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Lipoprotein Lipase Metabolism in Experimentally Nephrotic Rats.* (25402)

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Our earlier studies(1,2) strongly suggested that deficiency of circulating albumin in experimentally nephrotic rats is in some manner responsible for abnormal intravascular retention of lipid and consequent hyperlipemia. The possibility of an abnormality of heparin or lipoprotein lipase metabolism as an associated causal factor in experimental nephrotic hyperlipemia also was suggested by earlier studies(3). Since it has been proposed(4,5) that in humans, nephrotic hyperlipemia results from a deficiency of lipoprotein lipase, the following studies were conducted.

Methods. Young, adult male rats of Long-Evans strain were used (average weight: 200 g). The nephrotic state was induced by injection of potent rabbit anti-rat kidney serum (6) 5 to 7 days prior to study and confirmed by demonstration of plasma albumin(7) content less than 1.2 g/100 ml and by presence of gross plasma lactescence and of marked hypercholesteremia(8). Disappearance of heparin from plasma of nephrotic rats was assayed, albeit crudely, by determining clotting time and by protamine titration of plasma obtained from groups of nephrotic and control rats bled at intervals following heparin injection. Each of a series of 10 nephrotic and 18 control rats was injected intravenously with heparin (0.5 mg/100 g). Five nephrotic and 12 control rats were bled with siliconized syringes, from the aorta 2 hours after heparin injection, and the remaining 5 nephrotic and 6 controls after 3 hours. Immediately after bleeding, 0.5 ml of each sample was placed in each of 3 serology tubes for clotting time determination and also in each of a 4-tube system containing serial dilutions of 0.01% protamine sulfate (prepared 24 hours previously). All tubes were kept in water bath at 37°C, and clotting times recorded as the time of average occurrence of a fast clot during gentle tilting at 30-second intervals. Lipoprotein lipase activity of nephrotic postheparin plasma was determined by ability to increase light transmission and to release unesterified fatty acids (UFA)(9) when added to a triglyceride substrate in normal rat plasma. For light transmission studies, plasma samples were obtained from 19 nephrotic rats, 10 minutes after injection of either 0.05 mg (5 rats) or 0.3 mg (14 rats) of heparin/100 g of body weight. Similar postheparin plasma samples were obtained from 16 normal, control rats. For further control purposes plasma

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samples were obtained from an additional 5 nephrotic and 7 normal rats which did not receive heparin. In each instance 0.2 ml of postheparin (or untreated) plasma was added to the test system, which consisted of 1.5 ml pooled normal rat plasma, 1 ml of 0.1 M phosphate buffer (pH 7.2) and 0.3 ml of 1:100 dilution of 50% coconut oil emulsion (Ediol, Schenley Labs..) in 0.9% NaCl. The system was then incubated at 37°C in spectrophotometer cuvettes during 2-hour interval in which light transmission in the test system was measured at serial intervals in a Beckman model B spectrophotometer at 660 mμ. Distilled water was used as the blank. The results are expressed as percentage increases in light transmission which occurred during the experimental interval. For UFA studies, postheparin plasma samples were obtained as above from 22 nephrotic and 20 control rats after varying doses of heparin (0.02 to 1 mg 100 g body weight). For further control purposes, plasma of 4 untreated, normal rats also was obtained. UFA concentration of oil emulsion-normal rat plasma system described above was determined in each instance immediately and again 45 minutes after addition of 0.2 ml postheparin (or untreated) plasma, during which interval the samples were incubated at 37°C. All blood samples were obtained in chilled syringes from the aorta after a 30-hour fast, blood being collected in previously chilled tubes containing 1 10 volume of trisodium citrate, immediately separated by centrifugation in the cold for 15 minutes at 4,000 rpm and kept in an ice bath until use. No attempt was made to remove residual plasma lactescence from postheparin nephrotic rat plasma samples. To study rate of disappearance from plasma of lipoprotein lipase released in acute response to heparin injection, postheparin plasma samples were obtained 15, 45, 60 and 90 minutes after injection of heparin (0.4 mg 100 g) from 21 nephrotic and 19 control rats. These were then assayed by measuring the change in light transmission occurring in the test system described above, during a 60-minute interval following addition of postheparin plasma sample to be measured. Duration of lipolytic activity of postheparin nephrotic plasma also was studied in an additional series of 14 nephrotic and 15 control rats similarly injected with heparin and bled 10, 30, and 60 minutes later. In each instance the UFA content was determined immediately and 45 minutes after addition of postheparin plasma to the usual test system. To determine whether lipolytic activity would continue to be produced in plasma of nephrotic rats despite repeated heparin injection, 4 nephrotic and 4 control rats were each injected intravenously with 1 mg heparin at hourly intervals for 4 hours. One hour later each rat was injected with 0.1 mg heparin and postheparin plasma samples obtained 10 minutes later. In each instance ability of the postheparin plasma to increase light transmission and to release UFA in the same oil emulsion-normal rat plasma test system was determined as described above. The final experiment was concerned with determining ability of the same triglyceride substrate to be hydrolyzed by lipoprotein lipase when suspended in *nephrotic* rat plasma, the lipase being furnished by postheparin plasma obtained from normal rats. A series of 8 starved nephrotic rats was bled from the aorta using cold syringes, and the plasma immediately separated in a refrigerated centrifuge. All visible plasma lactescence was then removed by centrifuging at 4°C for 30 minutes at 28,000 rpm and the cleared subnatant plasma kept in ice bath until used. Each plasma sample was divided into paired aliquots of 3 ml and to each aliquot was added 0.35 ml of the 1:100 dilution of coconut oil emulsion and 0.25 ml of postheparin plasma, obtained 10 minutes after injection of a normal 30-hour-starved rat with 1 mg heparin. In addition, 60 mg of powdered bovine serum albumin (Armour Fraction V) was added to one of each paired aliquots. All samples were incubated at 37°C for 2 hours, during which light transmission was measured at intervals. UFA also was determined immediately and 45 minutes after addition of postheparin plasma. For control purposes, similar studies were done using plasma obtained from 5 starved, normal rats.

Results. 1. *Effect of heparin on blood clot-*

TABLE I. Effect of Intravenous Heparin on Clotting Time of Nephrotic Rats.

Type of rat	No. of rats	Avg wt, g	Total cholesterol, mg/100 ml	Clotting time, min.	Avg protamine-induced clotting time, min. .001 mg	.002 mg	.004 mg	.008 mg
*Rats bled 2 hr after heparin**								
Nephrotic	5	230	366 (159-502)†	20.5 (15-27)	17.0 (14.0-22.5)	14.7 (11.0-19.0)	11.0 (8.0-14.5)	7.0 (5.0-8.0)
Control	12	245	54 (41-69)	22.9 (10-35)	19.0 (12.5-23.0)	10.5 (7.3-16.5)	5.2 (4.0-6.3)	4.8 (3.5-6.3)
*Rats bled 3 hr after heparin**								
Nephrotic	5	210	417 (244-703)	17.2 (13-22)	14.5 (10.0-17.0)	10.1 (9.5-11.0)	6.5 (4.5-8.5)	5.2 (4.3-8.0)
Control	6	225	61 (42-74)	11.0 (7-17)	11.2 (8.3-21.0)	6.5 (5.0-8.3)	5.5 (4.0-6.5)	5.0 (3.3-6.5)

* 0.5 mg/100 g. † Range of values.

ting time of nephrotic rats. Table I presents results of blood clotting time of postheparin nephrotic and control rat blood. Blood clotting times of nephrotic rats averaged 20.5 minutes in those bled 2 hours, and 17.2 minutes in those bled 3 hours, after heparin injection, compared to an average of 22.9 minutes and 11 minutes in their respective controls. Table I also shows amount of protamine sulfate required to neutralize heparin-induced prolongation of clotting time of nephrotic rats' plasma at each sampling interval, was not less than that required for comparable effect in their respective control rats' plasma.

2. *Ability of nephrotic rat to release lipoprotein lipase following heparin administration.* The first experiment was concerned with ascertaining nephrotic rat's ability to release lipoprotein lipase into its plasma, immediately following heparin administration. Fig. 1 shows percentile average increase of light transmission in coconut oil emulsion-normal rat plasma test system, following addition of plasma obtained from groups of nephrotic and control rats. Whereas no change in light transmission was noted following addition either of preheparin nephrotic or of control rats' plasma, a progressive increase of light transmission was consistently observed following addition of postheparin nephrotic plasma, of considerably greater magnitude in instances where the latter was obtained from nephrotic rats injected with larger quantity of heparin. At both dosage levels, rate and magnitude of increase of light transmission in the test system following addition of postheparin nephrotic rats' plasma were similar to changes induced by addition of postheparin plasma obtained from their respective controls. The plasma total cholesterols of nephrotic rats averaged 255 mg/100 ml (range: 152-527).

Fig. 2 shows the increase of UFA in the same oil emulsion-normal rat plasma test system which occurred during 45-minute interval following addition of plasma obtained from nephrotic and control rats variously injected with heparin (0.02 to 1 mg/100 g weight). In each instance the average increase of UFA effected by postheparin nephrotic rats' plasma was not less than that induced by their respective control rats' postheparin plasma. As expected, in both groups the magnitude of UFA increase was progressively greater when postheparin plasma was obtained from rats given the larger dosage of heparin. On the other hand, no increase of UFA was noted in the test system following addition of plasma obtained from control rats not injected with heparin, the initial UFA content in these rats averaging 0.540 meq/l (range: 0.432-0.592) and 45 minutes later averaging 0.502 meq/l (range: 0.412-0.540). Plasma total cholesterol of nephrotic rats averaged 357 mg/100 ml (range: 163-457).

The second experiment compared *duration* of lipolytic activity of postheparin nephrotic and normal rat plasma. Fig. 3 shows percentage average increase of light transmission occurring in oil emulsion-normal rat plasma test system during 60-minutes following addition of test postheparin nephrotic and control rats' plasma, variously obtained at 15 to 90

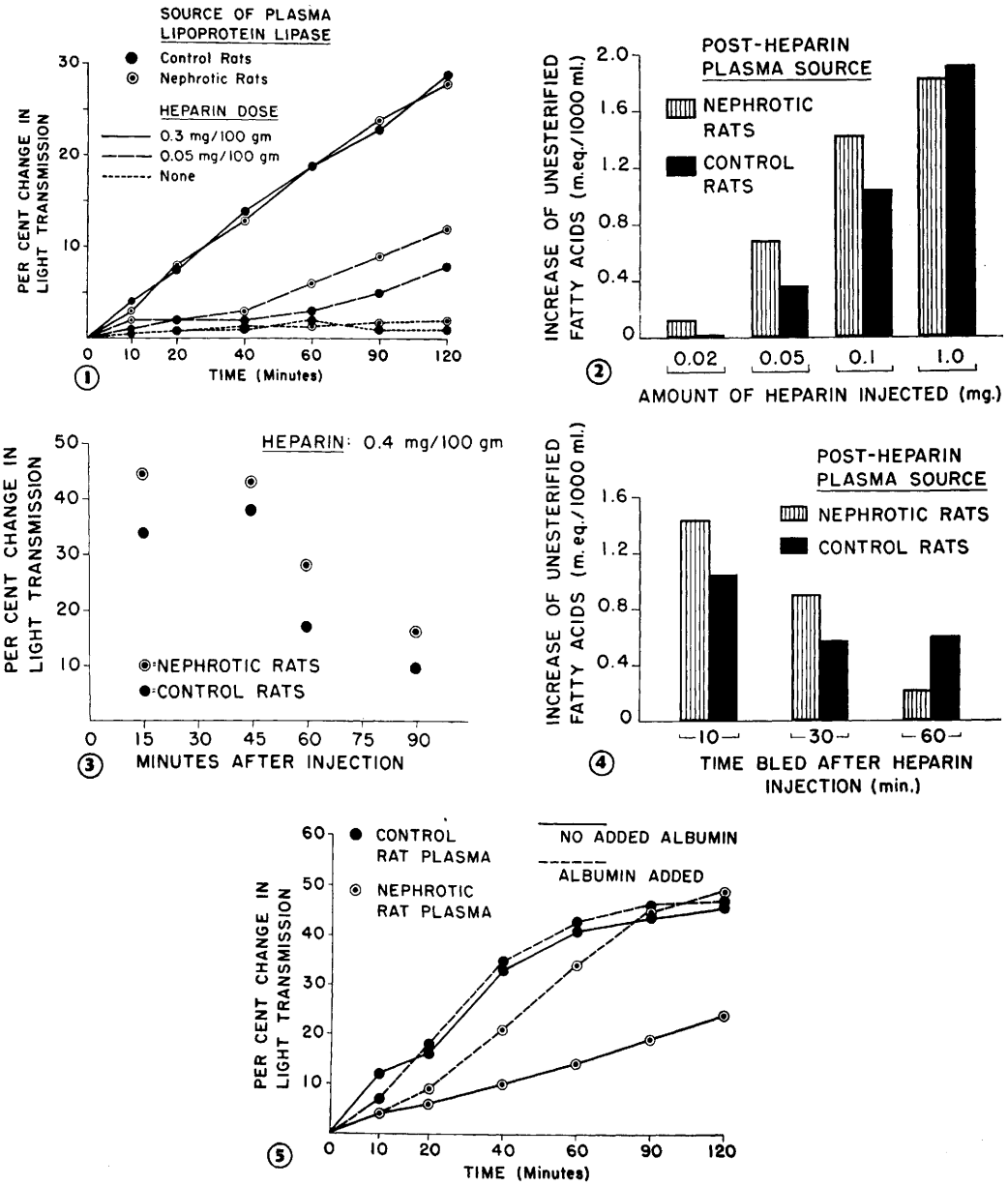

FIG. 1. Lipemia-clearing activity of post-heparin nephrotic rats' plasma.
FIG. 2. Lipolytic activity of post-heparin nephrotic rat's plasma.
FIG. 3. Lipemia-clearing activity of nephrotic rat's plasma at intervals after intrav. heparin.
FIG. 4. Lipolytic activity of nephrotic rat's plasma at intervals after intrav. heparin.
FIG. 5. Effect of albumin on lipemia-clearing of triglyceride in nephrotic rats' plasma.

minutes following heparin injection. At each interval, the lipolytic activity of postheparin nephrotic rats' plasma, thus assayed, was not less than that of control rats. Fig. 4 shows the average increase in UFA content induced in the same oil emulsion-normal rat plasma system by addition of postheparin plasma obtained from nephrotic and control rats again bled at various intervals following heparin administration. The postheparin plasma obtained from nephrotic rats bled 10 and 30 minutes after heparin injection effected com-

parable or slightly gerater increases of UFA than did their respective control rats' postheparin plasma, the converse being found among rats bled 60 minutes after heparin injection. However, the number of rats involved in this experiment was relatively small, and the observed differences are not significant. The plasma total cholesterols of nephrotic rats averaged 298 mg/100 mg (range: 163-457).

The third experiment compared lipolytic activity of plasma obtained from nephrotic and control rats after repeated injections of heparin. Postheparin plasma thus obtained from nephrotic rats increased light transmission an average of 28% (range: 21-34) during 60-minute interval following addition to the oil emulsion-normal rat plasma test system, and increased the UFA content of the test system from initial average of 0.485 meq/l (range: 0.304-0.577) to 1.949 meq/l (range: 1.533-3.010) 45 minutes later. Addition of control rats' postheparin plasma to the test system similarly increased light transmission an average of 27% (range: 22-30) and increased the UFA content from an initial average of 0.347 meq/l (range: 0.149-0.600) to an average of 1.662 meq/l (range: 1.209-2.124).

3. *Ability of nephrotic plasma to support lipolysis of a triglyceride substrate.* The final experiment was concerned with studying ability of normal rat postheparin plasma to effect lipolysis of the same triglyceride substrate used above when suspended in nephrotic plasma ultracentrifugally "cleared" of its own excess triglyceride content. Addition of normal rat postheparin plasma to the oil emulsion comparably increased the UFA content whether the triglyceride substrate was suspended in normal or nephrotic rat plasma, although the albumin content of the normal rat plasma averaged 2.74 g/100 ml (range: 2.01-2.51) compared to an average of 0.79 g/100 ml (range: 0.62-1.15) in the nephrotic plasma. Thus the UFA content increased an average of 1.305 meq/l (range: 0.517-2.074) in the oil-nephrotic rat plasma and 1.283 meq/l, range: 1.034-1.727) in the oil-normal rat plasma following addition of the normal rat postheparin plasma. These values were not significantly altered when albumin was added to the same test system, averaging 1.682 meq/l (range: 1.271-2.283) and 1.076 meq/l (range: 0.449-1.423), respectively, under these circumstances.

During the 2-hour experimental interval following addition of normal rat postheparin plasma, light transmission increased an average of 46% in the control, oil emulsion-*normal* rat plasma system, and an average of 47% in the paired aliquots also containing added albumin (Fig. 5). In contrast, light transmission increased an average of 24% during the same interval in the oil emulsion-nephrotic rat plasma test system following addition of the same postheparin plasma, and an average of 48% in paired aliquots containing added albumin.

Discussion. Since lipoprotein lipase production is not related to the anticoagulant action of heparin, the results of the first experiment suggest that the experimental nephrotic state in rats is not associated with any abnormality in plasma disappearance of exogenously administered heparin.† Moreover, the data can do no more than imply a similar normality in nephrotic rats' handling of endogenously derived heparin.

It is believed significant, however, that our studies failed to detect any abnormality in lipoprotein lipase response of the nephrotic rat to administered heparin. Thus, whether plasma was obtained from the nephrotic rat immediately, somewhat later, or even after repeated doses of heparin, it exhibited as much lipase activity as did plasma of rats similarly injected with heparin.

That nephrotic rat postheparin plasma effected release of UFA and optical "clearing" of the triglyceride substrate comparable to that induced by normal rat postheparin plasma also fails to support the contention that such experimental nephrotic rat plasma contains an inhibitor of lipoprotein lipase (12). The presence of such an inhibitor has

† Although the data actually suggest a delayed rate of heparin removal in the nephrotic rat, any statistical evaluation of such a conclusion did not appear warranted in view of the relative crudeness of the assay method employed.

not been ruled out, however, since in the human at least, it may be contained in the fatty layer of ultracentrifuged, hyperlipemic plasma (13), removed from the nephrotic plasma used in final experiment.

In the final experiment no abnormality was detected in ability of nephrotic plasma to support lipase-induced release of UFA from a triglyceride substrate suspended in such plasma. The fact that concomitant optical "clearing" of this triglyceride substrate proceeded at a slower rate and was of lesser magnitude when suspended in nephrotic plasma compared to normal rat plasma is not surprising since lipase-induced release of UFA is only poorly related to optical density changes and occurs even in absence of optical "clearing"(14). Addition of extra albumin to the hypoalbuminemic, nephrotic rat's plasma appeared to normalize the ability of such plasma to support continued lipase-induced "clearing" of the added triglyceride substrate. This would appear only to confirm the well-known role of albumin as an acceptor for UFA(10,11) and the fact that continued optical "clearing" of a triglyceride substrate by lipoprotein lipase is prevented by release of UFA in excess of that which can be bound by available albumin(10,11,14).

Although adequate albumin appears essential for sustained lipolysis *in vitro*(10,11,14) and for sustained lipase-induced, lipemia-clearing *in vivo* in the nephrotic rat(3), its known role as an acceptor of UFA(10,11) does not actually explain the mechanism by which severe renal loss of albumin and hypoalbuminemia appear sequentially to induce hypertriglyceridemia(1,2), hyperphospholipemia and hypercholesteremia(15). Thus our exeriments do not indicate any actual inability of lipase to release UFA in nephrotic plasma, and indeed, intravascular lipolysis of triglyceride may be neither a prerequisite nor a significant physiological mechanism for plasma egress of its fatty acid moiety(16,17).

Summary. Heparin-activated lipolytic mechanisms were studied *in vitro* in rats with nephrosis experimentally induced by injection of antikidney serum. The nephrotic rat was found normally capable of releasing lipoprotein lipase following heparin administration. The nephrotic rat's plasma also was found normally capable of supporting lipoprotein lipase-induced hydrolysis of a triglyceride substrate.

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