ON THE NATURE OF ADRENOCORTICOID-INDUCED INCREASE IN TYROSINE-α-KETOGLUTARATE TRANSAMINASE ACTIVITY OF RAT LIVER

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A rapid and specific increase in tyrosine-α-ketoglutarate transaminase activity of rat liver after substrate administration was first observed by Lin and Knox (1957). They also found that adrenalectomized rats fail to respond to tyrosine unless hydrocortisone is given. Response to steroid administration is augmented by tyrosine, but not by phenylalanine or glutamate, suggesting that a specific substrate-inducing effect of tyrosine is involved. In the rat, the activity of this transaminase increases sharply immediately after birth. This developmental increase is also dependent on adrenal steroids (Serini, Kenney, and Kretchmer, 1959). In adrenalectomized newborns, tyrosine neither substitutes for nor augments the effect of hydrocortisone in restoring transaminase development, but supplementation with methionine results in a higher level of activity than that obtained with hydrocortisone alone (Kenney and Kretchmer, 1959). A specific role of substrate per se is further rendered improbable by the observation that methionine is as effective as tyrosine in increasing the response to hydrocortisone in adrenalectomized adult rats (Kenney, 1960). It would thus seem that alteration in transaminase activity is actually a hormonal induction that can be influenced in some fashion by various amino acids.

The nature of the change in activity in "induced" adult rats as well as in newborns has been investigated with the aid of a highly specific antitransaminase from the sera of immunized rabbits. The enzyme was purified 500- to 600-fold from rat livers by modifications and extensions of methods described previously (Kenney, 1959) and was administered

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to rabbits in Freund's adjuvant. Antisera thus obtained precipitated and inactivated the enzyme, and a single band is found when the reaction between antibody and the purified enzyme is studied by double diffusion in agar gel. Analysis of the induction phenomenon was carried out using rats that had been adrenalectomized 13 days before the experiment. Those of the control group received 8μc of C^{14}-leucine intraperitoneally in two doses, 2 1/2 hr apart. Induced animals were similarly treated with isotopic leucine and, in addition, were given hydrocortisone (30 mg/kg) and tyrosine (600 mg/kg) with the first injection of isotope. The animals were killed 5 hr after the start of the experiment, and liver soluble fractions prepared. These were further cleared by heating at 37° for 30 min; α-ketoglutarate (5×10^{-3} M) was added to protect the enzyme from thermal denaturation. After removal of the resulting precipitate, transaminase activity and protein were determined, and aliquots taken for quantitative precipitin tests. Antibody was added in excess, and the mixtures were incubated for 30 min at 37°, then overnight at 3°. Protein content and radioactivity of the antigen-antibody precipitate were determined after extensive washing with 0.15 M NaCl, and that of the total protein of the soluble fraction after removal of acid-soluble, hot trichloroacetic acid-soluble, and ethanol-ether-soluble materials. The results are presented in Table 1. It is apparent that the marked increase in transaminase activity resulting from induction is not associated with a corresponding increase in antigenically reactive protein, and incorporation of isotopic amino acid into this protein is not appreciably affected by induction. In this and similar experiments, transaminase

Table 1

Immunochemical Assay of the Induction of Tyrosine-α-Ketoglutarate Transaminase in Rat Liver

Measurement*	Control	Induced
Transaminase activity (units)	7.7	248.8
Ag-Ab precipitate (μg)	117	128
Ag-Ab radioactivity (cpm)	49	55
Soluble protein radioactivity (cpm)	157	209

* All data are expressed in terms of a constant amount of the soluble fraction and are the averages of values obtained in determinations on 4 animals in each group. Protein content and radioactivity of antigen-antibody (Ag-Ab) precipitates were not corrected for antibody.

activity in the livers of noninduced adrenalectomized controls was much lower than that typical of intact rats, presumably reflecting the effect of prolonged adrenal deprivation.

Similarly, the natural increase in transaminase activity that occurs after birth is not associated with appreciable increase in antigen content of the liver (Table 2).

Table 2
Immunochemical Assay of the Developmental Increase in
Tyrosine-α-Ketoglutarate Transaminase*

Time after birth (hr)	Transaminase activity (units)	Ag-Ab protein (μg)
0.5	5.5	120
12.0	144.0	127

* The livers of six litter mates killed at the times indicated were pooled; other conditions and procedures as described for Table 1.

These results are clearly incompatible with a mechanism of induction involving de novo synthesis of enzyme protein and suggest that adrenal steroids promote either the activation of an antigenically similar but enzymically inactive precursor protein, or the release of an inhibitor. Mixing experiments fail to demonstrate any inhibition of enzyme in liver preparations from induced animals by extracts from noninduced intact or adrenalectomized rats. In support of the precursor hypothesis, analysis by the technique of double diffusion in agar gel of crude liver preparations reveals a strong band present in those from noninduced animals that is diminished or absent after induction. The possibility that this represents a precursor protein is now being investigated.

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