FACTORS INFLUENCING ACTIVITY OF TYROSINE-α-KETOGLUTARATE TRANSAMINASE IN ISOLATED RAT LIVER

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Increase of activities of some enzymes in mammalian liver, after administration of specific substrates or hormones has been well established (Knox and Behrman 1959; Pollock 1959; and Lin and Knox 1957). Previously, one of us (Sereni, Kenney, and Kretchmer 1959) observed that adrenalectomy of newborn rats at birth prevented the development of tyrosine transaminase activity which usually increases 5 to 10 fold during the first twelve hours of life. Cortisol when administered to the adrenalectomized newborn rats re-established the usual postnatal increase of tyrosine transaminase activity.

The present work demonstrates that tyrosine transaminase activity can be increased in isolated liver and evaluates the role of tyrosine and cortisol in inducing this increase. During the course of the present work (Barnabei and Sereni 1960) a report of a similar study has appeared (Goldstein, Stella, and Knox 1962).

Livers of fasted adult male rats, weighing 250 to 300 gm. were perfused by the technique of Miller, Bly, Watson and Bale (1951). Following 10 minutes of perfusion with the basic solution, a small lobe of liver was

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excised for estimation of the baseline tyrosine transaminase. Perfusion with the experimental solution was then initiated and continued for 3 hours at which time another lobe of liver was removed for assay of tyrosine transaminase activity. In one experiment, liver samples were also taken for analysis after one and two hours of perfusion.

Tyrosine transaminase activity was determined on 0.1 and 0.2 ml. of 10% isotonic sucrose homogenates using a modification (Sereni, Kenney, and Kretchmer 1959) of the procedure of Lin and Knox (1957). Nitrogen was estimated by a micro-Kjeldahl procedure.

When isolated rat liver was perfused for 3 hours with the basic medium, diluted rat blood supplemented with an acid hydrolysate of casein, a slight increase of tyrosine transaminase activity was found (Exp. 1-4). Addition of tyrosine to a concentration of 2.3 mM lead to no further increase of activity (Exp. 5-7). However, activity was considerably reduced if amino acids (casein hydrolysate) were omitted from the basic perfusate (Exp. 8,9). Cortisol when added to the basic medium at a concentration of 1.2 mM lead to a slight increase in activity above that obtained with the basic perfusate (Exp. 10-12) but a more striking increase in activity was obtained when both cortisol and tyrosine were added (Exp. 13-16). This enhancement of activity was almost completely blocked by anaerobiosis (Exp. 17), 10 mM chloramphenicol (Exp. 18,19), or 25 mM ethionine (Exp. 20, 21). The inhibition of ethionine could be reversed by concomitant addition of 30 mM methionine (Exp. 22). The rate of increase of tyrosine transaminase activity when the perfusate was supplemented with both tyrosine and cortisol (Exp. 16) is shown in Figure 1. During the first hour only a slight increase was observed but after one hour of perfusion the activity rose sharply.

The data demonstrate again the important role of the adrenal corticoids in inducing an increase of tyrosine transaminase (Lin and Knox 1957). The production of this effect in isolated liver suggests that the action of the hormone is directly on the parenchymal cell. Unlike the enhancement of

TABLE I

Change of Tyrosine Transaminase Activity in Perfused Rat Liver

Exp.	Supplements to Perfusate	Enzyme Activity			
		Perfusion		Δ	% Change
		10 mins.	3 hrs.		
1	None	115	173	+ 58	+ 50
2	"	175	167	- 8	- 5
3	"	161	275	+114	+ 70
4	"	322	371	+ 49	+ 15
5	l-tyrosine 2.3 mM	331	315	- 16	+ 5
6	"	231	348	+117	+ 51
7	"	366	408	+ 47	+ 15
8	Amino acids omitted	260	55	-205	- 79
9	"	207	37	-170	- 82
10	Cortisol 1.2 mM	300	394	+ 94	+ 31
11	"	196	415	+219	+111
12	"	198	372	+174	+ 88
13	Cortisol + l-tyrosine	96	191	+ 95	+ 99
14	"	116	480	+364	+314
15	"	213	413	+200	+ 94
16	"	93	354	+261	+281
17	Cortisol + l-tyrosine, anaerobiosis	161	190	+ 29	+ 18
18	Cortisol, l-tyrosine + chloramphenicol 10 mM	182	209	+ 27	+ 15
19	"	273	312	+ 39	+ 14
20	Cortisol, l-tyrosine + dl-ethionine 25 mM	168	132	- 36	- 21
21	"	198	218	+ 20	+ 10
22	Cortisol, l-tyrosine, dl-ethionine + methionine 30 mM	259	528	+269	+104

Enzyme activity is expressed as μmoles p-hydroxyphenylpyruvate formed per 10 minutes per mg. N by a 10% liver homogenate at 37°. The livers were perfused at 25-30 ml. per minute. Sixty ml. of the basic medium contained 30 ml. of rat blood, 10 ml. of 0.9% saline, 10 ml. of Krebs-Ringer bicarbonate and 10 ml. of a solution containing 400 mg. of an acid hydrolysate of casein, 5 mg. l-tryptophan and 100 mg. glucose. Supplements were dissolved in the 0.9% saline and/or the Krebs-Ringer bicarbonate.

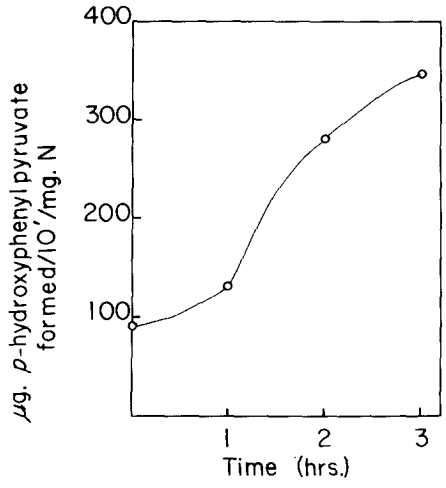

Fig. 1. Tyrosine transaminase activity of isolated rat liver in the course of experiment 16.

threonine dehydrase (Sayre, Jensen and Greenberg 1956) or tryptophan peroxidase (Price and Dietrich 1957) activities by their specific substrates, tyrosine transaminase activity was stimulated by tyrosine only in the presence of adrenal corticoids. Presumably adrenal hormones originally present in the perfusion blood were metabolized (Berliner and Daugherty 1960). The inhibition of induced activity by chloramphenicol and ethionine and especially the reversal of the latter inhibition by methionine are consistent with "de novo" synthesis of new enzyme (Kenney 1962).

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