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Thorotrast Inhibition of Amylase Synthesis by the Isolated, Perfused Rat Liver.* (30983)

N. HIATT, G. M. COVERDALE AND G. BONORRIS (Introduced by M. H. Maxwell)

Department of Surgery, Cedars-Sinai Medical Center, Medical Research Institute, Los Angeles, Calif.

The removal of intravenously injected crystalline hog amylase from the serum of dogs was previously investigated in this laboratory. It was found that at the end of 6 hours, the decrease of amylase in the serum was accompanied by an increase in the amylase activity of the liver equivalent to one-half to two-thirds of the enzyme injected. It was also found that the rise in amylase activity of the liver was almost completely inhibited in dogs with Thorotrast blockade of the reticulo-endothelial system (RES)—which includes the Kupfer cells. It seems possible that in dogs the RES is involved in the mechanism which regulates the amylase level of the serum and the amylase activity of the liver(1,2).

Several investigators have shown that the isolated, perfused rat liver synthesizes amylase and releases it into the perfusion medium(3,4). Many of the cells of the rat liver are RE cells, Kupfer cells. As in the dog, these may be involved in the mechanism that regulates the serum and liver amylase. The present study investigates the release of amylase into the perfusion medium by isolated livers from rats with Thorotrast blockade of the RES.

Methods. Rats of the Slonaker-Addis strain, weighing between 250 and 400 g were used in these experiments. The plasma amylase activity was determined by the method of Van Loon *et al*(5) and the plasma volume by the Evans blue method(6). The total circulating amylase activity was then calculated. The RES in the experimental group of rats was blocked by intravenous injection of 3 ml per kilo of Thorotrast (25% ThO_2) 4 to 6 hours before their use as liver donors(7). The control group was not treated. The liver was removed by the method of Brauer *et al* (8). It was weighed and a small biopsy taken for the determination of amylase activity by the method of McGeachin *et al*(9). The liver was then perfused on the apparatus developed in this laboratory by Rosenfeld *et al*(10), using 150 ml of perfusion fluid. This consisted of either 100 or 120 ml of heparinized rat blood diluted to 150 ml in Ringer's solution. A specimen of perfusion fluid was removed for hematocrit and amylase determination before the perfusion was begun and thereafter at half-hourly intervals for 3 hours. At the completion of the perfusion period, the liver was again weighed and another biopsy taken for determination of the amylase activity. The plasma volume of the perfusion fluid was calculated from the hematocrit.

Results. In 5 normal rats, the total circulating amylase activity (plasma volume $\times$ plasma amylase concentration) averaged 187 units (Table I).

The isolated livers of 6 normal rats were perfused. The total amylase in the perfusing medium increased by an average of 329 units after 2 hours, and by 376 units after 3 hours

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TABLE I. Total Units Circulating Plasma Amylase in Normal Rat.

Body wt (g)	Plasma vol (ml)	Plasma amylase (units/ml)	Total amylase (units)
326	10.7	19.2	204
318	9.5	18.3	170
300	10.6	19.5	207
285	9.8	18.3	180
250	9.6	17.9	173

(Table II). The amylase activity lost by the liver during this 3-hour perfusion was less than 5% of that gained by the perfusion medium during the same period. Liver weights did not change significantly during the perfusion.

The isolated livers of 5 rats which had been treated with Thorotrast were perfused. There was no significant change of amylase activity in the perfusing fluid (Table III). In these livers, total amylase activity increased by almost 30%: the weight by almost 10%.

None of the isolated, perfused livers from either group secreted more than negligible amounts of amylase into the bile.

Discussion. The procedure used in this investigation yields results for normal livers that are substantially the same as those obtained by other observers(3,4). That is, in 2 hours, the isolated, perfused liver from a normal rat releases into the perfusion medium an amount of amylase greater than the rat's total circulating enzyme. The decrease in amylase activity of the liver during the perfusion is only a small fraction of that gained by the perfusion medium (Table II).

With the identical procedure, perfusion of isolated livers from Thorotrast-treated rats results in no release of amylase into the perfusing medium. These livers gain a significant amount of amylase (30%) during the 3-hour perfusion. But this gain seems to be associated with the trapping of amylase-rich perfusion fluid, reflected as an increase in the weight of the liver. The gain in liver amylase activity (36 units) is almost equal to the loss of amylase in the perfusion medium (47 units) (Table III).

It is not clear why Thorotrast blockade of the RES inhibits the release of amylase into the perfusion medium. We found that Thorotrast added to crystalline hog amylase does not affect the activity of the enzyme. Blockade of the RES with Thorotrast causes the Kupfer cells to enlarge(11). It is conceivable that the failure of the plasma amylase to increase is due to interference with circulation through the liver by the enlarged Kupfer cells. However, microscopic examination of a section from a blockaded liver shows many open sinusoids and renders such an hypothesis unlikely (Fig. 1). In the previous papers on amylase synthesis by isolated, perfused livers(3,4) neither the hepatic cell nor the

TABLE II. Amylase Levels During Perfusion of Normal Rat Liver (6 Animals).

Time (hr)	Plasma amylase (units/ml)	Plasma vol‡ (ml)	Total units of plasma amylase	Liver wt (g)	Liver amylase (units/g)	Total liver amylase	Bile amylase (units/ml)	Bile vol (ml)
0	16.0 ± .8*	102 (96–106)†	1679 (1565–1793)*	10.5 (8.3–13.6)†	8.1 (6.9– 9.3)†	84.2 (68.2–98.6)†		
½	17.4 ± .1							
1	17.6 ± 1.1	102 (96–106)	1875 (1658–2016)					
1½	19.3 ± .1							
2	18.8 ± 1.1	103 (96–108)	2008 (1776–2163)					
2½	19.8 ± .1							
3	19.4 ± .8§	100 (96–105)	2055§ (1862–2247)	10.3 (8.9–11.7)	7.3 (6.1–10.6)	75.3 (63.9–92.5)	2.4 (1.2–3.1)	1.7 (1.6–1.8)

* Standard error.
† Range.
‡ Plasma volume = total perfusion volume minus the product of hematocrit and perfusion volume.
§ $P < .05$ compared with value at zero time.

TABLE III. Amylase Levels During Perfusion of Rat Liver with Thorotrast Blockade of RES (5 Animals).

Time (hr)	Plasma amylase (units/ml)	Plasma vol‡ (ml)	Total units of plasma amylase	Liver wt (g)	Liver amylase (units/g)	Total liver amylase	Bile amylase (units/ml)	Bile vol (ml)
0	18.4 ± 1.0*	102 (96–106)†	1868 (1658–2247)†	12.0 (10.7–14.6)†	8.9 (6.8–12.3)†	111.8 (76.8–150.4)†		
½	18.9 ± 1.2							
1	18.2 ± 1.1	100 (96–105)	1836 (1574–2174)					
1½	18.2 ± 1.0							
2	18.2 ± 1.0	102 (96–105)	1907 (1670–2226)					
2½	18.0 ± .9							
3	18.6 ± 1.0§	100 (96–106)	1821§ (1691–1919)	13.6 (11.1–16.7)	10.0 (7.1–14.9)	147.8 (78.8–207.1)	1.9 (.2–2.8)	1.7 (1.6–1.8)

* Standard error.
† Range.
‡ Plasma volume = total perfusion volume minus the product of hematocrit and perfusion volume.
§ No significant difference from value at zero time.

Kupfer cell was established as the source of the newly formed amylase. In view of the present study, the role of RE cells in the synthesis of amylase must be investigated further.

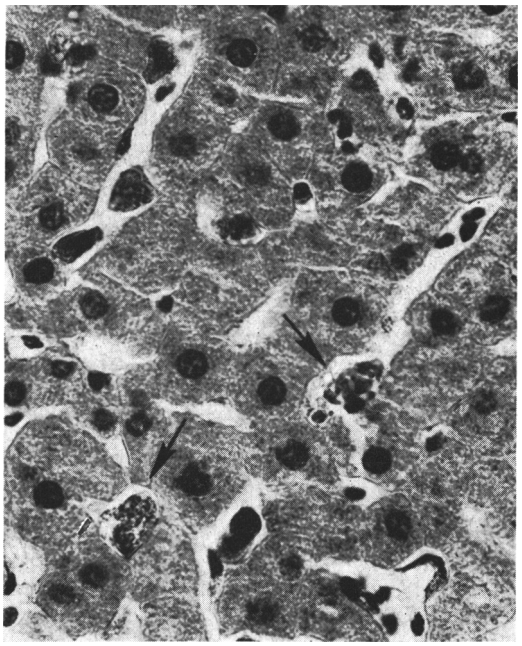

FIG. 1. Liver from rat with Thorotrast blockade of RES (×600). (Arrow points to the cells containing Thorotrast.)

Summary. Isolated, perfused livers from normal rats synthesize amylase and release it into the perfusion medium. Isolated, perfused livers from rats with Thorotrast blockade of the RES do not synthesize amylase and release it into the perfusion medium.

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