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Development of Phenylalanine Hydroxylase in Mammalian Liver

Recent studies of the mechanism of conversion of phenylalanine to tyrosine in the soluble fraction of liver homogenates have established that the reaction requires the participation of two discrete enzymes in addition to reduced pyridine nucleotide (*1, 2*) and an as yet unidentified cofactor (*3, 4*). One of the enzymes can be prepared from tissues that do not hydroxylate phenylalanine (for example, kidney, brain, spleen); this suggests that a reaction of more general nature is involved. The second enzyme, which is specific, has been found only in liver. Wallace *et al.* (*5*) and Mitoma *et al.* (*6*) have recently demonstrated that the livers of phenylketonuric individuals lack this specific enzyme, thus strengthening the prevailing concept regarding phenylketonuria—namely, that the syndrome is associated with a specific congenital inability to oxidize phenylalanine to tyrosine.

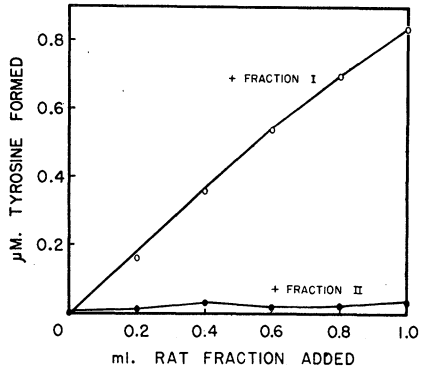

Fig. 1. Effect of rat liver fractions on phenylalanine hydroxylation in the liver of the fetal pig. Beakers containing 1.0 ml of the soluble fraction of fetal pig liver (17.2 mg of protein), 150 μmole of potassium phosphate buffer (*p*H 6.8), 2 μmole of DPNH, 2 μmole of L-phenylalanine, rat liver fractions as indicated, and 0.15*M* KCl to make a total volume of 3.0 ml were shaken for 60 minutes at 35°C. Tyrosine formed was measured colorimetrically. Rat fraction I, 3.3 mg of protein per milliliter; rat fraction II, 7.3 mg of protein per milliliter; these fractions individually formed only traces (less than 0.01 μmole) of tyrosine in 60 minutes.

Investigations on the development of this enzyme system in fetal, newborn, and adult rats have established the fact that the over-all reaction is virtually absent in fetal liver and in the livers of rats less than 24 hours old. In these experiments, 1.0 ml soluble fractions of liver (prepared from 33 percent homogenates in 0.15*M* KCl by centrifugation at 105,000*g* for 30 minutes) were shaken at 35°C for 60 minutes with 150 μmole of potassium phosphate buffer (*p*H 6.8), 2 μmole of L-phenylalanine, 2 μmole of reduced diphosphopyridine nucleotide (DPNH), and 5 μmole of nicotinamide in a total volume of 1.75 ml; the tyrosine formed was measured colorimetrically (*7*).

Under these conditions, preparations from adult animals and from animals 4 to 12 days of age converted all or most of the added phenylalanine to tyrosine, while preparations from fetal or newborn animals were essentially inactive. These inactive preparations could not be activated by substitution of TPNH (reduced triphosphopyridine nucleotide) for DPNH, or by increasing the amount of reduced pyridine nucleotide either by direct addition or by supplementing the reaction mixture with appropriate reducing systems (glucose-6-phosphate and yeast glucose-6-phosphate dehydrogenase with TPN; lactate and muscle lactic dehydrogenase with DPN). The ability to hydroxylate phenylalanine was also absent or negligible in livers of fetal pigs and fetal rabbits.

Experiments were carried out in order to determine which of the components is absent or inactive in fetal liver. Fractions I and II were prepared from adult rat livers by the procedure of Mitoma (*2*); each of these preparations alone was essentially inactive, while the two combined, when fresh, gave high rates of phenylalanine hydroxylation. Addition of rat fraction I, containing the specific enzymic component, to the inactive soluble fraction of fetal pig liver yielded a highly active combination, the level of activity being directly related to the amount of fraction I added (Fig. 1). Addition of fraction II from rat liver had essentially no effect on the fetal liver system. Similarly, supplementing the soluble fractions from livers of fetal rats or rabbits with fraction I resulted in high activity, while addition of fraction II was ineffective.

The absence of phenylalanine hydroxylation in fetal liver thus results from the same deficiency as that observed in the livers of patients with phenylpyruvic oligophrenia—that is, the specific enzymic component of the system, present in rat fraction I, is apparently not present in fetal liver.

Further, although fraction I has been described as being quite labile (*2*), it was found that aged or dialyzed preparations of this fraction, which were in fact inactive when assayed with fraction II, were still active in supplementing the fetal liver system. However, if the fetal liver preparation was well dialyzed, addition of aged or dialyzed fraction I was only slightly effective. These results indicate that fraction I contains two components, one of which is rapidly lost on aging or dialysis, and that this labile component is present in fetal liver. Loss of this component on dialysis, as well as on aging, suggests a factor of low molecular weight, which may be the auto-oxidizable cofactor described by Kaufman (*4*).

These findings lend support to the conclusion that the component missing in fetal liver, which is supplied by rat fraction I, is the specific hydroxylation enzyme. It is of interest to note that some other enzymes whose deficiency in postnatal life is associated with disorders of incomplete metabolism are also not active in fetal tissues; those investigated include the enzymes of tyrosine oxidation (*8*) and glucose-6-phosphatase (*9*). This relationship is being studied further in this laboratory (*10, 11*).

FRANCIS T. KENNEY
GABRIELLE H. REEM
NORMAN KRETCHMER

Department of Pediatrics,
New York Hospital–Cornell
Medical Center, New York

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10. Experiments similar to those described above were carried out with the soluble fractions of livers from two premature infants (liver samples obtained at autopsy, 9 and 2 hours after death). As with the fetal tissue, these preparations were inactive alone, active when rat fraction I was added, and not affected by rat fraction II. Although these results suggest that the liver of the premature infant also lacks the specific hydroxylation enzyme, the possibility exists that the time which elapsed between death and the enzyme assays was sufficient for specific autolysis of this enzyme.

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