On the Nature of Enzymatic Defect in Phenylpyruvic Oligophrenia. (23034)

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(Introduced by A. Meister)
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In a previous communication(1) from this laboratory, it was reported that phenylalanine hydroxylase consists of 2 protein fractions; one of which (Fraction I) is present only in the liver and the other (Fraction II) in almost all tissues, including brain.

Since patients with phenylpyruvic oligophrenia are essentially devoid of phenylalanine hydroxylase activity(2,3,4), the question arises as to which, if not both, of the 2 fractions may be absent in this disorder. In the present communication, data are presented to show that Fraction II is present in phenylketonuric patients and some of the implications of these findings are discussed.

Methods. Liver samples* from normals and phenylketonuric patients were obtained at autopsy, 4 hours after death, and kept in frozen state until use. Four grams of liver were thawed, minced and homogenized in 3 volumes of isotonic KCl. Fractions I and II from both human liver and rat liver were prepared, as described previously(1); the terms, purified enzyme fraction and crude enzyme fraction, used in the Tables, refer to terms used in that paper. The incubation conditions and the method for enzyme assay were also the same as used before(5).

Results. As shown in Table I, conversion of phenylalanine to tyrosine was catalyzed upon recombination of rat Fraction I with rat Fraction II. In these studies combination of Fractions I and II of human liver, both normal and phenylketonuric, failed to catalyze this reaction. This was apparently due to failure of Fraction I, in normal liver, to survive during the several hours of autolysis between death and autopsy, since fresh human liver can catalyze this reaction(4,5). However, stable Fraction II survived under these conditions. Thus, when rat Fraction I was incubated with Fraction II from the phenylketonuric liver, formation of tyrosine took place. Furthermore, as shown in Table II equal amounts of Fraction II, from both phenylketonuric and normal livers, catalyzed formation of tyrosine to the same degree, when they were combined with rat Fraction I.

It is apparent from this that Fraction II is present in phenylketonuric livers in normal amounts indicating that in these patients the block in tyrosine formation is more likely associated with absence of Fraction I. Unfortunately, the lability of Fraction I in the normal autopsy samples made it impossible to show this directly. Similar studies on fresh biopsy material will be required to establish

TABLE I. Presence of Fraction II in Phenylketonuric Liver.

Enzyme preparations*	μmole of tyrosine formed
RI	.0 †
RII	.0
RI + RII	.44
PI + PII	.0
PI + RII	.0
RI + PII	.40

* Purified enzyme fractions were used in these experiments. RI—Rat liver Fraction I, 13.5 mg/beaker. RII—Rat liver Fraction II, 6.9 mg/beaker. PI—Phenylketonuric liver Fraction I, 17 mg/beaker. PII—Phenylketonuric liver Fraction II, 15.8 mg/beaker.

† Limit of detection of enzymatically formed tyrosine by the colorimetric method used was 0.05 μmole of tyrosine.

All beakers contained 2 μmoles of L-phenylalanine, 0.5 μmole of DPN, 5 μmoles of nicotinamide, 400 μmoles of glucose, 325 units of glucose dehydrogenase and enzyme fractions in final vol of 2.2 ml. Incubation was carried out 15 min. at 37° in a Dubnoff metabolic shaking incubator under air. The data presented above represent typical values obtained after several experimental runs.

* The patient was a 2-year-old white female exhibiting characteristic chemical and clinical symptoms of phenylpyruvic oligophrenia. The normal liver, data for which are shown in Table II, was also obtained from a 2-year-old, white female. Another sample of liver was obtained from a 6-month-old, white male. The authors are indebted to Sister Fernandes of the Pathology Department of Georgetown Hospital for providing us with the autopsy samples.

TABLE II. Comparison of Fraction II Activity in Normal and Phenylketonuric Livers.

Enzyme preparations*	μmole of tyrosine formed
RI	.15
RI + RII	.98
RI + NII	.82
RI + PII	.81
RI + P**II	.15

* Crude enzyme fractions were used. RI—Rat Fraction I, 26.6 mg/beaker. RII—Rat Fraction II, 10.8 mg/beaker. NII—Normal human Fraction II, 15 mg/beaker. PII—Phenylketonuric Fraction II, 15 mg/beaker. P**II—Phenylketonuric Fraction II, heated 5 min. in boiling water bath, 15 mg/beaker.

Incubation conditions were as described under Table I. Similar results were obtained with NII from a second control patient.

this point. Such studies are reported by Wallace *et al.*, in the preceding paper(4). In that report as well as in this one, experimental findings on tissues from only one phenylketonuric patient are given. It would obviously be desirable to have these findings corroborated on more patients but liver samples from phenylketonuric patients are not readily obtainable.

Since the conversion of phenylalanine to tyrosine takes place only in the liver and Fraction I is found exclusively in this organ, the conclusion that Fraction I is the enzyme primarily concerned with the hydroxylation reaction seems justifiable. Fraction II which is found in many tissues, may then be looked upon as an enzyme concerned with an auxiliary reaction, the nature of which is still not understood. Had Fraction II been missing one might have inferred a disturbance in metabolism in many tissues, including brain. Since, as is suggested by these studies, Fraction I is absent, the primary defect in phenylketonuria must be one of liver function. The chemical anomalies manifested in these patients most probably result from failure to hydroxylate phenylalanine, resulting in an excess of phenylalanine in tissues and an overproduction of other intermediary metabolites of phenylalanine catabolism. Suggestions that the mental deficiency is caused by an overproduction of one of these metabolites or by excess phenylalanine itself(6,7) are supported by these enzymatic findings.

Summary. Evidence is presented to show the presence of the stable Fraction II of phenylalanine hydroxylase in the liver of a patient with phenylpyruvic oligophrenia. The biochemical significance of this finding is discussed.

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