbelastung und Vitamin B_6-Mangel. II. Mitteilung. Statistische Untersuchungen. Dtsch. Gesundheitswes. 16: 993–994, 1961.
273. KNAPP, A.: Tryptophanbelastung und Vitamin B_6-Mangel. III. Mitteilung. Hereditäre Faktoren für den Ausfall des Tryptophanbelastungstestes. Dtsch. Gesundheitswes. 16: 1041–1045, 1961.
274. KNOX, W. E.: Adaptive enzymes in animals. In: Enzymes and Drug Action, Ciba Foundation Symposium, ed. by J. L. Mongar, pp. 245–274. J. & A. Churchill, London, 1962.
275. KNOX, W. E.: An evaluation of the treatment of phenylketonuria with diets low in phenylalanine. Pediatrics 26: 1–11, 1960.
276. KNOX, W. E. AND MEHLER, A. H.: The conversion of tryptophan to kynurenine in liver. 1. The coupled tryptophan peroxidase-oxidase system forming formylkynurenine. J. biol. Chem. 187: 419–430, 1950.
277. KOBINGER, W.: Beeinflussung der Cardiazolkrampfschwelle durch veränderten 5-Hydroxytryptamingehalt des Zentralnervensystems. Arch. exp. Path. Pharmak. 233: 559–566, 1958.
278. KOCH, R.: Zur Strahlenschutzwirkung eines SH-haltigen Vitamin B_6-Derivates. Acta chem. scand. 12: 1873–1875, 1958.
279. KOCH, R., KLEMM, D. AND SEITER, I.: Zur Toxikologie schwefelhaltiger Pyridoxin-Derivate. Arzneim.-Forsch. 10: 683–686, 1960.
280. KOCH, R., LANGENDORFF, H. AND MELCHING, H.-J.: Der chemische Strahlenschutz. Chemotherapia 3: 153–175, 1961.
281. KOCH, R. AND SCHMIDT, U.: Untersuchungen über einen biologischen Strahlenschutz. XXXIV. Mitteilung: Weitere Untersuchungen zur Strahlenschutzwirkung SH-Gruppen-tragender Vitamin B_6-Derivate. Strahlentherapie 113: 89–99, 1960.
282. KOHN, R., HEILMEYER, L. AND CLOTTEN, R.: Reversible Pyridoxin-sensible symptomatische sideroachrestische Anämie unter Isoniazidbehandlung bei einer käsigen Lymphknotentuberkulose mit Pleuritis exsudativa. Dtsch. med. Wschr. 87: 1765–1768, 1962.
283. KOJIMA, K., MIZUNO, K. AND MIYAZAKI, M.: Gamma-amino-butyric acid in ocular tissue. Nature, Lond. 181: 1200–1201, 1958.
284. KOTAKE, Y. AND MASAYAMA, T.: Studien über den intermediären Stoffwechsel des Tryptophans. Hoppe-Seyl. Z. 243: 237–244, 1936.
285. KRAFT, H.-G., FIEBIG, L. AND HOTOVY, R.: Zur Pharmakologie des Vitamin B_6 und seiner Derivate. Arzneim.-Forsch. 11: 922–929, 1961.
286. KRAMER, W.: Cerebral complication following isoniazid administration. Acta tuberc. scand. 38: 51–57, 1960.
287. KREBS, H. A.: Untersuchungen über den Stoffwechsel der Aminosäuren im Tierkörper. Hoppe-Seyl. Z. 217: 191–227, 1933.
288. KRIEGER-THIEMER, E.: Akute Isoniazidvergiftung. Ärztl. Wschr. 13: 17–19, 1958.
289. KRÜCKE, W.: Erkrankungen der peripheren Nerven. IV. Die polyneuritischen Krankheitsbilder. In: Handbuch

der spez. path. Anatomie, ed. by G. Döring, B. Herzog, W. Krücke and H. Orthner, vol. 13, part 5, pp. 117–196. Springer Verlag, Berlin, 1955.

290. Kuchinskas, E. J., Horvath, A. and Du Vigneaud, V.: An antivitamin B_6-action of L-penicillamine. Arch. Biochem. **68**: 69–75, 1957.
291. Kuchinskas, E. J. and du Vigneaud, V.: An increased vitamin B_6-requirement in the rat on a diet containing L-penicillamine. Arch. Biochem. Biophys. **66**: 1–9, 1957.
292. Kuemmerle, H. P., Senn, A., Rentchnick, P. and Goosens, N.: Klinik und Therapie der Nebenwirkungen. Georg Thieme Verlag, Stuttgart, 1960.
293. Kuffler, S. W. and Edwards, C.: Mechanism of gamma aminobutyric acid (Gaba) action and its relation to synaptic inhibition. J. Neurophysiol. **21**: 589–610, 1958.
294. Kuntzman, R., Costa, E., Gessa, G. L., Hirsch, C. and Brodie, B. B.: Combined use of α-methyl metatyrosine (MMT) and reserpine to associate norepinephrine (NE) with excitation and serotonin (5-HT) with sedation. Fed. Proc. **20**: 308, 1961.
295. Kuriaki, K., Yakushiji, T., Noro, T., Shimizu, T. and Saji, S. H.: γ-Aminobutyrylcholine. Nature, Lond. **181**: 1336–1337, 1958.
296. Langendorff, H., Langendorff, M. and Koch, R.: Untersuchungen über einen biologischen Strahlenschutz. XXIV. Mitteilung. Überprüfung des spezifischen Strahlenschutzeffektes der Cystein-Cysteamingruppe unter Berücksichtigung eines Mercapto-Pyridin-Derivates. Strahlentherapie **107**: 121–126, 1958.
297. Langendorff, H. and Melching, H.-J.: Untersuchungen über einen biologischen Strahlenschutz. XXXIII. Mitteilung. Weitere Untersuchungen über den Wirkungsmechanismus des 5-Hydroxy-Tryptamin im Strahlenschutzversuch. Strahlentherapie **110**: 505–509, 1959.
298. Langendorff, H., Melching, H.-J. and Ladner, H. A.: Untersuchungen über einen biologischen Strahlenschutz. XXXI. Mitteilung. Zum Wirkungsmechanismus des 5-Hydroxy-Tryptamin im Strahlenversuch. Strahlentherapie **109**: 554–560, 1959.
299. Langendorff, M., Melching, H.-J., Langendorff, H., Koch, R. and Jaques, R.: Untersuchungen über einen biologischen Strahlenschutz. XXI. Mitteilung. Weitere Untersuchungen zur Wirkung zentral-erregender und -dämpfender Pharmaka auf die Strahlenempfindlichkeit des Tieres. Strahlentherapie **104**: 338–344, 1957.
300. Langendorff, H., Melching, H.-J. and Rösler, H.: Untersuchungen über einen biologischen Strahlenschutz. XXXVII. Mitteilung. Über den Anteil des Adenylsäuresystems und des Pyridoxal-5-Phosphats am Strahlenschutzeffekt des Serotonins. Strahlentherapie **113**: 603–609, 1960.
301. Langendorff, H., Melching, H.-J. and Streffer, C.: Untersuchungen über einen biologischen Strahlenschutz. XLII. Mitteilung. Der Einfluss des 5-Hydroxy-Tryptamin auf strahlenbedingte Veränderungen des Aminosäurestoffwechsels. Strahlentherapie **116**: 1–14, 1961.
302. Lauwers, P., Verstraete, M. and Joossens, J. V.: Methyldopa in the treatment of hypertension. Brit. med. J. 295–300, 1963.
303. Laver, W. G., Neuberger, A. and Udenfriend, S.: Initial stages in the biosynthesis of porphyrins: 1. The formation of δ-aminolaevulic acid by particles obtained from chicken erythrocytes. Biochem. J. **70**: 4–14, 1958.
304. Leitch, I. and Hepburn, A.: Pyridoxine: Metabolism and requirement. Nutr. Abstr. Rev. **31**: 389–401, 1961.
305. Lepkovsky, S., Roboz, E. and Haagen-Smit, A. J.: Xanthurenic acid and its role in the tryptophan metabolism of pyridoxine-deficient rats. J. biol. Chem. **149**: 195–201, 1943.
306. Leuthardt, F. M. and Greenstein, J. P.: Degradation of cystine peptides by tissues. Science **101**: 19–21, 1945.
307. Levine, R. R., Smith, E. R. and Clark, B. B.: The effect of pyridoxal and other compounds on the mechanical activity and the sodium and potassium content of isolated rabbit atria. J. Pharmacol. **128**: 159–167, 1960.
308. Lewis, W. C., Caldon, G., Thurston, J. R. and Gilson, W. E.: Psychiatric and neurological reactions to cycloserine in the treatment of tuberculosis. Dis. Chest **32**: 172–182, 1957.
309. Loefer, J. B.: Effect of pyridoxine and desoxypyridoxine on rat fibrosarcoma grafts. Cancer Res. **11**: 481–484, 1951.
310. Loew, O.: Giftwirkung des Diamids. Ber. dtsch. chem. Ges. **23**: 3203–3206, 1890.
311. Louttit, R. T.: Effect of phenylalanine and isocarboxazid feeding on brain serotonin and learning behavior in the rat. J. comp. Physiol. Psychol. **55**: 425–428, 1962.
312. Lovenberg, W., Weissbach, H. and Udenfriend, S.: Aromatic L-aminoacid decarboxylase. J. biol. Chem. **237**: 89–93, 1962.
313. Lowe, I. P., Robins, E. and Eyerman, G. S.: The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. J. Neurochem. **3**: 8–18, 1958.
314. Lyon, J. B., Bain, J. A. and Williams, H. L.: The distribution of vitamin B_6 in the tissue of two inbred strains of mice fed complete and vitamin B_6-deficient rations. J. biol. Chem. **237**: 1989–1991, 1962.
315. Lyon, J. B. and Porter, J.: The effect of pyridoxine deficiency on muscle and liver phosphorylase of two inbred strains of mice. Biochim. biophys. Acta **58**: 248–254, 1962.
316. Mackay, D.: The mechanism of the reaction of cysteine with pyridoxal 5'-phosphate. Arch. Biochem. **99**: 93–100, 1962.
317. Makino, K., Kinoshita, T., Aramaki, Y. and Shintani, S.: Atoxopyrimidine action of vitamins of the B_6-group. Nature, Lond. **174**: 275–276, 1954.
318. Makino, K. and Koike, M.: Competitive inhibition of pyridoxal phosphate action by toxopyrimidine phosphate in the tyrosine decarboxylase system (anti-coenzyme). Enzymologia **17**: 157–163, 1954.
319. Manthei, R. W.: Vitamin B_6 and isoniazid toxicity. Fed. Proc. **19**: 414, 1960.
320. Marco, C. de and Bognolo, D.: The reaction between cysteamine and pyridoxal phosphate. Arch. Biochem. Biophys. **98**: 526–527, 1962.

321. MARIE, J., HENNEQUET, A., LYON, G., DEBRIS, P. AND LEBALLE, J. C.: Pyridoxine dependence, a metabolic disease manifested by pyridoxine-sensitive convulsive seizures. Rev. Neurol. **105:** 406–419, 1961.
322. MASCITELLI-CORIANDOLI, E. AND BOLDRINI, R.: Pyridoxine and pyridoxal phosphate concentrations in the liver and heart of thyroxinized animals. Experientia **15:** 229–230, 1959.
323. MASCITELLI-CORIANDOLI, E., BOLDRINI, R. AND CITTERIO, C.: Different effects exerted by isoniazid upon the coenzyme A and pyridoxine contents of the host's tumor and tissues. Biochem. Pharmacol. **1:** 238, 1958.
324. MASKE, H.: Untersuchungen über einen Test zum Nachweis des Vitamin B_6-Mangels. Klin. Wschr. **35:** 561–565, 1957.
325. MASON, M. AND GULLEKSON, E.: Inhibition of pyridoxal phosphate-dependent enzymes by sulfate esters of estradiol, estrone and diethylstilbestrol. J. Amer. chem. Soc. **81:** 1517, 1959.
326. MASON, M. AND GULLEKSON, E.: Estrogen enzyme interactions: inhibition and protection of kynurenine transaminase by the sulfate esters of diethylstilbestrol, estradiol and estrone. J. biol. Chem. **235:** 1312–1316, 1960.
327. MASON, M. AND SCHIRCH, L.: Inhibition of B_6 enzymes by free and conjugated estrogens. Fed. Proc. **20:** 200, 1961.
328. MASSIEU, G. H., TAPIA, R. AND ORTEGA, B. G.: Free amino acids in brain of mice treated with L-glutamic acid-γ-hydrazide. Biochem. Pharmacol. **11:** 976–979, 1962.
329. MATSUDA, M. AND MAKINO, K.: Effects of pyridoxal 5-phosphate analogue, pyridoxal 5-sulfate, on glutamic acid decarboxylase. Biochim. biophys. Acta **48:** 194–195, 1961.
330. MAURER, H. J. AND DITTMEYER: Zur Wirkung der ionisierenden Strahlen auf die Vitamine der B-Gruppe. Fortschr. Röntgenstr. **86:** 28P, 1957.
331. MAXFIELD, J. R., MCILWAIN, A. J. AND ROBERTSON, J. E.: Treatment of radiation sickness with vitamin B_6 (pyridoxine-hydrochloride). Radiology **41:** 383–388, 1943.
332. MAY, C. D.: Vitamin B_6 in human nutrition: a critique and object lesson. Pediatrics **14:** 269–279, 1954.
333. MAYNERT, E. W. AND KAJI, H. K.: On the relationship of brain γ-aminobutyric acid to convulsions. J. Pharmacol. **137:** 114–121, 1962.
334. MCCALL, K. B., WAISMAN, H. A., ELVEHJEM, C. A. AND JONES, S. E.: A study of pyridoxine and pantothenate deficiencies in the monkey (Macaca mulatta). J. Nutr. **31:** 685–697, 1946.
335. MCCORMICK, D. B., GREGORY, M. E. AND SNELL, E. E.: Pyridoxal phosphokinases. I. Assay, distribution, purification and properties. J. biol. Chem. **236:** 2076–2084, 1961.
336. MCCORMICK, D. B., GUIRARD, B. M. AND SNELL, E. E.: Comparative inhibition of pyridoxal kinase and glutamic acid decarboxylase by carbonyl reagents. Proc. Soc. exp. Biol., N. Y. **104:** 554–557, 1960.
337. MCCORMICK, D. B. AND SNELL, E. E.: Pyridoxal kinase of human brain and its inhibition by hydrazine derivatives. Proc. nat. Acad. Sci., Wash. **45:** 1371–1379, 1959.
338. MCCORMICK, D. B. AND SNELL, E. E.: Pyridoxal phosphokinase. II. Effect of inhibitors. J. biol. Chem. **236:** 2085–2088, 1961.
339. MCFARLAND, M. L., PETERS, M. V., BALLANTYNE, R. M. AND MCHENRY, E. W.: Pyridoxine and experimental X-radiation injury in rats. Amer. J. Physiol. **163:** 394–399, 1950.
340. MCGEER, E. G. AND TISCHLER, B.: Vitamin B_6 and mental deficiency. The effects of large doses of B_6 (pyridoxine) in phenylketonuria. Canad. J. Biochem. Physiol. **37:** 485–491, 1959.
341. MCGEER, P. L., BOULDING, J. E., GIBSON, W. C. AND FOULKES, R. G.: Drug induced extrapyramidal reactions. Treatment with diphenhydramine hydrochloride and dihydroxyphenylalanine. J. Amer. med. Ass. **177:** 665–670, 1961.
342. MCGRATH, F. P., COMSTOCK, C. C. AND OBERST, F. W.: Acute and subacute inhalation toxicity of hydrazine vapors to rats. Fed. Proc. **11:** 374, 1952.
343. MCKEAN, C. M., SCHANBERG, S. M. AND GIARMAN, N. J.: A mechanism of the indole defect in experimental phenylketonuria. Science **137:** 604–605, 1962.
344. MCKHANN, G. M., ALBERS, R. W., SOKOLOFF, L., MICKELSEN, O. AND TOWER, D. B.: The quantitative significance of the gamma-aminobutyric acid pathway in cerebral oxidative metabolism. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 169–181. Pergamon Press, New York, 1960.
345. MCKHANN, G. M., MICKELSEN, O. AND TOWER, D. B.: Oxidative metabolism of incubated cerebral cortex slices from pyridoxine-deficient kittens. Amer. J. Physiol. **200:** 34–38, 1961.
346. MCKHANN, G. M. AND TOWER, D. B.: Gamma aminobutyric acid: a substrate for oxidative metabolism of cerebral cortex. Amer. J. Physiol. **196:** 36–38, 1959.
347. MCKIBBIN, J. M., SCHAEFER, A. E., FROST, D. V. AND ELVEHJEM, C. A.: Studies on anemia in dogs due to pyridoxine deficiency. J. biol. Chem. **142:** 77–84, 1942.
348. MEISTER, A.: Transamination. Advanc. Enzymol. **16:** 185–246, 1955.
349. MELCHING, H.-J.: Untersuchungen über den chemischen Strahlenschutz. Dtsch. med. Wschr. **85:** 2284–2288, 1960.
350. MELCHING, H.-J. AND LANGENDORFF, M.: Das Rauwolfia-Alkaloid Reserpin als wirksame Strahlenschutzsubstanz. Naturwissenschaften **44:** 377, 1957.
351. MELCHING, H.-J., MESSERSCHMIDT, O., STREFFER, C. AND SHIBATA, K.: Untersuchungen über einen biologischen Strahlenschutz. XLIV. Mitteilung. Der therapeutische Einfluss der Splenektomie auf den Strahlenschaden. Strahlentherapie **116:** 395–403, 1961.
352. METZLER, D. E., IKAWA, M. AND SNELL, E. E.: A general mechanism for vitamin B_6-catalyzed reactions. J. Amer. chem. Soc. **76:** 648–652, 1954.
353. MICKELSEN, O.: Intestinal synthesis of vitamins in the nonruminant. Vitam. & Horm. **14:** 1–95, 1956.

354. MIELKE, H. G.: Aplastische Anämie (Erythroblastophthise) nach INH-Behandlung. Folia haemat., Frankf. **2**: 1–10, 1958.
355. MIHICH, E. AND NICHOL, C. A.: The effect of pyridoxine deficiency on mouse sarcoma 180. Cancer Res. **19**: 279–284, 1959.
356. MIHICH, E. AND NICHOL, C. A.: Development of a subline of mouse sarcoma-180 capable of growing in pyridoxine deficient animals. Nature, Lond. **188**: 379–382, 1960.
357. MIHICH, E., ROSEN, F. AND NICHOL, C. A.: The effect of pyridoxine deficiency on a spectrum of mouse and rat tumors. Cancer Res. **19**: 1244–1248, 1959.
358. MILLICHAP, J. G., WOODBURY, D. M. AND GOODMAN, L. S.: Mechanism of the anticonvulsant action of acetazoleamide, a carbonic anhydrase inhibitor. J. Pharmacol. **115**: 251–258, 1955.
359. MINER, D. L., MILLER, J. A., BAUMANN, C. A. AND RUSCH, H. P.: The effect of pyridoxine and other B vitamins on the production of liver cancer with p-dimethylaminoazobenzene. Cancer Res. **3**: 296–302, 1943.
360. MITOMA, C.: An evaluation of evidence for beta-hydroxy-gamma-aminobutyric acid as a brain metabolite. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 236–237. Pergamon Press, New York, 1960.
361. MOLDAVE, K.: The effect of vitamin B_6 on the in vivo utilization of radioactive phenylalanine. Arch. Biochem. Biophys. **75**: 418–424, 1958.
362. MOLONY, C. J. AND PARMELEE, A. H.: Convulsions in young infants as a result of pyridoxine (vitamin B_6) deficiency. J. Amer. med. Ass. **154**: 405–406, 1954.
363. MORCZEK, A. AND NEUMEISTER, K.: Behandlung der strahleninduzierten Blutbildschädigungen mit Vitamin B_6. Strahlentherapie **120**: 398–404, 1963.
364. MÜLLER-CALGAN, H. AND HOTOVY, R.: Der Einfluss von Pyridoxol und von zwei Pyridoxoldisulfiden auf das Verhalten von Katzen und Rhesusaffen. Arch. exp. Path. Pharmak. **243**: 314, 1962.
365. MURPHY, G. F. AND SOURKES, T. L.: Effect of catecholamino acids on the catecholamine content of rat organs. Rev. canad. Biol. **18**: 379–388, 1959.
366. MURPHY, G. F. AND SOURKES, T. L.: The action of antidecarboxylases on the conversion of 3,4-dihydroxyphenylalanine to dopamine in vivo. Arch. Biochem. Biophys. **93**: 338–343, 1961.
367. MUSHETT, C. W.: Discussion note. Amer. J. clin. Nutr. **4**: 326–327, 1956.
368. MUSHETT, C. W. AND EMERSON, G.: Arteriosclerosis in pyridoxine deficient monkeys and dogs. Fed. Proc. **16**: 367, 1957.
369. NADLER, H. L. AND YI-YUNG HSIA, D.: Epinephrine metabolism in phenylketonuria. Proc. Soc. exp. Biol., N. Y. **107**: 721–723, 1961.
370. NAKKEN, K. F.: The action of ionizing radiation on pyridoxal-5-phosphate (PLP) and pyridoxine-phosphate. Acta physiol. scand. **50**: suppl. 175, 108–109, 1960.
371. NARUSE, H., KATO, M., KUROKAWA, M., HABA, R. AND YABE, T.: Metabolic defects in a convulsive strain of mouse. J. Neurochem. **5**: 359–369, 1959–60.
372. NEAME, K. D.: Phenylalanine as inhibitor of transport of amino acids in brain. Nature, Lond. **192**: 173–174, 1961.
373. NEUBERGER, A.: Aspects of metabolism of glycine and porphyrins. Biochem. J. **78**: 1–10, 1961.
374. NICKERSON, W. J. AND ROMANO, A. H.: Enzymatic reduction of cystine by coenzyme I (DPNH). Science **115**: 676–678, 1952.
375. NISHIZAWA, Y., KODAMA, T. AND SUGAHARA, S.: Brain γ-aminobutyric acid during and on suppressing of the running fit. J. Vitaminol., Kyoto **6**: 236–239, 1960.
376. NOALL, M. W., RIGGS, T. R., WALKER, L. M. AND CHRISTENSEN, H. N.: Endocrine control of amino acid transfer. Distribution of an unmetabolizable amino acid. Science **126**: 1002–1005, 1957.
377. NORDIO, S., SEGNI, G., ROMANO, C. AND GROSSI-BIANCHI, M. L.: Unusual diseases which are susceptible to treatment with pyridoxine. Mschr. Kinderheilk. **110**: 116–118, 1961.
378. NORIMUTSU, K.: The interrelationship between xanthurenic acid formation and amino acid metabolism in the rat. Kumamoto med. J. **12**: 39–63, 1959.
379. OATES, J. A., GILLESPIE, L., UDENFRIEND, S. AND SJOERDSMA, A.: Decarboxylase inhibition and blood pressure reduction by α-methyl-3,4-dihydroxy-*dl*-phenylalanine. Science **131**: 1890–1891, 1960.
380. OEHME, P.: Die Aktivität verschiedener Transaminasen unter Hydroxylamin, *d*-Cycloserin und *d,l*-Cycloserin. Naturwissenschaften **50**: 20–21, 1963.
381. OHARA, K., SANO, I., KOIZUMI, H. AND NISHINUMA, K.: Free β-hydroxy-γ-aminobutyric acid in brain. Science **129**: 1225–1226, 1959.
382. OLIVARD, J. AND SNELL, E. E.: Growth and enzymatic activities of vitamin B_6 analogues. J. biol. Chem. **213**: 203–228, 1955.
383. OLIVEIRA, J. E. D. DE, PEARSON, W. N., MISRA, K. P., HUDSON, G. W. AND DARBY, W. J.: The effect of X-irradiation on the vitamin excretion of the rhesus monkey (*Macaca mulatta*). J. Nutr. **59**: 527–537, 1956.
384. OLSON, N.: Discussion note. Amer. J. clin. Nutr. **4**: 327–328, 1956.
385. OPPENHEIM, A. AND LIH, B.: Peroral administration of vitamin B_6 (pyridoxine hydrochloride) in the treatment of radiation sickness. Radiology **47**: 381–385, 1946.
386. OTT, W. H.: Antipyridoxine activity of 2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine in the chick. Proc. Soc. exp. Biol., N. Y. **61**: 125–127, 1946.
387. OXENDER, D. L. AND CHRISTENSEN, H. N.: Transcellular concentration as a consequence of intracellular accumulation. J. biol. Chem. **234**: 2321–2324, 1959.
388. OXENDER, D. L. AND ROYER, M.: Action of pyridoxal-phosphate and related agents on uptake of amino acids by Ehrlich ascites cells. Fed. Proc. **20**: 140, 1961.

389. PAL, P. R. AND CHRISTENSEN, H. N.: Interrelationships in the cellular uptake of amino acids and metals. J. biol. Chem. **234**: 613–617, 1959.
390. PAL, P. R. AND CHRISTENSEN, H. N.: Uptake of pyridoxal and pyridoxal phosphate by Ehrlich ascites tumor cells. J. biol. Chem. **236**: 894–897, 1961.
391. PALM, D.: Über die Hemmung der Dopa-Decarboxylase durch Isonicotinsäurehydrazid. Arch. exp. Path. Pharmak. **234**: 206–209, 1958.
392. PARE, C. M. B., SANDLER, M. AND STACEY, R. S.: 5-Hydroxytryptamine deficiency in phenylketonuria. Lancet **1**: 551–553, 1957.
393. PARE, C. M. B., SANDLER, M. AND STACEY, R. S.: Decreased 5-hydroxytryptophan decarboxylase activity in phenylketonuria. Lancet **2**: 1099–1101, 1958.
394. PARE, C. M. B., SANDLER, M. AND STACEY, R. S.: The relationship between decreased 5-hydroxyindole metabolism and mental defect in phenylketonuria. Arch. Dis. Childh. **34**: 422–425, 1959.
395. PARKS, R. E., KIDDER, G. W. AND DEWEY, V. C.: Thiosemicarbazide toxicity in mice. Proc. Soc. exp Biol., N. Y. **79**: 287–289, 1952.
396. PERAULT, A.-M., PULLMAN, B. AND VALDEMORO, C.: Electronic aspects of the reactions of pyridoxal phosphate enzymes. Biochim. biophys. Acta **46**: 555–575, 1961.
397. PISANO, J. J., MITOMA, C. AND UDENFRIEND, S.: Biosynthesis of γ-aminobutyric acid and arginine. Nature, Lond. **180**: 1125–1126, 1957.
398. PISANO, J. AND UDENFRIEND, S.: Formation of γ-guanidinobutyric and guanidinoacetic acids in brain. Fed. Proc. **17**: 403, 1958.
399. PLETSCHER, A.: On the mode of action of monoamine oxidase inhibitors in the brain. In: Biochemistry of the Central Nervous System, ed. by F. Brücke, vol. 3, pp. 124–130. Pergamon Press, New York, 1960.
400. PLETSCHER, A., GEY, K. F. AND ZELLER, P.: Monoaminoxydase-Hemmer. Chemie, Biochemie, Pharmakologie, Klinik. In: Fortschritte der Arzneimittelforschung, ed. by E. Zucker, vol. 2, pp. 417–590. Birkhäuser Verlag, Basel, 1960.
401. POPPEN, K. J., GREENBERG, L. D. AND RINEHART, J. F.: The blood picture of pyridoxine deficiency in the monkey. Blood **7**: 436–444, 1952.
402. PORTER, C. C., CLARK, I. AND SILBER, R. H.: The effect of B-vitamin deficiency on tryptophan metabolism in the rat. Arch. Biochem. **18**: 339–343, 1948.
403. PORTER, C. C. AND TITUS, D. C.: Distribution and metabolism of methyldopa in the rat. J. Pharmacol. **139**: 77–87, 1963.
404. PRICE, J. M., BROWN, R. R. AND LARSON, F. C.: Quantitative studies on human urinary metabolites of tryptophan as affected by isoniazid and deoxypyridoxine. J. clin. Invest. **36**: 1600–1607, 1957.
405. PRICE, J. M. AND DODGE, L. W.: Occurrence of the 8-methyl-ether of xanthurenic acid in normal human urine. J. biol. Chem. **223**: 699–704, 1956.
406. PRICE, J. M., MILLER, E. C. AND MILLER, J. A.: Intracellular distribution of vitamin B_6 in rat and mouse livers and induced rat liver tumors. Proc. Soc. exp. Biol., N. Y. **71**: 575–578, 1949.
407. PULT, N. AND BARANDUN, S.: Erfahrungsbericht des Team der Gesellschaft Schweizerischer Tuberkulosearzte zur klinischen Erprobung von Cycloserin. Schweiz. Z. Tuberk. **15**: 417–468, 1958.
408. PURPURA, D. P., BERL, S., GONZALEZ-MONTEAGUDO, O. AND WYATT, A.: Brain amino acid changes during methoxypyridoxine-induced seizures (cat). In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 331–335. Pergamon Press, New York, 1960.
409. PURPURA, D. P. AND CARMICHAEL, M. W.: Characteristics of blood-brain barrier to gamma-aminobutyric acid in neonatal cat. Science **131**: 410–412, 1960.
410. PURPURA, D. P., GIRADO, M. AND GRUNDFEST, H.: Selective blockade of excitatory synapses in the cat brain by γ-aminobutyric acid. Science **125**: 1200–1202, 1957.
411. PURPURA, D. P., GIRADO, M., SMITH, T. G., CALLAN, D. A. AND GRUNDFEST, H.: Structure-activity determinants of pharmacological effects of amino acids and related compounds on central synapses. J. Neurochem. **3**: 238–268, 1959.
412. QUACKENBUSH, F. W., STEENBOCK, H., KUMMEROW, F. A. AND PLATZ, B. R.: Linoleic acid, pyridoxine and pantothenic acid in rat dermatitis. J. Nutr. **21**: 225–234, 1942.
413. QUADBECK, G. AND SARTORI, G. D.: Über den Einfluss von Pyridoxin und Pyridoxal-5-Phosphat auf den Thiosemicarbazid-Krampf der Ratte. Arch. exp. Path. Pharmak. **230**: 457–461, 1957.
414. RABINOWITZ, J. C. AND SNELL, E. E.: The Vitamin B_6 group; microbiological activity and natural occurrence of pyridoxamine phosphate. J. biol. Chem. **169**: 643–650, 1947.
415. RABINOWITZ, J. C. AND SNELL, E. E.: The vitamin B_6 group. Distribution of pyridoxal, pyridoxamine and pyridoxine in some natural products. J. biol. Chem. **176**: 1157–1167, 1948.
416. RANKE, B., RANKE, E. AND CHOW, B. F.: The interrelationship between vitamin B_6 and B_{12} deficiencies in rats. J. Nutr. **71**: 411–415, 1960.
417. READ, M. S.: A summary of the wholesomeness of gamma-irradiated foods. Fed. Proc. **19**: 1055, 1960.
418. REID, J. D. AND SHEPHERD, D. M.: Inhibition of histidine-decarboxylases. Life Sci. **1**: 5–8, 1963.
419. REILLY, R. H., KILLAM, K. F., JENNEY, E. H., MARSHALL, W. H., TAUSIG, T., APTER, N. S. AND PFEIFFER, C. C.: Convulsant effects of isoniazid. J. Amer. med. Ass. **152**: 1317–1321, 1953.
420. REILLY, R. H., GUNTHER, M. W., TIENSTRA, H., SAJJADI, M., WEINER, P. S., ELLERD, A., TAUSIG, T. AND PFEIFFER, C. C.: Therapy of resistant schizophrenia with semicarbazide and photic stimulation. Fed. Proc. **12**: 359–360, 1953.
421. RICHARDSON, L. R., WILKES, S. AND RITCHEY, S. J.: Comparative vitamin B_6-activity of frozen, irradiated and heat-processed foods. J. Nutr. **73**: 363–368, 1961.

422. RICHTERICH, R.: Pathophysiologie und klinische Bedeutung der Plasma-Transaminasen. Röntgen- u. Lab.-Prax. **13:** L75–81, L91–99, L117–126, 1960.
423. RIGGS, T. R., COYNE, B. AND CHRISTENSEN, H. N.: Intensification of the cellular accumulation of amino acids by pyridoxal. Biochim. biophys. Acta **11:** 303–304, 1953.
424. RIGGS, T. R. AND WALKER, L. M.: Diminished uptake of C^{14}-α-aminoisobutyric acid by tissues of vitamin B_6-deficient rats. J. biol. Chem. **233:** 132–137, 1958.
425. RINDI, G. AND FERRARI, G.: The gamma-aminobutyric acid and glutamic acid content of brains of rats treated with toxopyrimidine. Nature, Lond. **183:** 608–609, 1959.
426. RINEHART, J. F. AND GREENBERG, L. D.: Vitamin B_6 deficiency in the rhesus monkey with particular reference to the occurrence of arteriosclerosis, dental caries and hepatic cirrhosis. Amer. J. clin. Nutr. **4:** 318–328, 1956.
427. ROBERTS, E. AND BAXTER, C. F.: γ-Aminobutyric acid in brain. In: Biochemistry of the Central Nervous System, ed. by F. Brücke, vol. 3, pp. 268–278. Pergamon Press, New York, 1960.
428. ROBERTS, E. AND EIDELBERG, E.: Metabolic and neurophysiological roles of γ-aminobutyric acid. Int. Rev. Neurobiol. **2:** 279–332, 1960.
429. ROBERTS, E. AND FRANKEL, S.: γ-Aminobutyric acid in brain: its formation from glutamic acid. J. biol. Chem. **187:** 55–63, 1950.
430. ROBERTS, E. AND FRANKEL, S.: Glutamic acid decarboxylase in brain. J. biol. Chem. **188:** 789–795, 1951.
431. ROBERTS, E. AND FRANKEL, S.: Further studies of glutamic acid decarboxylase in brain. J. biol. Chem. **190:** 505–512, 1951.
432. ROBERTS, E., ROTHSTEIN, M. AND BAXTER, C. F.: Some metabolic studies of γ-aminobutyric acid. Proc. Soc. exp. Biol., N. Y. **97:** 796–802, 1958.
433. ROBITZEK, E. H. AND NENASHEV, P. K.: Toxicidad de la cicloserina en el tratamiento de la tuberculosis en humanos, con referencia al uso de la piridoxina. Rev. mex. Tuberc. **18:** 553–557, 1957.
434. RODWELL, V. W., VOLCANIN, B. E., IKAWA, M. AND SNELL, E. E.: Bacterial oxidation of vitamin B_6. I. Isopyridoxal and 5-pyridoxic acid. J. biol. Chem. **233:** 1548–1554, 1958.
435. ROSE, W. C.: Nutritive significance of amino acids and certain related compounds. Science **86:** 298–300, 1937.
436. ROSEN, F.: Effect of isonicotinic acid hydrazide on niacin and pyridoxine metabolism in rats. Proc. Soc. exp. Biol., N. Y. **88:** 243–246, 1955.
437. ROSEN, F., BUDNICK, L. E., SOLOMON, D. K. AND NICHOL, C. A.: Corticosteroids and transaminase activity. III. A relationship between changes in alanine transaminase activity and the growth of Walker carcinosarcoma 256. Cancer Res. **21:** 620–626, 1961.
438. ROSEN, F., MILHOLLAND, R. J. AND SOLOMON, D.: Relation of pyridoxine to the basal and adaptive levels of tyrosine-α-ketoglutarate transaminase (TKT) in rat liver. Fed. Proc. **20:** 225, 1961.
439. ROSIE, R. H.: Vitamin B_6. Forschung, Klinik, Praxis. Wissenschaftl. Ber. E. Merck AG, Darmstadt, 1959.
440. ROSS, R. R.: Use of pyridoxine hydrochloride to prevent isoniazid toxicity. J. Amer. med. Ass. **168:** 273–275, 1958.
440a. ROZE, U. AND STROMINGER, J. L.: The non-enzymatic reaction between D-cycloserine and pyridoxal phosphate. Fed. Proc. **22:** 423, 1963.
441. RUBIN, B., HASSERT, G. L., THOMAS, B. G. H. AND BURKE, J. C.: Pharmacology of isonicotinic acid hydrazide (Nydrazid). Amer. Rev. Tuberc. **65:** 392–401, 1952.
442. RUDESILL, C. L. AND WEIGAND, C. G.: The treatment of Parkinson's disease with pyridoxine hydrochloride (vitamin B_6 hydrochloride). J. Ind. med. Ass. **34:** 355–360, 1941.
443. SALDIAS, C., CABIESES, F. AND EIDELBERG, E.: Electroencephalographic changes induced by the intravenous administration of acetazolamide (Diamox) in epileptic patients. Electroenceph. clin. Neurophysiol. **9:** 333–336, 1957.
444. SALVADOR, R. A. AND ALBERS, R. W.: The distribution of glutamic-γ-aminobutyric transaminase in the nervous system of the rhesus monkey. J. biol. Chem. **234:** 922–925, 1959.
445. SANDMANN, R. AND SNELL, E. E.: Some effects of ω-methyl analogues of vitamin B_6 in rats. Proc. Soc. exp. Biol., N. Y. **90:** 63–67, 1955.
446. SANO, I., GAMO, T., KAKIMOTO, Y., TANIGUCHI, K., TAKESADA, M. AND NISHINUMA, K.: Distribution of catechol compounds in human brain. Biochim. biophys. Acta **32:** 586–587, 1959.
447. SANO, K. AND ROBERTS, E.: Binding of γ-aminobutyric acid by brain preparations. Biochem. biophys. Res. Comm. **4:** 358–361, 1961.
448. SARRETT, H. P.: A study of the measurement of 4-pyridoxic acid in urine. J. biol. Chem. **189:** 769–777, 1951.
449. SCHAUMANN, W.: Beeinflussung der analgetischen Wirkung des Morphins durch Reserpin. Arch. exp. Path. Pharmak. **235:** 1–9, 1958.
450. SCHEIER, G. E. AND WILLIAMS, M. A.: Sequential changes in liver fatty acids of rats fed methyl-linoleate with and without vitamin B_6. Fed. Proc. **21:** 393, 1962.
451. SCHEINBERG, I. H. AND STERNLIEB, I.: Copper metabolism. Pharmacol. Rev. **12:** 355–381, 1960.
452. SCHLENK, F. AND FISCHER, A.: Studies on glutamic-aspartic acid transminase. Arch. Biochem. **12:** 69–78, 1947.
453. SCHLICHTERGROLL, A. VON: Personal communication.
454. SCHNEIDER, J. A.: Reserpine antagonism of morphine analgesia in mice. Proc. Soc. exp. Biol., N. Y. **87:** 614–615, 1954.
455. SCHOTT, H. F. AND CLARK, W. G.: Dopadecarboxylase inhibition through the interaction of coenzyme and substrate. J. biol. Chem. **196:** 449–462, 1952.
456. SCHÜMANN, H. J.: Formation of adrenergic transmitters. In: Adrenergic Mechanisms, ed. by J. R. Vane, G. E. W. Wolstenholme and C. M. O'Connor, pp. 6–16. J. & A. Churchill, Ltd., London, 1960.

457. SCHULMAN, M. P. AND RICHERT, D. A.: Heme synthesis in vitamin B_6 and pantothenic acid deficiencies. J. biol. Chem. **226**: 181–189, 1957.
458. SCHWABE, U.: Vorkommen und Bedeutung der γ-Aminobuttersäure im Zentralnervensystem. Dtsch. med. Wschr. **86**: 2235–2239, 1961.
459. SCOTT, L. D. AND TARLETON, G. J.: Pyridoxine hydrochloride (vitamin B_6) in the control of radiation sickness. Radiology **47**: 386–391, 1946.
460. SCRIVER, C. R.: Vitamin B_6-dependency and infantile convulsions. Pediatrics **26**: 62–74, 1960.
461. SCRIVER, C. R. AND HUTCHISON, J. H.: The vitamin B_6 deficiency syndrome in human infancy: Biochemical and clinical observations. Pediatrics **31**: 240–250, 1963.
462. SHAH, S. N., JOHNSTON, P. V. AND KUMMEROW, F. A.: The effect of pyridoxine on cholesterol metabolism. J. Nutr. **72**: 81–86, 1960.
463. SHAPIRO, D. M., SHILS, M. E. AND DIETRICH, L. S.: Quantitative biochemical differences between tumor and host as a basis for cancer chemotherapy. I. Vitamin B_6. Cancer Res. **13**: 703–708, 1953.
464. SHERMAN, H.: Pyridoxine and related compounds (Vitamin B_6 group). X. Effects of deficiency. In: The Vitamins, ed. by W. H. Sebrell, Jr. and R. S. Harris, vol. 3, pp. 264–276. Academic Press, New York, 1954.
465. SIEBENMANN, C. O. AND BARBARA, C. B.: Some observations regarding hydrazides and convulsions: Anticonvulsant properties of 2-formyl-phenoxyacetic acid towards hydrazides. Fed. Proc. **19**: 24, 1960.
466. SIEGEL, D.: Cell nucleus morphological changes in Ehrlich ascites tumor cells by isonicotinic hydrazide (INH). Arch. Geschwulstforsch. **18**: 295–298, 1962.
467. SILVERMAN, A., KLIGERMAN, M. M., FERTIG, J. W. AND ELLIS, K.: The effectiveness of pyridoxine and dramamine on clinical radiation sickness. Radiology **66**: 403–407, 1956.
468. SISKEN, B. S., SANO, K. AND ROBERTS, E.: γ-Aminobutyric acid content and glutamic acid decarboxylase and γ-aminobutyric acid transaminase activities in the optic lobe of the developing chicken. J. biol. Chem. **236**: 503–507, 1961.
469. SJOERDSMA, A.: Newer biochemical approaches to the treatment of hypertension. Ann. N. Y. Acad. Sci. **88**: 933–938, 1960.
470. SJOERDSMA, A., OATES, J. A., ZALTZMAN, P. AND UDENFRIEND, S.: Serotonin synthesis in carcinoid patients. Its inhibition by α-methyldopa with measurement of associated increases in urinary 5-hydroxy-tryptophan. New Engl. J. Med. **263**: 585–588, 1960.
471. SMITH, S. E.: Action of α-methyldopa on brain and intestinal 5-hydroxytryptamine in mice. J. Physiol. **148**: 18–19P, 1959.
472. SMITH, S. E.: The pharmacological actions of 3,4-dihydroxy-phenyl-α-methylalanine (α-methyldopa), an inhibitor of 5-hydroxytryptophan decarboxylase. Brit. J. Pharmacol. **15**: 319–327, 1960.
473. SNELL, E. E.: The vitamin activities of "pyridoxal" and "pyridoxamine." J. biol. Chem. **154**: 313–314, 1944.
474. SNELL, E. E.: Discussion note. Amer. J. clin. Nutr. **4**: 374–376, 1956.
475. SNELL, E. E.: Chemical structure in relation to biological activities of vitamin B_6. Vitam. & Horm. **16**: 77–125, 1958.
476. SNELL, E. E.: Some aspects of the metabolism of vitamin B_6. In: Vitamin Metabolism, ed. by W. Umbreit and H. Molitor, vol. 11, pp. 250–262. Pergamon Press, New York, 1960.
477. SNELL, E. E., GUIRARD, B. M. AND WILLIAMS, R. J.: Occurrence in natural products of a physiologically active metabolite of pyridoxine. J. biol. Chem. **143**: 519–530, 1942.
478. SNELL, E. E. AND RANNEFELD, A. N.: The vitamin B_6 group. The vitamin activity of pyridoxal and pyridoxamine for various organisms. J. biol. Chem. **157**: 475–489, 1945.
479. SNYDERMAN, S. E., CARRETERO, R. AND HOLT, L. E.: Pyridoxine deficiency in the human being. Fed. Proc. **9**: 371–372, 1950.
480. SOKOLOFF, L., LASSEN, N. A., MCKHANN, G. M. AND TOWER, D. B.: Effects of pyridoxine withdrawal on cerebral circulation and metabolism in a pyridoxine-dependent child. Nature, Lond. **183**: 751–753, 1959.
481. SOURKES, T. L.: Inhibition of dihydroxyphenylalanine decarboxylase by derivatives of phenylalanine. Arch. Biochem. Biophys. **51**: 444–456, 1954.
482. SOURKES, T. L., MURPHY, G. F., CHAVEZ, B. AND ZIELINSKA, M.: The action of some α-methyl and other amino acids on cerebral catecholamines. J. Neurochem. **8**: 109–115, 1961.
483. SOURKES, T. L., MURPHY, G. F. AND WOODFORD, V. R.: Effects of deficiencies of pyridoxine, riboflavin and thiamine upon the catecholamine content in rat tissues. J. Nutr. **72**: 145–152, 1960.
484. SPIES, T. D., HIGHTOWER, D. P. AND HUBBARD, L. H.: Some recent advances in vitamin therapy. J. Amer. med. Ass. **115**: 292–297, 1940.
485. SPRINCE, H., LOWY, R. S., FOLSOME, C. E., BEHRMANN, J. S. AND RARITAN, C. B.: Studies on the urinary excretion of "xanthurenic acid" during normal and abnormal pregnancy: a survey of the excretion of xanthurenic acid in normal nonpregnant, normal pregnant, preeclamptic, and eclamptic women. Amer. J. Obstet. Gynec. **62**: 84–92, 1951.
486. SPRINGSON, D. B. AND COULON, A.: The precursors of sphingosine in brain tissue. J. biol. Chem. **207**: 585–592, 1954.
487. STEKOL, J. A., WEISS, S. AND ANDERSON, E. I.: On the activity of "cystine desulfurase" in mouse tumors. J. biol. Chem. **233**: 936–938, 1958.
488. STENGER, K. AND WOLF, H.: Krämpfe im Säuglingsalter und Vitamin B_6-Stoffwechsel. Mschr. Kinderheilk. **108**: 98–100, 1960.
489. STOERK, H. C.: The regression of lymphosarcoma implants in pyridoxine deficient mice. J. biol. Chem. **171**: 437–438, 1947.

490. STREET, H. R., COWGILL, G. R. AND ZIMMERMAN, H. M.: Some observations of vitamin B_6 deficiency in dog. J. Nutr. 21: 275–290, 1941.
491. SWELL, L., LAW, M. D., SCHOOLS, P. E. AND TREADWELL, C. R.: Tissue lipid fatty acid composition in pyridoxine deficient rats. J. Nutr. 74: 148–156, 1961.
492. TABOR, C. W., TABOR, H. AND ROSENTHAL, S.: Purification of amine oxidase from beef plasma. J. biol. Chem. 208: 645–661, 1954.
493. TAKAHASHI, H., NAGASHIMA, A., KOSHINO, C. AND TAKAHASHI, H.: Effects of γ-aminobutyric acid (Gaba), γ-aminobutyryl choline (Gaba-CH) and their related substances on the cortical activity. Jap. J. Physiol. 9: 257, 1959.
494. TALLAN, H. H.: Free amino acids in brain after administration of imipramine, chlorpromazine and other psychotropic drugs. In: Amino Acid Pools. Distribution, Formation and Function of Free Amino Acids, ed. by J. T. Holden, pp. 465–470. Elsevier Publ. Co., Amsterdam, 1962.
495. TERROINE, T. AND ADRIAN, J.: Les interrelations vitaminiques. Carence en pyridoxine et teneurs des tissus en Riboflavine, Niacine et acide Pantothénique. Arch. Sci. physiol. 4: 435–444, 1950.
496. THOMSON, J. F. AND MIKUTA, E. T.: Effect of total body x-irradiation on tryptophan peroxidase activity of rat liver. Proc. Soc. exp. Biol., N. Y. 85: 29–32, 1954.
497. THOMPSON, R. Q. AND GUERRANT, N. B.: Effect of dietary protein and vitamin B_6 on hepatic thionase activity. J. Nutr. 50: 161–174, 1953.
498. TOWER, D. B.: Glutamic acid metabolism in the mammalian central nervous system. In: Biochemistry of the Central Nervous System, ed. by F. Brücke, vol. 3, pp. 213–250. Pergamon Press, New York, 1960.
499. TOWER, D. B.: Neurochemistry of Epilepsy. C. C Thomas, Springfield, 1960.
500. TSUKADA, Y., HIRANO, S., NAGATA, Y. AND MATSUTANI, T.: Metabolic studies of gamma-aminobutyric acid in mammalian tissues. In: Inhibition in the Nervous System and γ-Aminobutyric Acid, ed. by E. Roberts, pp. 163–168. Pergamon Press, New York, 1960.
501. UDENFRIEND, S., LOVENBERG, W. M. AND WEISSBACH, H.: L-Amino acid decarboxylase activity in mammalian tissues and its inhibition by α-methyl dopa. Fed. Proc. 19: 7, 1960.
502. UEDA, K., AKEDO, H. AND SUDA, M.: Intestinal absorption of amino acids. IV. Participation of pyridoxal phosphate in the active transfer of *l*-amino acids through the intestinal wall. J. Biochem., Tokyo 48: 584–592, 1960.
503. UMBREIT, W. W. AND WADDELL, J. G.: Mode of action of desoxypyridoxine. Proc. Soc. exp. Biol., N. Y. 70: 293–299, 1949.
504. UMBREIT, W. W., WOOD, W. A. AND GUNSALUS, I. C.: The activity of pyridoxal phosphate in tryptophane formation by cell-free enzyme preparations. J. biol. Chem. 165: 731–732, 1946.
505. UNNA, K. R.: Pyridoxine and related compounds. In: The Vitamins, ed. by W. H. Sebrell, Jr. AND R. S. Harris, vol. 3, pp. 290–293. Academic Press, New York, 1954.
505a. VAN SLYKE, D. D. AND MEYER, G. M.: The fate of protein digestion products in the body. III. The absorption of amino acids from the blood by the tissues. J. biol. Chem. 16: 197–212, 1913.
506. VATES, T., AGRANOFF, B. AND SOKOLOFF, L.: Glutamic decarboxylase activity in human cerebrospinal fluid. Fed. Proc. 18: 164, 1959.
507. VERLOOP, M. C. AND RADEMAKER, W.: Anemia due to pyridoxine deficiency in man. Brit. J. Haemat. 6: 66–80, 1960.
508. VICTOR, M. AND ADAMS, R. D.: Symposium on role of some newer vitamins in human metabolism and nutrition: The neuropathology of experimental vitamin B_6 deficiency in monkeys. Amer. J. clin. Nutr. 4: 346–353, 1956.
509. VILTER, R. W.: Symposium on role of some newer vitamins in human metabolism and nutrition: The metabolism of vitamin B_6 in human beings. Amer. J. clin. Nutr. 4: 378–385, 1956.
510. VILTER, R. W., MUELLER, J. F., GLAZER, H. S., JARROLD, T., ABRAHAM, J., THOMPSON, C. AND HAWKINS, V. R.: The effect of vitamin B_6 deficiency induced by desoxypyridoxine in human beings. J. Lab. clin. Med. 42: 335–357, 1953.
511. VOGT, M.: The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. 123: 451–481, 1954.
512. WACHSTEIN, M.: Evidence for abnormal vitamin B_6 metabolism in pregnancy and various disease states. Amer. J. clin. Nutr. 4: 369–377, 1956.
513. WACHSTEIN, M. AND GUDAITIS, A.: Detection of vitamin B_6-deficiency. Utilization of an improved method for rapid determination of xanthurenic acid in urine. Amer. J. clin. Path. 22: 652–655, 1952.
514. WACHSTEIN, M. AND GUDAITIS, A.: Disturbance of vitamin B_6 metabolism in pregnancy. J. Lab. clin. Med. 40: 550–557, 1952.
515. WACHSTEIN, M. AND GUDAITIS, A.: Disturbance of vitamin B_6 metabolism in pregnancy; influence of various amounts of pyridoxine hydrochloride upon abnormal tryptophane load test in pregnant women. J. Lab. clin. Med. 42: 98–107, 1953.
516. WACHSTEIN, M., KELLNER, J. D. AND ORTITZ, J. M.: Pyridoxal phosphate in plasma and leukocytes of normal and pregnant subjects following B_6 load test. Proc. Soc. exp. Biol., N. Y. 103: 350–353, 1960.
517. WACHSTEIN, M. AND LOBEL, S.: Relation between tryptophane metabolism and vitamin B_6 in various diseases as studied by paper chromatography. Amer. J. clin. Path. 26: 910–925, 1956.
518. WACHSTEIN, M., MOORE, C. AND GRAFFEO, L. W.: Pyridoxal phosphate (B_6-al-PO_4) levels of circulating leukocytes in maternal and cord blood. Proc. Soc. exp. Biol., N. Y. 96: 326–328, 1957.
519. WADA, H. AND SNELL, E. E.: The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. J. biol. Chem. 236: 2089–2095, 1961.
520. WAKIL, S. J.: Mechanism of fatty acid synthesis. J. Lipid Res. 2: 1–24, 1961.

521. WALLACH, D. P.: Studies on the GABA pathway. II. The lack of effect of pyridoxal phosphate on GABA-KGA transaminase inhibition induced by aminooxy acetic acid. Biochem. Pharmacol. **8:** 328-331, 1961.
522. WANG, H. L., HARWALKER, V. R. AND WAISMAN, H. A.: Influence of dietary phenylalanine and tryptophan on tissue serotonin. Fed. Proc. **20:** 6, 1961.
523. WEIL-MALHERBE, H.: Effect of convulsive therapy on plasma adrenaline and noradrenaline. J. ment. Sci. **101:** 156-162, 1955.
524. WEIR, D. R. AND MORNINGSTAR, W. A.: Effect of pyridoxine deficiency induced by desoxypyridoxine on acute lymphatic leukemia of adults. Blood **9:** 173-182, 1954.
525. WEISSBACH, H., BOGDANSKI, D. F., REDFIELD, B. G. AND UDENFRIEND, S.: Studies on the effect of vitamin B_6 on hydroxytryptamine (serotonin) formation. J. biol. Chem. **227:** 617-624, 1957.
526. WEISSBACH, H., LOVENBERG, W. AND UDENFRIEND, S.: Enzymatic decarboxylation of α-methyl amino acids. Biochem. biophys. Res. Comm. **3:** 225-227, 1960.
527. WEISSBACH, H., LOVENBERG, W. AND UDENFRIEND, S.: Characteristics of mammalian histidine decarboxylating enzymes. Biochim. biophys. Acta **50:** 177-179, 1961.
528. WELLER, H. AND FICHTENBAUM, M.: Der Tryptophanbelastungstest als Nachweis eines Vitamin B_6-Mangels bei Arteriosklerose. Klin. Wschr. **39:** 1275-1280, 1961.
529. WERLE, E.: Hemmung der Histidindecarboxylase durch α-Methyl-dopa. Naturwissenschaften **48:** 54-55, 1961.
530. WERLE, E. AND HERRMANN, H.: Über die Bildung von Histamin aus Histidin durch tierisches Gewebe. Biochem. Z. **291:** 105-121, 1937.
531. WERLE, E. AND KOCH, W.: Zur Kenntnis der Aminosäuredecarboxylasen und ihres Wirkungsmechanismus. Biochem. Z. **319:** 305-322, 1949.
532. WERLE, E. AND MENNICKEN, G.: Über die Bildung von Tryptamin aus Tryptophan und von Tyramin aus Tyrosin durch tierisches Gewebe. Biochem. Z. **291:** 325-327, 1937.
533. WERLE, E. AND PALM, D.: Histamin in Nerven II. Biochem. Z. **320:** 322-334, 1950.
534. WERLE, E. AND PECHMANN, E. VON: Über die Diaminooxydase der Pflanzen und ihre adaptive Bildung durch Bakterien. Liebig's Ann. **562:** 44-60, 1949.
535. WEST, G. B.: Formation of adrenaline and noradrenaline in the body. J. Pharm., Lond. **5:** 311-314, 1953.
536. WESTERMANN, E., BALZER, H. AND KNELL, J.: Hemmung der Serotoninbildung durch α-Methyldopa. Arch. exp. Path. Pharmak. **234:** 194-205, 1958.
537. WHIPPLE, G. H. AND ROBSCHEIT-ROBBINS, F. S.: Amino acids (natural and synthetic) as influencing hemoglobin production in anemia. Proc. Soc. exp. Biol., N. Y. **36:** 629-632, 1937.
538. WIEGAND, R. G.: The formation of pyridoxal and pyridoxal-5-phosphate hydrazones. J. Amer. chem. Soc. **78:** 5307-5309, 1956.
539. WIELAND, T. AND LÖW, I.: Zur Biochemie der Vitamin B-Gruppe (Pantothensäure und Vitamin B_6). Fortschr. Chem. org. Naturst. **4:** 28-63, 1945.
540. WILBRANDT, W. AND ROSENBERG, T.: The concept of carrier transport and its corollaries in pharmacology. Pharmacol. Rev. **13:** 109-183, 1961.
541. WILEY, R. H. AND CLEVENGER, R. L.: Aldehyde hydrazone derivatives in cancer chemotherapy. J. med. pharm. Chem. **5:** 1367-1371, 1962.
542. WILEY, R. H. AND IRICK, G.: Pyridoxal hydrazine derivatives for cancer chemotherapy. J. med. pharm. Chem. **5:** 49-53, 1962.
543. WILLIAMS, H. L. AND ABDULIAN, D. H.: The effects of convulsant hydrazides including INH on blood levels and urinary excretion. J. Pharmacol. **116:** 62-63, 1956.
544. WILLIAMS, H. L. AND BAIN, J. A.: Convulsive effect of hydrazides: relationship to pyridoxine. Int. Rev. Neurobiol. **3:** 319-348, 1961.
545. WILLIAMS, H. L. AND WIEGAND, R.: Xanthurenic acid excretion and possible pyridoxine deficiency produced by isonicotinic acid hydrazide and other convulsant hydrazides. J. Pharmacol. **128:** 344-348, 1960.
546. WILLIAMS, M. A., GUNNING, B. AND PERTEL, R.: Liver pyridine nucleotide synthesis in the vitamin B_6-deficient rat. Proc. Soc. exp. Biol., N. Y. **109:** 442-445, 1962.
547. WILLIAMS, M. A. AND HATA, B.: Liver coenzyme A levels in the vitamin B_6-deficient rat. Arch. Biochem. Biophys. **80:** 367-371, 1959.
548. WILLIAMS, M. A. AND SCHEIER, G. E.: Relation of pyridoxine (vitamin B_6) intake to liver fatty acids in rats fed methyl linoleate. Fed. Proc. **21:** 393, 1962.
549. WILSON, J. E. AND DU VIGNEAUD, V.: L-Penicillamine as a metabolic antagonist. Science **107:** 653, 1948.
550. WILSON, J. E. AND DU VIGNEAUD, V.: Inhibition of the growth of the rat by L-penicillamine and its prevention by aminoethanol and related compounds. J. biol. Chem. **184:** 63-70, 1950.
551. WINSTEIN, W. A. AND EIGEN, E.: Paper partition chromatographic analysis and microbial growth factors: The vitamin B_6 group. Proc. Soc. exp. Biol., N. Y. **67:** 513-517, 1957.
552. WINTROBE, M. M., ROLLIS, R. H., MILLER, M. H., STEIN, J. H., ALCAYGA, R., HUMPHREYS, S., SUKSTA, A. AND CARTWRIGHT, G. E.: Pyridoxine deficiency in swine. With particular reference to anemia, epileptiform convulsions and fatty liver. Johns Hopk. Hosp. Bull. **72:** 1-25, 1943.
553. WITTEN, P. W. AND HOLMAN, R. T.: Polyethenoid fatty acid metabolism. VI. Effect of pyridoxine on essential fatty acid conversions. Arch. Biochem. Biophys. **41:** 266-273, 1952.
554. WOHL, M. G., LEVY, H. A., SZUTKA, A. AND MALDIA, G.: Pyridoxine deficiency in hyperthyroidism. Proc. Soc. exp. Biol. N. Y. **105:** 523-527, 1960.
555. WOOD, W. A., GUNSALUS, I. C. AND UMBREIT, W. W.: Function of pyridoxal phosphate; resolution and purification of tryptophanase enzyme of *Escherichia coli*. J. biol. Chem. **170:** 313-321, 1947.

556. WOODRING, M. J. AND STORVICK, C. A.: Vitamin B_6 in milk: Review of literature. J. Ass. off. agric. Chem., Wash. **43**: 63–80, 1960.
557. YAMADA, H. AND YASUNOBU, K. T.: Monoamine oxidase. I. Purification, crystallisation and properties of plasma monoamine oxidase. J. biol. Chem. **237**: 1511–1516, 1962.
558. YAMADA, H. AND YASUNOBU, K. T.: The nature of the prosthetic groups of plasma amine oxidase. Biochem. biophys. Res. Comm. **8**: 387–390, 1962.
559. YAMADA, K. AND NAGAI, M.: The effect of vitamin B_6 on the production of experimental liver cancer. J. Vitaminol., Osaka **5**: 188–209, 1959.
560. YAMADA, K. AND OGAWA, S.: Effect of vitamin B_6 and its antagonists on hemebiosynthesis in the culture of rat bone marrow. Vitamins **13**: 595–598, 1957.
561. YAMADA, K., SAWAKI, S. AND HAYAMI, S.: Participation of vitamin B_6 in the biosynthesis of coenzyme A. J. Vitaminol., Osaka **2**: 296–298, 1956.
562. YAMADA, K., SAWAKI, S. AND HAYAMI, S.: Inhibitory effect of cycloserine on some enzymic activities related to vitamin B_6. J. Vitaminol., Osaka **3**: 68–72, 1957.
563. YUWILER, A. AND LOUTTIT, R. T.: Effects of phenylalanine diet on brain serotonin in the rat. Science **134**: 831–832, 1961.
564. ZBINDEN, G. AND STUDER, A.: Vergleichende Untersuchungen über die Wirkung von Pyridoxin, Pyridoxal-5'-phosphat und Pyridoxalisonicotinyl-hydrazon auf die experimentelle Isoniazid-"Neuritis" der Ratte. Int. Z. Vitaminforsch. **26**: 130–137, 1955.
565. ZBINDEN, B. AND STUDER, A.: Experimenteller Beitrag zur Frage der Isoniazid-Neuritis und ihrer Beeinflussung durch Pyridoxin. Z. Tuberk. **107**: 97–107, 1955.
566. ZBINDEN, G. AND STUDER, A.: Zur Wirkung von Vitaminen der B-Gruppe auf die experimentelle Isoniazid-"Neuritis." Schweiz. Z. allg. Path. **18**: 1198–1213, 1955.
567. ZELLER, E. A.: Oxidation of amines. In: The Enzymes, ed. by J. B. Sumner and K. Myrbäck, vol. 2, part 1, pp. 536–558. Academic Press, New York, 1951.
568. ZORINI, A. O., SPINA, G. AND SIMONI, G. E. DE: Clinical and biological investigations on the new antituberculosis drugs (pyrazinamide and cycloserine). Dis. Chest **34**: 27–46, 1958.