Inhibition of DOPA Decarboxylase by Aromatic Acids Associated with Phenylpyruvic Oligophrenia. (22773)

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It has been demonstrated(1) that the biochemical lesion associated with mental deficiency in phenylpyruvic oligophrenia is a failure of an enzyme system in the liver of these patients to hydroxylate phenylalanine to tyrosine. This condition results in the appearance of abnormal oxidation products of aromatic amino acids in the blood and urine(2). The light hair and skin color associated with this disease has suggested an impairment of tyrosine metabolism. Experimental support for this suggestion has recently been brought forth in the report, which shows that phenylalanine and the related aberrant aromatic acids associated with this disease, competitively inhibit plant as well as mammalian tyrosinase*(3). Further the repigmentation which has been observed when these patients are placed on a low phenylalanine diet(4) is consistent with this notion.

The recent finding that patients suffering with this affliction have low epinephrine plasma values(5) suggests a further interference with enzyme systems as a consequence of the appearance of abnormal amounts of phenylalanine and its related metabolites. These low plasma levels of epinephrine led us to examine the effects of these aromatic acids on the beef adrenal medulla DOPA decarboxylase, an enzyme which functions in the metabolic pathway for epinephrine synthesis.

Methods. DOPA decarboxylase was obtained from beef adrenal medulla as described by Langemann(6). The tissue was ground with sand in a mortar, suspended in an equal amount of 0.1 M phosphate buffer, pH 6.8 and centrifuged. The rate of decarboxylation was determined with a Warburg respirometer at 37°C. The gas phase was nitrogen. The main compartment contained 0.8 ml enzyme, 40 γ pyridoxal-5-phosphate and inhibitor. One side arm contained 20 μmoles D-L DOPA while the other contained 3.6 N H_2SO_4. After the removal of the air with nitrogen the vessels were closed and equilibrated for 10 minutes. The substrate was tipped into the main compartment and readings were made at 5 and 10 minute intervals. After 10 minutes the acid was tipped in, and a final reading was taken. Sodium phenylpyruvate, phenyllactic acid, and L-phenylalanine were obtained from Nutritional Biochem. Inc., phenylacetic acid was obtained from Matheson, Coleman and Bell Inc.; pyridoxal-5-phosphate was obtained from Mann Biochem. Inc., and D-L dihydroxyphenylalanine was obtained from Eastman Kodak Co.

TABLE I. Inhibition of Adrenal Medulla DOPA Decarboxylase by Phenylketonuria Metabolites.

Additions	Conc., μmoles/3 ml	μl CO_2/hr	% inhibition
Control	—	160	—
Phenylpyruvic acid	100	0	100
	50	0	100
	10	36	77
Phenyl-lactic acid	100	0	100
	50	36	77
	10	82	50
Phenylacetic acid	100	80	50
	50	163	0
L-phenylalanine	100	164	0

Each flask contained 800 mg adrenal medulla, 40 γ pyridoxal phosphate. Phosphate buffer pH 6.8. The gas phase was nitrogen.

Results. The results of these experiments on the inhibition of adrenal DOPA decarboxylase are given in the Table. Phenylpyruvate and phenyllactate were the most effective inhibitors of the series, while phenylacetic acid was poor. L-phenylalanine showed no inhibitory effect. It is interesting to compare these results with those obtained by Hartman *et al.*(7) in their study using DOPA decarboxylase from hog kidney cortex. They obtained comparable inhibitions of their enzyme with phenylpyruvate, and phenylacetic acid, but obtained no inhibitions with phenyllactate.

* To be published.

These data suggest an explanation for the low plasma levels of epinephrine in patients suffering from phenylpyruvic oligophrenia(5) in that these aromatic acids which accumulate as a result of biochemical lesion may inhibit the synthesis of epinephrine in the adrenal gland.

The importance of epinephrine in normal nerve function is yet to be elaborated; however, its presence in brain tissue is well established(8). Weil-Malherbe has shown that epinephrine plasma levels were significantly lower in a group of mental defectives than in a group of mixed patients(5). As yet, information is not available which would allow us to speculate on the influence of a deficiency of epinephrine on the etiology of mental deficiency.

Summary. Phenylpyruvic acid, phenyllactic acid and phenylacetic acid inhibit beef adrenal medulla DOPA decarboxylase. The implications of these data in phenylpyruvic oligophrenia are discussed.

1. Jervis, G. A., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 514.
2. Folling, A., *Nord. med. tidskr.*, 1934, v8, 1054. Woolf, L. I., *Biochem. J.*, 1951, v49, LX.
3. Dancis, J., and Balis, M. E., *Pediatrics*, 1955, v15, 63.
4. Armstrong, M. D., and Tyler, F. H., *J. Clin. Invest.*, 1955, v34, 565.
5. Weil-Malherbe, H., and Bone, A. D., *J. Mental Sci.*, 1955, v101, 733.
6. Langemann, H., *Brit. J. Pharm. and Chem.*, 1951, v6, 318.
7. Hartman, W. J., Akawie, R. I., and Clark, W. G., *J. Biol. Chem.*, 1955, v216, 507.
8. Vogt, M., *J. Physiol. (London)*, 1954, v123, 451.

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Stability of the Activator of Bovine Plasminogen. (22774)

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Mullertz and Lassen(1) first reported that a mixture of streptokinase and human plasminogen acquired a characteristic which neither the streptokinase nor the plasminogen exhibited alone, namely, the ability to activate bovine plasminogen. They concluded that bovine blood lacks a factor (proactivator) which is present in human blood and suggested that this proactivator combines with streptokinase to form an activator of bovine plasminogen. The studies of Troll and Sherry(2) and Sherry(3) indicated that the activator of bovine plasminogen was also the activator of human plasminogen. It was further demonstrated(2) that the activator which appears after adding streptokinase to human plasminogen was lost by acidification and regained by the re-addition of streptokinase after neutralization of the fraction, indicating that streptokinase was required for activator activity.

This communication calls attention to the observation that a loss of activator can occur during the incubation of a mixture of streptokinase and human plasminogen.* The rate of disappearance of the activator has been found to be affected by the duration of incubation, the initial streptokinase concentration and the particular plasminogen preparation. These observations have significant bearing on the interpretation of the *in vitro* and *in vivo* activation of plasminogen by streptokinase.

Materials. Streptokinase (SK), Varidase (Lederle) was dialyzed before use. Bovine plasminogen was made according to Milstone (4) and then lyophylized. Lysine ethyl ester (LEE) was synthesized according to Werbin and Palm(5) and stored over phosphorous

* During preparation of this manuscript, Mullertz (*Biochem. J.*, v61, p424, Nov. 1955) reported on similar observations.