Studies on Conversion of Phenylalanine to Tyrosine in Phenylpyruvic Oligophrenia.* (23033)

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Recent studies(1,2) indicate that patients with phenylpyruvic oligophrenia have markedly reduced ability to convert phenylalanine to tyrosine. The enzyme system that catalyzes this conversion was demonstrated in mammalian liver preparations by Udenfriend and Cooper(3), and subsequently Jervis(4) reported that this enzyme activity was lacking in livers of 2 patients with phenylpyruvic oligophrenia, whose tissues became available for study at autopsy. No studies on freshly obtained liver have apparently been reported; the lability of this enzyme system suggests the desirability of such study. Mitoma(5) recently made the interesting observation that rat phenylalanine oxidizing system can be separated into two protein fractions; one of these (Fraction I) was relatively labile and was found solely in liver, while the other (Fraction II) was more stable and found also in certain other tissues. We have been fortunate in obtaining a biopsy specimen of liver from a patient† with phenylpyruvic oligophrenia, and have carried out studies on the ability of this preparation to catalyze conversion of phenylalanine to tyrosine in the presence and absence of the 2 purified rat liver fractions.

Methods. Liver samples were placed in cracked ice within a few minutes of excision, and homogenized within 10 minutes with 2 parts of ice-cold 0.15 M KCl. Rat Fractions I and II were obtained as described(5). The reaction mixtures consisted of diphosphopyridine nucleotide (0.06 μM), nicotinamide (0.5 μM), ethanol (2 μM), L-phenylalanine-3-C^{14} (0.4 μM; 56,000 cpm), and 0.1 ml of homogenate (or 0.05 ml of rat fraction); final volume was 0.20 ml. The mixtures were incubated 30 minutes with shaking at 37°. After incubation, one-third of the reaction mixture was transferred to a paper chromatographic strip (30 x 2 cm; Whatman No. 3), developed in a solvent consisting of n-butanol, acetic acid, and water (4:1:1). After ascending chromatography for 12 hours, the strips were cut into one cm sections and counted in a flow-gas tube. Under these conditions, the R_F values for tyrosine and phenylalanine were, respectively, 0.38 and 0.66. The % conversion was calculated from the cpm in the tyrosine area and the total cpm on each strip. No significant radioactive material was found in other than the phenylalanine and tyrosine areas. The values given in Table I represent the averages of 2 closely agreeing duplicate experiments. Homogenates used in these experiments were frozen immediately after use; repetition of experiments 3 weeks later with freshly-prepared purified rat fractions gave very similar results.

Results. As indicated in Table I, the homogenate prepared from the liver of the phenylketonuric patient did not catalyze detectable conversion of phenylalanine to tyrosine. Under experimental conditions employed, a conversion of 0.5% could have been

TABLE I.

Tissue preparation	% conversion
Phenylketonuric liver homogenate	0
Idem + rat Fraction I	76.4
" + " " II	12.3
Rat Fraction I	9.2
" " II	3.3
" " I + rat Fraction II	24.0
Rat liver homogenate	21.9
Normal human liver homogenate	7.8
Idem	10.2
"	14.1

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† The patient was a 4-year-old white male exhibiting characteristic biochemical and clinical signs of phenylpyruvic oligophrenia; the authors are indebted to Dr. Marshall Kreidberg of Boston Floating Hospital for making this patient available for study and for his cooperation and interest.

detected. This result contrasts with those obtained with homogenates prepared from several normal human livers‡ and livers of rats. Rat liver Fractions I and II, in confirmation of Mitoma, catalyzed much lower conversion when studied alone than when assayed together; values for rat Fractions I and II together were about twice the sum of the individual values for the 2 fractions. When rat Fraction I and the phenylketonuric liver preparation were incubated together, a striking increase in conversion of phenylalanine to tyrosine was observed; much less activity was noted when the phenylketonuric preparation and rat Fraction II were incubated together. These observations are consistent with the conclusion that the liver of the phenylketonuric patient did not catalyze conversion of phenylalanine to tyrosine. They also suggest that the more labile protein fraction, apparently present only in liver is lacking in phenylketonuric liver. The smaller activation observed with rat Fraction II may probably be ascribed to incomplete separation of the fractions; it is evident that neither of the rat fractions is completely free of the other.

The striking activation observed with phenylketonuric liver homogenate and rat Fraction I together suggests not only that a component corresponding to rat Fraction I is lacking, but that considerable quantities of the component corresponding to rat Fraction II are present in the phenylketonuric liver. Similar studies carried out with normal human liver homogenates and homogenates of rat liver indicated that rat Fraction I produced activation, although less than that observed with the phenylketonuric liver preparation. The latter experiments are consistent with the belief that in crude homogenates the component corresponding to Fraction II is present in large amounts, and also with the observed lability(5,6) of Fraction I. However, other explanations are not excluded, and it is evident that final conclusions cannot be made until the detailed mechanism of this enzymatic reaction is elucidated. The present findings and those reported in the accompanying paper(6) suggest that the defect in phenylpyruvic oligophrenia is related to the absence of a specific hepatic factor rather than one which is present in a number of tissues including brain.

Summary. A homogenate of a biopsy specimen of liver obtained from a patient with phenylpyruvic oligophrenia did not catalyze detectable conversion of phenylalanine to tyrosine, under conditions whereby liver homogenates obtained from normal individuals were active in catalyzing such conversion. Addition of a fraction prepared from rat liver (which exhibited relatively low activity alone) to the inactive phenylketonuric liver homogenate resulted in striking activation of the conversion of phenylalanine to tyrosine.

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