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The Functional Status of Lipoprotein Lipase in Rat Liver

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1. Acetone-dried powders of liver and heart tissues from rats given a high-carbohydrate diet or a fat meal were assayed for lipoprotein lipase activity. Heart tissue showed typical lipoprotein lipase activity, whereas none was detected in liver by the usual assay procedures. 2. When mixed acetone-dried powders were prepared from heart plus liver, there was a marked suppression of the expected activity, indicating that an inhibitor was present in the liver. This inhibition was partially overcome in the presence of relatively large amounts of heparin. 3. Lipoprotein lipase was also detected in liver alone when large quantities of heparin were added to the assay system. 4. No increase in lipoprotein lipase activity in either liver or heart was detected when rats were given a fat meal. 5. It is concluded that the liver of the rat contains lipoprotein lipase that is normally present in an inactive state. The results imply that a heparinase is the agent responsible for the inactivation. 6. The significance of the non-functional status of lipoprotein lipase in the liver is discussed. The results support the view that direct hydrolysis of plasma triglycerides by the liver is not a significant physiological process.

There is evidence that the endothelium of certain capillary beds contains the enzyme LPL,† which is concerned with the hydrolysis of triglycerides of chylomicrons and lipoproteins to FFA and glycerol before their utilization by tissues (Robinson, 1963*a*). The enzyme is specific for triglycerides contained in lipoproteins and is activated by heparin and inhibited by protamine and 0·35–1·0M-sodium chloride. By using these criteria, it has been demonstrated clearly in heart and lung tissue (Anfinsen, Boyle & Brown, 1952), in adipose tissue (Hollenberg, 1959; Cherkes & Gordon, 1959), in muscle (Hollenberg, 1960) and in the lactating mammary gland (McBride & Korn, 1963; Robinson, 1963*b*). It is generally considered, however, to be absent from (or not demonstrated convincingly in) liver (Green & Webb, 1964; Robinson, 1965; Olson & Alaupovic, 1966; Fredrickson, Levy & Lees, 1967).

In the original measurements of lipase activity in extracts of acetone-dried powders from rat liver (Korn, 1955), LPL activity was not established since the lipase present was not stimulated by heparin. However, Barclay *et al.* (1962) have indicated the presence of a lipase that is stimulated by heparin. Jeffries (1954*b*) and Morris & French (1958) were unable to demonstrate LPL activity in the perfusate of isolated rat livers to which heparin had been added. Indeed, Jeffries (1954*b*) observed a net decrease in activity after post-heparin blood had traversed the liver. Spitzer & Spitzer (1956) obtained similar results with the perfused rat liver; they observed an increase in lipase activity of the perfusate, but this lipase was not LPL. Nevertheless, LeQuire, Hamilton, Adams & Merrill (1963) have demonstrated that administration of heparin will release a lipase from dog liver, and Condon, Tobias & Datta (1965) have shown that LPL may be released from the liver of man after the administration of heparin. Boberg, Carlson & Normell (1964) have also shown the release of LPL after adding heparin to a perfused dog liver. Thus there is good evidence for the presence of the enzyme in the liver of man and dog, but evidence for its presence in rat liver is still equivocal.

There is also conflicting evidence on the possible role of a lipase in facilitating the direct utilization of triglycerides by the liver. Studies with the perfused rat liver (Morris & French, 1958; Hillyard, Cornelius & Chaikoff, 1959; Kay & Entenman, 1961; Heimberg, Weinstein, Klausner & Watkins, 1962; Morris, 1963; Rodbell, Scow & Chernick, 1964) all showed uptake and oxidation of triglycerides. Heparin was used in all of these studies

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† Abbreviations: LPL, lipoprotein lipase (clearing-factor lipase, EC 3.1.1.3); FFA, free fatty acids (non-esterified long-chain fatty acids).

as an anticoagulant. In experiments with perfused rat livers carried out without the use of heparin (Felts & Mayes, 1965) little uptake or oxidation of chylomicron triglyceride was observed. These differences may be explained if it is postulated that LPL is active in the isolated rat liver only in the presence of exogenous heparin. In the present work, a preliminary report of which has appeared (Mayes & Felts, 1966), this possibility is examined.

MATERIALS AND METHODS

Animals. Male Wistar rats (250–300g.; Scientific Animal Service, Elstree, Herts.), maintained on M.R.C. diet 41B, were transferred to a high-carbohydrate diet 1 week before the experiment. This diet consisted of: vitamin-free casein, 20%; starch, 62%; salt mixture (Hubbel, Mendel & Wakeman, 1937), 3%; brewer's yeast, 5%; cellulose powder, 10%; cod-liver oil (2 drops/100g. of diet). Rats designated 'carbohydrate-fed' were used without further treatment. Rats designated 'fat-fed' were taken off the carbohydrate diet and given 4ml. of cream (approx. 1g. of fat) by intubation 4–6hr. before the experiment. All rats were decapitated with a guillotine and exsanguinated. The blood was allowed to clot at room temperature and the serum collected by centrifuging.

Preparation of homogenates of acetone-dried powders of rat tissues and their assay for LPL activity. Tissues (heart or liver) were removed rapidly, and approx. 1g. portions were weighed and homogenized immediately in 100ml. of ice-cold acetone in an all-glass homogenizer. The homogenate was filtered on a Buchner funnel and the precipitate washed with 25ml. of diethyl ether and dried by air. It was then transferred to a glass–Teflon homogenizer and suspended in 6·0 or 8·0ml. of cold aq. 0·025N-NH_3 soln. adjusted to pH8·5 with HCl. After 5min. the suspension was filtered through Pyrex wool to remove larger connective-tissue particles and portions of the filtrate were taken for lipase assay. The assay system was a modification of that described by Robinson (1963*a*) and consisted of 0·5ml. of 1·35M-tris buffer, pH8·1, 1·5ml. of 15% (w/v) bovine albumin (fraction V; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) in 0·15M-NaCl, 0·2ml. of substrate, consisting of activated triglyceride emulsion (10mg./ml.), 1·0ml. of serum or homogenate of tissue acetone-dried powder and 0·3ml. of 0·15M-NaCl containing additions where indicated. The substrate used was Intralipid (A.B. Vitrum, Stockholm, Sweden) that had been 'activated' by incubation at 37° for 30min. with an equal volume of fresh rat serum. Such activated Intralipid has substrate properties very similar to those of chylomicrons (Boberg & Carlson, 1964). Additions to the assays consisted of heparin powder (Evans Medical Supplies Ltd., Liverpool) dissolved in 0·15M-NaCl, protamine sulphate (Sigma Chemical Co. Ltd., London, S.W. 6) dissolved in 0·15M-NaCl, or NaCl (0·6M). The concentrations indicated in the Tables are final concentrations of the added compounds in the assay system. Incubations were carried out for 1hr. at 37°. Lipase activity was assayed by titrating in duplicate the FFA liberated during the incubation by using the procedure of Dole as modified by Trout, Estes & Friedberg (1960) and Salaman & Robinson (1961). A.R.-grade reagents were used wherever possible.

Acetone-dried powders prepared from post-heparin plasma retained essentially all of the original lipase activity, demonstrating that the method of extracting LPL from tissues did not lead to destruction of the enzyme.

Results were analysed statistically by Student's *t* test or by analysis of variance and changes were considered statistically significant when a value of $P<0·05$ was attained.

RESULTS AND DISCUSSION

Initial experiments were carried out to compare the lipase activity in liver with that in heart and serum. Since LPL activity might be induced in the liver by variations in diet, or the enzyme might be

Table 1 *Effect of diet on lipase activity in serum and in homogenates of acetone-dried powders from liver and heart tissue of rats*

The dietary treatments and method of assay for lipase activity are described in the text. The results are expressed as means ± S.E.M. with the numbers of experiments in parentheses. Statistical significance was tested by comparing the mean change in activity of the individual experiments, due to each addition, with zero. The significance of differences is designated as follows: N.S., no significant difference ($P>0·05$); *P 0·05–0·01; **P 0·01–0·001; ***$P<0·001$.

		Lipase activity (μmoles of FFA released/ml. or g./hr.)			
Tissue	Dietary treatment	No addition	With NaCl (0·6M)	With protamine (0·3mg./ml.)	With heparin (0·4i.u./ml.)
Serum	Carbohydrate-fed (9)	0·02±0·10	0·01±0·10 (N.S.)	0·00±0·05 (N.S.)	0·43±0·17 (N.S.)
	Fat-fed (9)	0·03±0·25	0·14±0·10 (N.S.)	0·14±0·03 (N.S.)	1·10±0·35*
Liver	Carbohydrate-fed (7)	6·9 ±2·4	8·2 ±1·0 (N.S.)	4·4 ±1·3*	7·2 ±0·7 (N.S.)
	Fat-fed (7)	8·3 ±1·2	7·6 ±1·1 (N.S.)	6·7 ±1·0*	8·8 ±0·5 (N.S.)
Heart	Carbohydrate-fed (3)	14·6	3·2	7·3	41·3
	Fat-fed (3)	15·1	1·5	3·8	50·8
	Carbohydrate-fed and fat-fed, combined results (6)	14·9 ±2·7	2·4 ±0·9**	5·6 ±1·6*	46·0±6·6***

transported to the liver from other tissues attached to its chylomicron substrate, the experiments were carried out with rats that had been maintained on a high-carbohydrate diet or given a fat meal (Table 1).

Serum from both carbohydrate- and fat-fed animals showed a very low lipase activity and no significant change was noted in the presence of either sodium chloride or protamine. On the addition of heparin, lipase activity increased in serum from both groups but the increase reached statistical significance only in serum from the fat-fed group, confirming the findings of Jeffries (1954*a*).

Homogenates of acetone-dried powders from heart contained more lipase activity than did either serum or liver. Both sodium chloride and protamine inhibited the lipase markedly, and heparin caused a highly significant increase in activity. No differences were detected between dietary groups.

Homogenates of acetone-dried powders from liver showed considerable lipase activity, but there was no significant difference between the carbohydrate-fed and fat-fed animals. Addition of sodium chloride had no significant effect on the activity in either group. However, protamine inhibited activity significantly in both groups. The addition of heparin (0·4 i.u./ml.) did not alter the lipase activity in either group.

The above experiments confirm earlier studies showing that LPL is present in heart. However, though the liver contains a lipase capable of hydrolysing triglyceride emulsions, it does not have all the characteristic properties of LPL. Thus either LPL is absent from the liver of the rat, or, if it is present, it is inhibited by some factor.

The possibility that rat liver contained an inactivating system for LPL was investigated. Acetone-dried powders were prepared from heart and liver tissue separately, and from the two tissues together, and the powders were assayed for lipase activity (Table 2). The addition of sodium chloride, protamine or heparin (0·4 i.u./ml.) to homogenates of acetone-dried powders of liver did not alter the lipase activity significantly. The lipase activity of homogenates of acetone-dried powders of heart were affected by sodium chloride, protamine and heparin in a manner similar to that noted in Table 1. When heart and liver tissues were mixed before preparation of the acetone-dried powder, the activity of the powder was markedly depressed below that expected from the addition of the units of activity assayed in each tissue alone. As with liver, the addition of sodium chloride, protamine or heparin (0·4 i.u./ml.) had no significant effect on the activity of the combined preparation.

The most likely explanation of these results is that a factor is present in liver tissue that is inhibitory to the LPL activity in heart. The enzyme might be destroyed by liver tissue, or a cofactor or prosthetic group might be removed or inhibited by the liver, rendering the enzyme inactive. Heparin has been considered to be a specific activator or prosthetic group of LPL (Korn, 1955). In addition, Jacques (1940) has described a heparinase in liver. From these considerations, we postulated that in the liver preparations heparin might be removed from its active site on the enzyme, and that if sufficient heparin were added it might counteract this effect. Indeed, when the heparin concentration was increased tenfold (4·0 i.u./ml.) in the assay of the combined heart and liver homogenate, the lipase activity was restored to over 50% of the total of that of the two tissues assayed separately (Table 2). Moreover, in the presence of a similar concentration of heparin, lipase activity increased significantly in homogenates of acetone-dried powders of liver alone; homogenates prepared from

Table 2. *Lipase activity in homogenates of acetone-dried powders of liver and heart and of combined liver and heart tissue from rats*

Tissues were obtained from rats maintained on the stock laboratory diet. The method of assay of lipase activity is described in the text. The results are expressed as means ± S.E.M. with the numbers of experiments in parentheses. Statistical significance was tested by comparing the mean change in activity of the individual experiments, due to each addition, with zero. The significance of differences is designated as follows: N.S., no significant difference ($P > 0.05$); *P 0·05–0·01; **P 0·01–0·001; ***$P < 0.001$.

Tissue	Lipase activity (μmoles of FFA released/hr.) No addition (6)	With NaCl (0·6M) (6)	With protamine (0·3mg./ml.) (6)	With heparin (0·4i.u./ml.) (6)	With heparin (4·0i.u./ml.) (4)
Liver (1g.)	9·7±0·9	10·1±0·7 (N.S.)	8·7±0·5 (N.S.)	9·5±0·9 (N.S.)	12·1±1·2*
Heart (1g.)	25·3±4·9	3·8±1·0*	13·8±6·0***	76·7±14·6**	82·4±7·1***
Liver (1g.) plus heart (1g.)					
Expected	35·0	13·9	22·5	86·2	94·5
Found	18·8±2·8	14·8±4·3 (N.S.)	17·8±2·3 (N.S.)	20·6±4·8 (N.S.)	51·1±8·9*

Table 3. *Effect of diet on heparin-stimulated lipase activity in homogenates of acetone-dried powders from rat livers*

The dietary treatments and method of assay of lipase activity are described in the text. The results are expressed as means of ten experiments ± S.E.M. Statistical significance was tested by comparing the mean activity of the individual experiments, due to the presence of heparin, with zero. The significance of differences is designated as follows: N.S., no significant difference ($P > 0.05$); *P 0·05–0·01; **P 0·01–0·001; ***$P < 0.001$.

Concn. of heparin (i.u./ml.)	Further addition	Heparin-stimulated lipase activity (μmoles of FFA released/g./hr.)	
		Carbohydrate-fed	Fat-fed
0·4	None	0·90 ± 0·25**	0·69 ± 0·63 (N.S.)
4·0	None	3·3 ± 0·7 **	4·2 ± 0·9***
40·0	None	6·3 ± 0·8***	6·1 ± 0·5***
40·0	NaCl (0·6 M)	1·2 ± 0·5*	0·8 ± 0·8 (N.S.)
40·0	Protamine (0·3 mg./ml.)	0·12 ± 0·66 (N.S.)	0·8 ± 0·6 (N.S.)

acetone-dried powders of heart alone did not exhibit any significant additional activity, showing that the lipase was fully activated before the further addition of heparin.

The possible effect of a fat meal on LPL activity in the liver was reinvestigated with high concentrations of heparin in the assay system. Homogenates of acetone-dried powders were assayed in the presence of 0·4 i.u., 4·0 i.u. or 40·0 i.u. of heparin/ml. Homogenates from carbohydrate-fed animals showed a statistically significant increase in lipase activity with each successive addition of heparin (Table 3). Addition of the lowest concentration of heparin to the extracts from fat-fed rats did not result in a significant increase in lipase activity; however, the two higher concentrations did. In both groups, lipase activity increased nearly tenfold as the heparin concentration was increased 100-fold. The heparin-stimulated lipase was sensitive to the inhibitory action of both sodium chloride and protamine, thus fulfilling the criteria necessary to establish the presence of LPL. No statistical differences were detected between carbohydrate-fed or fat-fed rats, demonstrating that it is unlikely that new synthesis of LPL is induced in the liver by the feeding of fat or that the enzyme is taken up from the circulation after a fat meal.

Other investigators have found that high concentrations of heparin are inhibitory to LPL (Korn, 1955; Boberg *et al.* 1964). In our experiments no inhibition was noted. The solutions employed were freshly prepared from powdered heparin and it is possible that inhibition may be the result of contaminants present in some preparations of heparin.

Experiments have been reported with perfused rat livers (Felts & Mayes, 1967) that confirm and extend the observations discussed above. When heparin was present in the perfusion fluid, appreciable release of LPL occurred only when the concentration was 4 i.u./ml. or above, in agreement with the present findings with extracts of acetone-dried powders of liver.

These observations raise the question of the functional significance of LPL in the liver. The source of the enzyme in the circulation is probably the capillary beds of the extrahepatic tissues (Robinson & Harris, 1959). Jeffries (1954*b*) and Yoshitoshi *et al.* (1963) suggested that the liver was the physiological site of inactivation of such circulating LPL. The mechanism of this inactivation is not understood, but the present results suggest that heparinase is involved. It is not known whether the LPL that is present in liver is derived from extrahepatic sources or whether all or some portion of it is synthesized in the liver itself. Also, it is not known whether the LPL is located in the same cellular compartment as heparinase. The finding that LPL activity is released from the liver by large concentrations of heparin does not show, necessarily, that it is normally active in this organ. Indeed, the evidence presented here, and in studies showing that the perfused liver does not hydrolyse appreciably the triglycerides of either chylomicrons (Felts & Mayes, 1965) or of very low-density lipoproteins (Mayes & Felts, 1967), supports the view that the hepatic LPL is normally in an inactive state.

The liver is probably the principal organ of the body that synthesizes and secretes into the circulation endogenous triglyceride as lipoproteins (Havel, Felts & Van Duyne, 1962). The presence in the liver of an inactivating system for LPL may prevent interference of this function by nullifying the potential hydrolytic action of LPL.

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