* Research supported by the Research Corporation and the National Cancer Institute (National Institutes of Health grant C-3304 BBC), and by the University of Nebraska Research Council.

† Permanent address.

[1] We shall refer to this note as part III. Part I by Yos, Jerrold M., William L. Bade, and Herbert Jehle, these PROCEEDINGS, **43**, 341 (1957); part II by the present author, these PROCEEDINGS **43**, 847 (1957), "Specificity of Intermolecular Forces due to Charge Fluctuations."

[2] Muller, H. J., *Am. Naturalist*, **56**, 32 (1922), *Cold Spring Harbor Symp. Quant. Biol.*, **9**, 290 (1941), and *Proc. Roy. Soc.*, **B 134**, 1 (1947); Haurowitz, F., *Chemistry and Biology of Proteins*, (Acad. Press, 1950), p. 340 ff.; Hamaker, H. C., *Physica*, **4**, 1058 (1937); Jehle, H., J. M. Yos and W. L. Bade, *Phys. Rev.*, **119**, 793 (1958); Yos, J. M., *Phys. Rev.*, **119**, 800 (1958); Yos, J. M., W. L. Bade, and H. Jehle, *in Symposium on Molecular Structure and Biological Specificity*, ed. L. Pauling and H. Itano, (Am. Inst. of Biol. Sciences, 1957), and *Proceedings of the First National Biophysics Conference* (Yale University Press, 1959).

[3] Cf. the remarks in part IV.

[4] Hamaker, H. C., *Rec. Trav. Chim. Pays Bas*, **56**, 3 (1937); *Chem. Weekblad*, **35**, 47 (1938); Overbeek, J. Th. G., in Kruyt, H. R., *Colloid Science*, (Elsevier, 1952) **1**, pp. 276, 277; Derjgauin, B., and L. D. Landau, *Acta Physicochim. USSR*, **14**, 663 (1941); Lifshitz, E. M., *JEPT*, **2**, 73 (1956); Debye, P., and E. Hueckel, *Phys. Z.*, **24**, 185 (1923); Onsager, L., *Chem. Revs.*, **13**, (1933); Klotz, I. M., in *The Proteins*, ed. Neurath and Bailey (Academic Press, 1953), p. 727 ff.; Prigogine, I., and A. Bellemans, *Disc. Faraday Soc., Nonelectrolytes*, **15**, 80 (1953).

[5] $H^{+}_{R}1a$ stands for "protein chain *H*ydrogen bonded to the $C_2'OH$ of the hydrogen donor (+) *R*iboses of a *single* (*1*) strand RNA whose bases are stretched out *a*lternately left and right from the phosphate-ribose backbone, like the oars of a racing boat." C_P2p stands for "individual amino acids *C*ovalently bonded to the *P*hosphates of a *double* (*2*) strand RNA, the bases of each strand being stacked-up *p*arallel as in a Watson-Crick-Wilkins-Rich helix." The schemes to be considered are: $H^{+}_{R}1a$ protein H bonded to H donor riboses of single strand alternate base RNA, $H^{-}_{P}1a$ protein H bonded to H acceptor phosphates of single strand alternate base RNA, $H^{\pm}_{B}1a$ protein H bonded to H donor or acceptor bases of single strand alternate base RNA, $H^{+}_{B}1p$ protein H bonded to H donor bases of single strand parallel base RNA, $H^{-}_{B}1p$ protein H bonded to H acceptor bases of single strand parallel base RNA. The first, third, and fourth of these schemes are represented in the illustrations.

[6] I am indebted to Dr. E. T. Bolton and his colleagues in the Department of Terrestrial Magnetism of the Carnegie Institution, Washington, D. C., for telling me about the not yet published results which they obtained. They have found a ratio of two amino acids per nucleotide in ribonucleoproteins from *E. coli*, and by means of a strong Na^{+} solution they have been splitting off one protein chain, the remainder being constituted in a protein-RNA chain with one amino acid per nucleotide. Subsequent treatment with urea resulted in splitting off the second protein chain from the RNA.

[7] I am indebted to Dr. Chr. Jardetzky who has drawn my attention to this stabilizing hydrogen bond.

---

*PYRIDOXAL KINASE OF HUMAN BRAIN AND ITS INHIBITION BY HYDRAZINE DERIVATIVES**

BY DONALD B. McCORMICK† AND ESMOND E. SNELL

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY

Communicated July 13, 1959

Although the mechanism of conversion of vitamin B_6 to pyridoxal phosphate is not fully known for any tissue,[1] it requires in every case participation of a kinase. Such kinases must be of almost ubiquitous occurrence and, indeed, have been detected in brain,[2] liver,[3] *Streptococcus faecalis*,[4, 5] *Escherichia coli*,[6] and yeast[6]. Despite the

central role of the ultimate product, pyridoxal phosphate, in amino acid metabolism only the kinase from yeast has been purified significantly and its properties studied in reasonable detail.[6] During a comparative study of pyridoxal kinases from different organisms,[7] brain tissue was found to be among the richest sources of this enzyme. This finding was of particular interest because of the intimate relationship of vitamin B_6 to brain metabolism as evidenced by the occurrence of convulsive seizures during vitamin B_6 deficiency, whether induced by restricted intake of the vitamin,[8-10] by administration of analogues of the vitamin,[9, 11] or by administration of carbonyl reagents such as isonicotinyl hydrazide and related compounds.[12, 13]

We have now partially purified the brain kinase and examined some of its properties. Carbonyl reagents proved to be potent inhibitors of the enzyme, which was maximally activated only by Zn^{++}. Some details of these studies are presented herein.

Methods.—Pyridoxal phosphate formation was followed by the method of Wada *et al.*,[14] with slight modifications. This involves colorimetric determination[15] of indole production from tryptophan in the presence of apotryptophanase from *E. coli* and is a considerable simplification over previously used methods. Protein was determined by the method of Lowry *et al.*[16] and by optical density measurement at 280 mμ.

TABLE 1

PURIFICATION OF PYRIDOXAL KINASE FROM HUMAN BRAIN

Fraction	Specific Activity*	Total Activity†
(1) Brain homogenate	1.5	22,100
(2) Supernatant solution from (1)	14.2‡	47,000
(3) Dialyzed fraction of (2) soluble in 40, insoluble in 60% $(NH_4)_2SO_4$	26.7	28,000
(4) Peak cut from DEAE-cellulose column, dialyzed	250	...

* Mμmoles of pyridoxal phosphate formed per mg of protein per hr at 37°C. The incubation mixture contained 5×10^{-4} *M* pyridoxal, 5×10^{-4} *M* ATP, 1×10^{-5} *M* Zn^{++}, 0.1 *M* potassium phosphate buffer at pH 6.5, and enzyme in 2.5 ml total volume. Incubation was at 37° for 1 hr.

† From 200 gm of cerebral tissue.

‡ An independent preparation from an autopsy specimen held in the deep freeze for two years prior to fractionation showed a specific activity of 300 at this point and was used (following dialysis) in some of the experimental work.

The semicarbazone, hydrazone and α-methylphenethylhydrazone of pyridoxal were synthesized by heating approximately equimolar quantities of pyridoxal hydrochloride with the appropriate carbonyl reagent in 0.1 *M* potassium acetate buffer, pH 4.5, filtering, and washing. The 2,4-dinitrophenylhydrazone was produced from a similar reaction in ethanolic HCl. All compounds yielded the expected analysis for C, H, and N.

Pyridoxal kinase was purified as described below. All operations were carried out at 0°C. (1) Cerebral cortex was homogenized in a Tenbroeck apparatus with three to four volumes of 0.1 *M* potassium phosphate buffer, pH 6.5, and the suspension centrifuged at 18,500 × gm for 30 minutes. (2) The clear, reddish-yellow supernatant solution was decanted and brought to 40 per cent saturation with ammonium sulfate. The mixture was centrifuged at 18,500 × gm for 15 minutes, and the precipitate discarded. (3) To the supernatant solution from step 2, ammonium sulfate was added to 60 per cent saturation. The precipitate

formed contains the kinase and was collected by centrifuging for 15 minutes. It was dissolved in a small volume of distilled water and dialyzed against 0.05 *M* potassium phosphate buffer, pH 6.5, to remove ammonium sulfate. (4) The dialyzed solution was again centrifuged. The active yellowish, supernatant solution was poured over 5 to 20 times its weight (cellulose/protein) of diethylaminoethylcellulose and eluted fractionally in a linear gradient fashion with potassium phosphate buffer between 0.05 *M*, pH 6.5, and 0.2 *M*, pH 5.5. (5) Fractions containing kinase activity were pooled. The protein was precipitated at 60 per cent saturation of ammonium sulfate, collected by centrifugation, dissolved in a small volume of 0.05 *M* potassium phosphate buffer, pH 7.0, and dialyzed against this buffer.

Results.—Purification and properties of the kinase: The purification of enzyme during the fractionation is shown in Table 1. Figure 1 shows the elution pattern of the material over DEAE-cellulose. The kinase activity of brain tissue does not decrease, and may even increase, on storage in the frozen state for as long as two years. Concentrates of the kinase at stage (3), Table 1, are also quite stable to storage at deep-freeze temperatures for long periods of time, but more highly purified fractions from the cellulose column are much less stable. Unlike crude fractions of the pyridoxal kinase of liver,[7] most of the activity of the brain kinase is destroyed by heating at 55°C for 5 minutes.

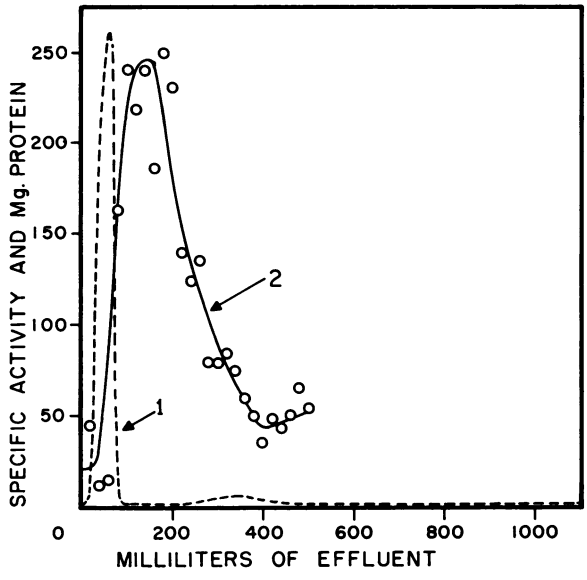

FIG. 1.—Fractionation of pyridoxal kinase from human brain on DEAE-cellulose. One gram of Fraction 3, Table 1 (sp. act. 26.7) was applied to five grams of the cellulose derivative. Assay conditions as in Table 1. Curve 1, protein; curve 2, specific activity of kinase.

The relation of pyridoxal phosphate formation to concentration of pyridoxal, adenosine triphosphate, and Zn^{++} is shown in Figure 2. The K_M value for pyridoxal was 10^{-4} to 10^{-5} *M*; the smaller value was obtained with more highly purified preparations. K_M values for ATP and for Zn^{++} were 5×10^{-5} *M* and 1×10^{-6} *M*, respectively. The pH optimum (Fig. 3) is approximately 6.5 with Zn^{++} as the activating metal ion, but is nearer 7.0 and much broader with Mg^{++} as the activating metal ion. At pH 6.5 the activity of the Zn^{++}-activated enzyme is at least twice that of the Mg^{++}-activated enzyme. Separate trials in which the concentrations of these ions were varied in systems containing optimal amounts of pyridoxal and ATP confirmed this superiority of Zn^{++}; at no concentration did Mg^{++} elicit the same high activities as did Zn^{++}. The K_M value for Mg^{++} (1×10^{-5} *M*) was approximately ten times that for Zn^{++} (1×10^{-6} *M*). From these and other experiments, near optimal conditions of assay for the kinase were established as those specified in Table 1.

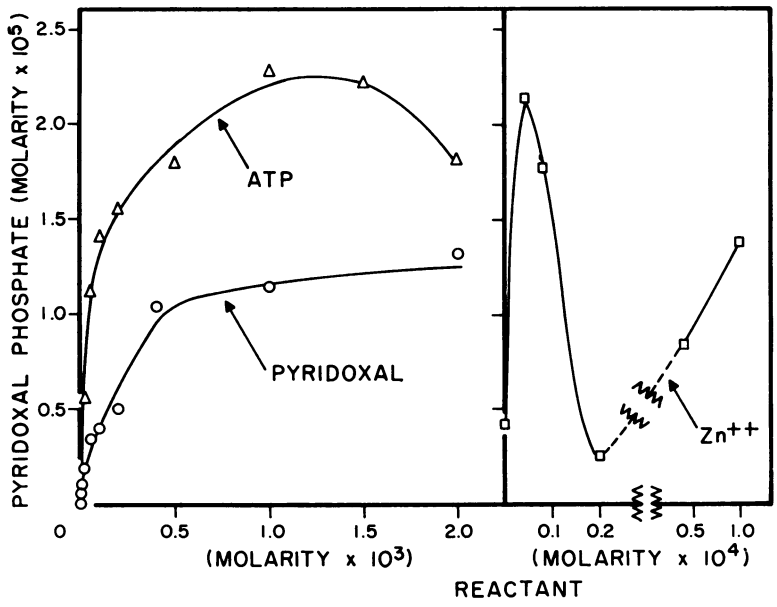

FIG. 2.—Relation of pyridoxal phosphate formation to concentration of pyridoxal, ATP and Zn^{++}. Assay conditions as in Table 1 with appropriate omission, and 0.2 mg. of kinase (sp. act. 300) per 2.5 ml. Precipitation of zinc phosphate begins at the point of decline in the activity-concentration curve.

Inhibition of the kinase by carbonyl reagents: The effects of several psychoactive drugs on activity of the kinase are shown in Figure 4. Chlorpromazine and 1-(1-phenylcyclohexyl)piperidine did not inhibit but rather stimulated the enzyme mildly, whereas the converse was observed with iproniazid phosphate. α-Methyl-

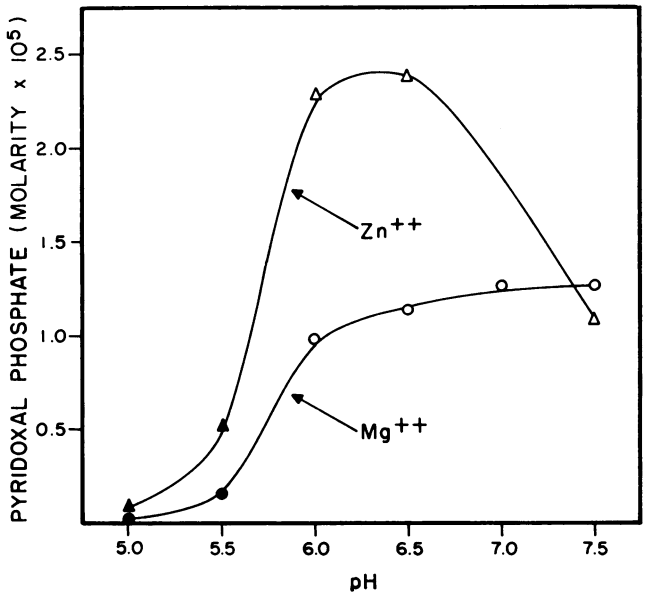

FIG. 3.—Relation of pH and activating metal ion to activity of the brain kinase. Assay conditions as in Table 1, but with Mg^{++} (1×10^{-4} *M*) substituted for Zn^{++} where indicated. 0.4 mg. of kinase (sp. act. 150 (calculated with Zn^{++} at pH 6.5)) per 2.5 ml.

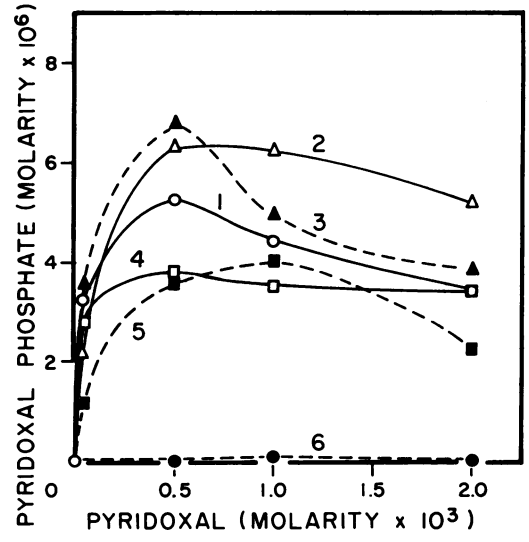

FIG. 4.—Effects of various psychoactive drugs on the activity of the brain kinase. Assay conditions as in Table 1, but with pyridoxal varied and 2×10^{-4} *M* drug added as indicated below. 0.2 mg. of kinase (sp. act. 150) per 2.5 ml. assay tube. Curve 1, control; 2, chlorpromazine; 3, 1-(1-phenylcyclohexyl)piperidine; 4, iproniazid phosphate; 5, 4-(o-(propylthio)-phenyl-1-piperazine pentanol)ethylcarbamate; 6, α-methylphenethylhydrazine.

phenethylhydrazine was an extremely potent inhibitor. The activity of the hydrazine-inhibited system was not restored by as much as a tenfold excess of pyridoxal, ATP or Mg^{++} (Fig. 5). Substrate removal or binding of essential metal ions was not, therefore, responsible for the inactivation.

That the actual inhibitor was the hydrazone, formed between pyridoxal and α-

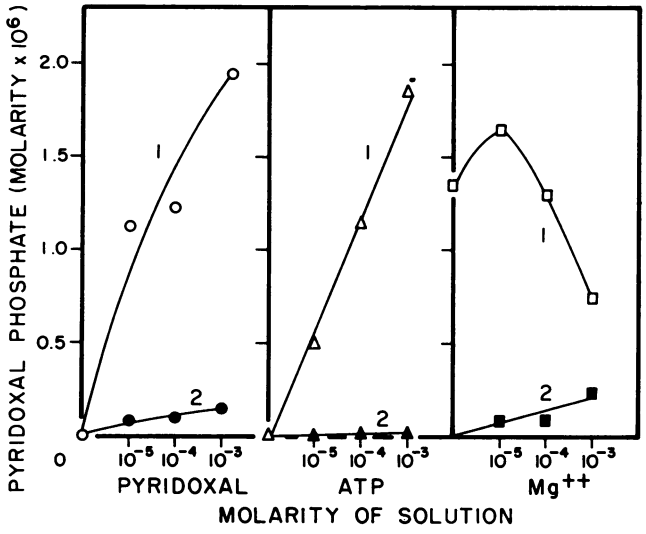

FIG. 5.—Effect of variation in reactant concentrations on the inhibitory action of α-methylphenethylhydrazine. Assay conditions as in Table 1, but with Zn^{++} replaced by Mg^{++} (1×10^{-4} *M*) and pyridoxal, ATP, or Mg^{++} varied as indicated. 0.2 mg. kinase (sp. act. 150) per 2.5 ml. assay tube. Curve 1, control; curve 2, plus 1×10^{-4} M α-methylphenethylhydrazine.

methylphenethylhydrazine, was shown by tests of the synthetic compound, which proved slightly more potent as an inhibitor than the parent hydrazine. In the presence of 5×10^{-4} *M* pyridoxal, 50 per cent inhibition of pyridoxal phosphate formation occurred with approximately 3×10^{-6} *M* hydrazone. The affinity of the kinase for this inhibitor is thus well over one hundred times that for pyridoxal itself. The severe and partially competitive nature of this inhibition is seen in Figure 6.

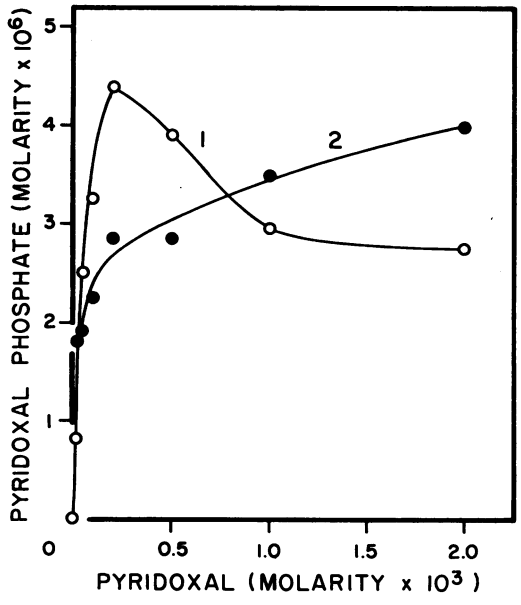

FIG. 6.—Inhibition of pyridoxal phosphate formation by low concentrations of pyridoxal α-methylphenethylhydrazone. Assay conditions as in Table 1, but with pyridoxal varied. 0.2 mg. of kinase (sp. act. 150) per 2.5 ml. assay tube. Curve 1, control; curve 2, plus 9×10^{-7} *M* hydrazone.

Similar inhibition of the purified kinase was exhibited by two other hydrazones of pyridoxal tested (Table 2) and also by pyridoxal oxime and pyridoxal semicarbazone. Suitable controls showed in all cases that the inhibition was of the kinase, and not of the tryptophanase system used for assay of pyridoxal phosphate formation. For example, pyridoxal α-methylphenethylhydrazone at concentrations that inhibited the kinase completely had no effect on the tryptophanase of *E. coli*.

Discussion.—The information available on most kinases has led to the tacit assumption that Mg^{++} is the preferred activator for all such enzymes. This assump-

TABLE 2

COMPARATIVE INHIBITORY POTENCIES OF CERTAIN CARBONYL REAGENTS AND THEIR CONDENSATION PRODUCTS WITH PYRIDOXAL FOR THE PYRIDOXAL KINASE OF HUMAN BRAIN

Compound Tested	Concentration at 50% Inhibition,* *M*
Isonicotinylhydrazide (isoniazid)	4.8×10^{-6}
Semicarbazide	1.5×10^{-5}
Pyridoxal semicarbazone	4.0×10^{-7}
Pyridoxal hydrazone	5.0×10^{-7}
Pyridoxal α-methylphenethylhydrazone	3.0×10^{-6}
Pyridoxal 2,4-dinitrophenylhydrazone	1.5×10^{-5}
Pyridoxal oxime	5.0×10^{-7}

* Testing conditions as in Table 1. 0.2 mg of kinase (sp. act. 150) per 2.5 ml assay tube.

tion appears to be based principally on extrapolation from a few carefully studied systems and data indicating that intracellular Mg^{++} is more plentiful than other divalent ions. Only infrequently have careful comparisons of the activities of different activating ions been reported; in some of these, indeed, Mn^{++} has proved as active as Mg^{++}. For the brain kinase Mg^{++}, though active, is definitely inferior as an activator to Zn^{++} under conditions optimal for enzymatic activity. Whether only one, or both of these ions functions with the kinase *in situ* is not determined by these data. Reported concentrations of both zinc[17] and magnesium[18] in brain tissue are above those required for maximum activation of the kinase in the *in vitro* system.

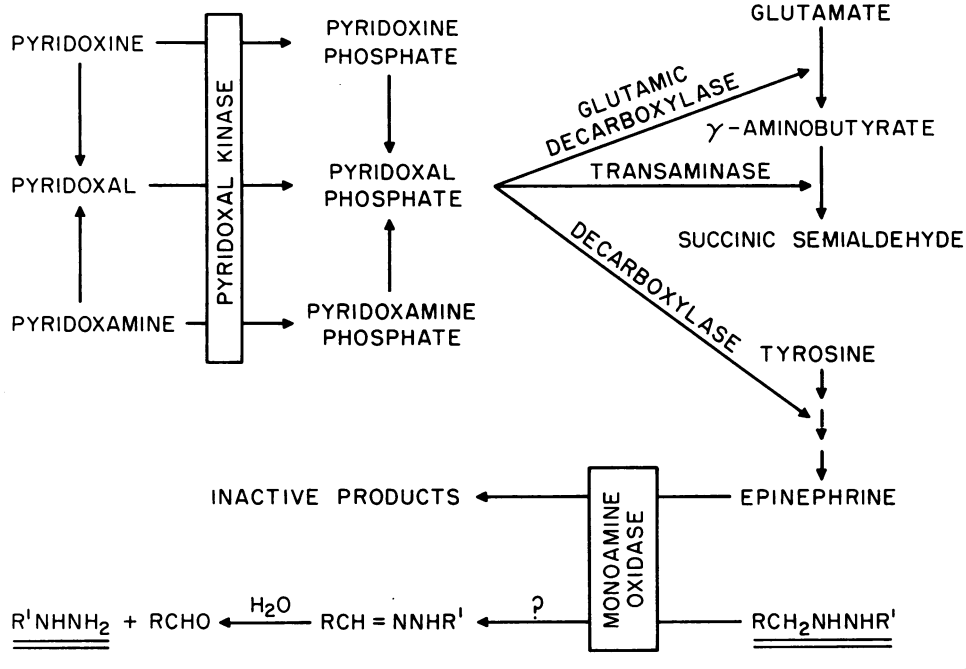

FIG. 7.—Some interrelationships among pyridoxal kinase, pyridoxal phosphate enzymes, and monoamine oxidase of importance for assessment of analeptic and convulsive actions of hydrazines and their derivatives.

Carbonyl reagents are known to inhibit most pyridoxal phosphate enzymes through formation of a coenzymatically inactive derivative with the cofactor.[19] Many of these reagents are now recognized as stimulants of the central nervous system ("psychic energizers") which, in sufficient excess, produce epileptiform seizures that may be alleviated by administration of pyridoxine.[13,20] The localization of glutamic decarboxylase in brain,[21] together with an observed reduction in levels of γ-aminobutyrate following isoniazid treatment[13] have been interpreted to mean that both the analeptic and convulsive activities of active carbonyl reagents may result from interference with production of this inhibitor of neuronal transmission by the glutamic decarboxylase.[22] The data presented here indicate strongly that this decrease in concentration of γ-aminobutyrate results from lowered pyridoxal phosphate levels as a consequence of inhibition of the pyridoxal kinase,

rather than from inhibition of the glutamic decarboxylase *per se*. A comparison of the sensitivity of kinase and decarboxylase to the hydrazine derivatives is required to settle this point;[23] however, the sensitivity of the kinase to isoniazid demonstrated here is far greater than that of the pyridoxal phosphate enzymes so far studied. Since pyridoxal kinase phosphorylates each of the three forms of vitamin B_6,[24] it is clear that pyridoxal phosphate formation from all forms of the vitamin will be inhibited by these carbonyl reagents, whichever of the possible alternate routes to the coenzyme may be followed *in vivo* (cf. Fig. 7). The sole prerequisite for such inhibition would be sufficient quantities of free pyridoxal in the tissues to form the actual inhibitor from the carbonyl reagent. That this is present is obvious from the increased excretion of vitamin B_6 that results from feeding isonicotinylhydrazide[25] and semicarbazide.[26] In the latter instance, pyridoxal semicarbazone was detected in the urine.

Several of the hydrazine derivatives are potent inhibitors of monoamine oxidase, an action thought to contribute to their analeptic action by preventing rapid removal of the sympathomimetic amines.[27] If they inhibit by serving as competitive substrates for the amine oxidase, a possible mechanism for formation of monosubstituted from disubstituted hydrazines, and hence of potent pyridoxal kinase inhibitors from compounds such as iproniazid, would be present *in vivo* (cf. Fig. 7). A few of the many possible sites at which hydrazines may interfere with known reactions of importance in brain metabolism are illustrated in Figure 7. The complexities of the possible interactions are such that more detailed speculations concerning their nature is unprofitable at this time.

Summary.—Brain tissue was found to be an unusually rich source of pyridoxal phosphokinase. A procedure for purification of this enzyme approximately 200-fold from human brain tissue is described. At its optimum pH of 6.5, the purified kinase was preferentially activated by Zn^{++} (K_M, 10^{-6} *M*) and less effectively by Mg^{++} (K_M, 10^{-5} *M*). Values of K_M for pyridoxal and adenosine triphosphate were 10^{-5} *M* and 5×10^{-5} *M*, respectively.

Because of its indirect role in formation of γ-aminobutyrate, the effects of various tranquilizers and psychic energizers on activity of the kinase were determined. Chlorpromazine and 1-(1-phenylcyclohexyl)piperidine showed a slight stimulatory action of uncertain significance; iproniazid inhibited moderately. α-Methylphenethylhydrazine and isoniazid were extremely effective inhibitors of the kinase; the actual inhibitors were found to be the corresponding hydrazones formed between these agents and pyridoxal. The hydrazone, oxime, and semicarbazone of pyridoxal are all similarly potent inhibitors, with affinities for the enzyme well over one hundred times that of the substrate, pyridoxal. Because of the low concentrations at which they exert this effect, it is considered that the physiological effects of these carbonyl reagents in bacteria and mammals result in part from their capacity to lower or eliminate production of pyridoxal phosphate by a given tissue.

* This investigation was supported in part by a grant (A-1448) from the U. S. Public Health Service. We are indebted to Parke, Davis & Co. for certain of the compounds tested.

† Post-doctoral research fellow of the U. S. Public Health Service.

[1] Snell, E. E., *Vitamins and Hormones*, **16**, 77 (1958).

[2] Roberts, E., and S. Frankel, *J. Biol. Chem.*, **190**, 505 (1951).

[3] Trufanov, A. F., and J. A. Kirsanoba, *Byull. Eksp. Biol. i Med.*, **22**, No. 6 (1946); *Chem. Abst.*, **41**, 7485 (1947).

[4] Gunsalus, I. C., and W. D. Bellamy, *J. Biol. Chem.*, **155**, 357 (1944).

[5] Bellamy, W. D., W. W. Umbreit, and I. C. Gunsalus, *J. Biol. Chem.*, **160**, 461 (1945).

[6] Hurwitz, J., *J. Biol. Chem.*, **205**, 935 (1953); **217**, 513 (1955); *Biochim. et Biophys. Acta*, **9**, 496 (1952); *Nutrition Symposium Series*, No. 13 National Vitamin Foundation, Inc., New York, 1956, p. 49.

[7] McCormick, D. B., M. E. Gregory, and E. E. Snell (to be published).

[8] Sherman, H., in *The Vitamins*, Vol. 3, ed. W. H. Sebrell, Jr., and R. S. Harris (New York Academic Press, Inc., 1954), pp. 265–276.

[9] Coursin, D. R., *J. Amer. Med. Assoc.*, **154**, 406 (1954).

[10] Maloney, C. J., and A. H. Parmelee, *J. Amer. Med. Assoc.*, **154**, 405 (1954).

[11] Sandman, R., and E. E. Snell, *Proc. Soc. Exptl. Biol. Med.*, **90**, 63 (1955).

[12] Jenny, E. H., and C. C. Pfeiffer, *J. Pharmacol. and Exper. Therap.*, **122**, 110 (1958).

[13] Killam, K. F., and J. A. Bain, *J. Pharmacol. and Exper. Therap.*, **119**, 225 (1957).

[14] Wada, H., T. Morisue, Y. Sakamoto, and K. Ichihara, *J. of Vitaminology* (Japan), **3**, 183 (1957).

[15] Wood, W. A., I. C. Gunsalus, and W. W. Umbreit, *J. Biol. Chem.*, **170**, 313 (1947).

[16] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[17] Eggleton, W. G. E., *Biochem. J.*, **34**, 991 (1940).

[18] Shohl, A. T., *Mineral Metabolism*. Am. Chem. Soc. Monograph No. 82, New York: (Reinhold Pub. Co., 1939).

[19] Greenberg, L. D., *Ann. Rev. Biochem.*, **26**, 219 (1957).

[20] Jenny, E. H., R. P. Smith, and C. C. Pfeiffer, *Fed. Proc.* **12**, 333 (1953).

[21] Roberts, E., and S. Frankel, *J. Biol. Chem.*, **187**, 55 (1950).

[22] Killam, K. F., *Fed. Proc.*, **17**, 1018 (1958).

[23] Preliminary comparisons have shown that the glutamic decarboxylase of beef brain requires almost 1000-fold greater concentration of carbonyl reagents for inhibition than does the pyridoxal kinase of this tissue.

[24] Like the yeast kinase,[6] we find the brain kinase also phosphorylates pyridoxine and pyridoxamine. Affinities of these substrates for the kinase are similar to that of pyridoxal.

[25] Vilter, R. W., J. P. Biehl, J. F. Mueller, and B. T. Friedman, *Fed. Proc.*, **13**, 776 (1954).

[26] Williams, H. L., and D. H. Abdullian, *J. Pharmacol. Exptl. Therap.*, **116**, 62 (1956).

[27] Biel, J. H., A. E. Drukker, T. F. Mitchell, E. P. Sprengeler, P. A. Nuhfer, A. C. Conway, and A. Harita, *J. Am. Chem. Soc.*, **81**, 2805 (1959).

INHIBITION OF IODIDE OXIDATION BY THYROXINE AND OTHER ANTIOXIDANTS

BY S. M. SIEGEL AND P. FROST

UNION CARBIDE RESEARCH INSTITUTE, WHITE PLAINS, NEW YORK

Communicated by J. Holtfreter, August 6, 1959

Among the physicochemical properties exhibited by many bioregulators is their ability to interact with oxidation-reduction systems. A study of this function in indole-3-acetic acid,[1] serotonin, and other indoles showed them to be highly efficient antioxidants. The oxidation of iodide to iodine with hydrogen peroxide was inhibited as well by nonindolic organic compounds such as thyroxine, suggesting a regulatory role for thyroid hormones in a primary step[2] in their own formation.

This paper deals with the antioxidant activities of selected organic substances and some physical and biological implications of this property.

Methods.—The iodide oxidation system has been employed in two modifications.