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Removal of Chylomicron Lipid from Plasma in Experimental Nephrosis.* (25766)

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Hyperlipemia is a characteristic feature of experimental nephrosis produced in rats by injection of rabbit anti-rat kidney serum. One factor contributing to the hyperlipemia is a decrease in rate of removal of chylomicron lipid from the circulation(1,2). Two possible explanations for this abnormality are suggested. First, the delayed removal may be dependent on presence in nephrotic plasma of an inhibitor to lipoprotein lipase which is responsible for intravascular lipolysis of chylomicrons(3). Second, the suggestion has been made that nephrotic hyperlipemia is due to retention of lipid in the plasma as the result of hypoalbuminemia, on the assumption that the amount of serum albumin is insufficient to transfer fatty acids at a normal rate from lipoproteins to the tissues(4). The following studies were undertaken to assess the effects of serum albumin concentration and of lipoprotein lipase inhibitor on rate of chylomicron removal from the circulation of rats made nephrotic by antikidney serum.

Methods. Nephrosis was produced in rats by injection of rabbit anti-rat kidney serum (5). Rat serum albumin was obtained from normal rat serum after precipitation of globulins by third-saturated ammonium sulfate. It was dialyzed against normal saline to remove excess sulfate, lyophilized, and made up in concentrated solution for administration. On electrophoretic examination the albumin was 87% pure. For *in vitro* studies on clearing of lipemic serum, the source of lipoprotein lipase was post-heparin plasma, obtained from the aorta of fasted normal rats 15 minutes after intravenous injection of heparin (4 mg/kg body weight). Post-heparin plasma was incubated with normal rat serum previously made lipemic by addition of rat chyle sufficient to give an optical density (O.D.) of 0.450-0.700 when read at 550 mμ or 750 mμ. In a preliminary experiment the O. D. of lipemic serum was found to be a linear function of amount of rat chyle added to it. To measure rate of removal of chylomicrons from the circulation, 1.0 ml of C^{14}-labeled chyle, prepared according to French and Morris(6), was injected intravenously; radioactivity was measured in whole blood at intervals over the next 30 minutes, by plating aliquots of 0.05 ml of tail vein blood and counting in a windowless proportional gas flow counter. The fractional rate constant of chylomicron re-

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moval (fraction/min.) was then taken as the slope of the log of radioactivity (counts/min/ml) plotted against time. Freshly prepared chyle was used for each experiment; lipid concentrations varied from one preparation to another, but in each experiment all animals received the same amount of lipid. Chylomicron counts were made on serum in a standard field under dark ground illumination. Animals were fed a standard laboratory chow and given tap water to drink, unless otherwise noted.

Results. Effect of glucose feeding on chylomicronemia. It was previously reported(2) that withdrawal of food and giving of 50% glucose to drink for 16 hours resulted in low levels of endogenous chylomicronemia in both normal and nephrotic rats. To confirm this finding, chylomicron counts were made on 4 normal and 4 nephrotic rats prepared in this way. In addition, 1 normal animal so prepared was given 1.0 ml of chyle intravenously, and serum was taken at once for a chylomicron count. Counts in the standard field were: normal, 3-5; nephrotic, 5-10; normal chyle-injected, 550. Thus the levels of endogenous chylomicronemia were insignificant in the presence of the level produced by injection of chyle; consequently in all subsequent experiments based on measurement of chylomicron removal rates, animals were prepared in this manner to minimize dilution of exogenous with endogenous chylomicrons.

Effect of endogenous hyperlipemia on rate of disappearance of chylomicrons from blood. Two rats were fed a diet containing 60% butter-fat and 5% cholesterol; this produced fasting serum lipid levels of 5.4 and 4.9 g/100 ml. Rate of removal of chylomicrons from the circulation was then measured in these animals and in 2 control animals given the standard diet. The fractional rate constants of removal were essentially the same for the 2 pairs of rats: 0.038 and 0.053 for control animals and 0.042 and 0.043 for hyperlipemic animals.

Effect of albumin administration on rate of disappearance of chylomicrons from blood of nephrotic rats. Six nephrotic rats were used, each serving as its own control. Labeled chyle was injected into each rat intravenously twice at an interval of 2-3 days, and disappearance rate of radioactivity from the blood was measured. Ten minutes before the second injection, each rat received 1 ml of rat serum albumin (9.0 g/100 ml), sufficient to increase serum albumin by approximately 1.5 g/100 ml. Fractional rate constants before and after albumin administration are listed in Table I. Results indicate that except in one rat (#6) raising the levels of albumin failed to raise fractional rate constant of removal to a significant degree.

TABLE I. Effect of Albumin on Fractional Rate Constant of Chylomicron Disappearance in Nephrotic Rats.

	Fraction/min.			Fraction/min.	
No.	Control	Post albumin inj.	No.	Control	Post albumin inj.
1	.0014	.0037	4	.0003	.0011
2	.0082	.0113	5	.0018	.0056
3	.0022	.0027	6	.0070	.0554

Susceptibility of chylomicrons from normal and nephrotic rats to post-heparin clearing factor in vitro. To aliquots of normal rat serum was added chyle collected from normal or nephrotic rats to give "lipemic sera" of equal O.D. To 0.9 ml aliquots of these sera was added 0.1 ml of normal post-heparin plasma, and mixtures were incubated at 35°C for 90 minutes, with readings of O.D. made every 30 minutes. The results (Table II) fail to show any differences in rates of clearing of chylomicrons obtained from normal and nephrotic rats.

Effect of nephrotic plasma on post-heparin clearing factor in vitro. To determine the effect of nephrotic plasma on clearing rate of lipemic serum by post-heparin plasma, aliquots of 0.1 ml or 0.3 ml of nephrotic and control sera were added to 1 ml of lipemic rat serum before addition of 0.1 ml of post-heparin plasma. The O.D. was read at 0, 30, 60 and 120 minutes. The results (Table III)

TABLE II. Clearance of Normal and Nephrotic Chylomicrons by Post-Heparin Plasma *In Vitro.*

	30 min.	60 min.	90 min.
Normal	.106	.165	.170
Nephrotic	.099	.164	.191

Values represent mean decreases in O.D. in 3 experiments.

TABLE III. Effect of Normal and Nephrotic Sera on Lipemia Clearing *In Vitro*.

Exp.		Conc. of test serum, % of total vol	% change in O.D. 30′	% difference	60′	% difference	120′	% difference
1	Control (6)	10			27.0	−18.5	34.4	−18.6
	Nephrotic (6)	10			22.0		28.0	
2	Control (4)	10			45.3	−30.0		
	Nephrotic (6)	10			31.7			
3	Control (6)	10	17.2	27.9	22.3	−24.7	19.5	−24.1
	Nephrotic (5)	10	12.4		16.8		14.8	
	Control (6)	25	16.4	40.8	22.8	−31.1	26.0	−53.1
	Nephrotic (5)	25	9.7		15.7		12.2	
4	Control (6)	10	10.7	50.5	18.2	−37.9	26.2	−33.6
	Nephrotic (8)	10	5.3		11.3		17.4	
	Control (6)	25	6.0	66.6	12.2	−62.3	19.8	−54.0
	Nephrotic (8)	25	2.0		4.6		9.1	

Concentration of 10% of test serum obtained by adding 0.1 ml of serum to 0.9 ml of lipemic plasma.

Concentration of 25% by addition of 0.3 ml of test serum to 0.9 ml of lipemic plasma.

indicate that nephrotic plasma in a concentration of 10% by volume caused a 30-50% inhibition of lipemia clearing. An increase in concentration of nephrotic plasma to 25% by volume caused an inhibition of somewhat greater magnitude.

Effect of nephrotic plasma on disappearance rate of chylomicrons from blood of normal rats. Three normal rats were injected with .5 ml of labeled chyle and disappearance rate of radioactivity in blood was measured. Three days later 1 ml of nephrotic serum was given to the same animals, followed in 10 minutes by a second injection of labeled chyle; radioactivity in blood was again measured. The results (Table IV) show that no inhibition of removal occurred in any of the animals as a result of administration of nephrotic serum.

Discussion. Deficiency of serum albumin in nephrotic rats has been suggested as the cause of nephrotic hyperlipemia on the assumption that the available albumin cannot remove the freed fatty acids which then inhibit further lipolysis(4). In support of this hypothesis, it was shown that continuous intravenous infusion of bovine serum albumin lowered the concentration of lipids in the blood of nephrotic rats to nearly normal values(7). However, Morris and French(1) found that administration of 200 mg (equal to the total serum albumin of a normal rat weighing 120 g) of bovine serum albumin produced no acceleration in rate of disappearance of chylomicrons from the blood of normal or nephrotic rats. The present work was based on use of homologous serum albumin in preference to bovine material in order to evaluate the role of albumin under more physiologic conditions. The results, showing failure of an increase in serum albumin concentration to increase rate of removal of chylomicrons, confirm the findings of Morris and French(1), and lead to the conclusion that hypoalbuminemia is not the cause of retention of chylomicron fat in the blood in nephrosis.

TABLE IV. Effect of Nephrotic Plasma on Fractional Rate Constant of Chylomicron Removal in 3 Animals.

(Fraction/min.)

Control	After inj. of nephrotic plasma
.158	.163
.193	.177
.190	.188

It has been postulated that the delayed removal of chylomicrons is the result of isotope dilution by endogenous plasma lipids released from fat depots(1). This thesis makes the assumptions that the endogenous lipemia is due to increased mobilization and that the serum lipids are present in the form of chylomicrons. Our results indicate that the dietary preparation used caused a fall in chylomicronemia to very low levels in both normal and nephrotic rats; though the level was slightly

higher in nephrotic animals, the difference was insignificant in the presence of the marked rise in chylomicronemia produced by injection of the test load of chyle. Furthermore, the presence of massive endogenous hyperlipemia in non-nephrotic rats did not alter rate of removal of administered chylomicrons. It is therefore concluded that the decreased rate of chyle removal from nephrotic plasma was not due to isotope dilution.

An inhibitor of lipoprotein lipase has been demonstrated in the serum of nephrotic rats (3), and the possibility was thus raised that the decreased rate of removal of chylomicrons is a consequence of this inhibition. Our results confirm the observation that nephrotic plasma inhibits the clearing action *in vitro* of post-heparin plasma. However, when normal rats were injected with nephrotic serum to obtain a concentration in their vascular system comparable to that used in the *in vitro* system, no inhibitory effect on rate of chyle removal was observed. This suggests that the clearing process may not play a major role in chylomicron removal. There is evidence that chylomicrons can be removed as particulate matter without prior hydrolysis by serum lipoprotein lipase(8), so that even if the degree of inhibition of lipoprotein lipase observed *in vitro* had obtained *in vivo,* it might not have been apparent in overall estimates of the chylomicron removal rate.

Summary. 1. Carbohydrate feeding reduced chylomicronemia to similarly low levels in normal and nephrotic rats; following such dietary preparation the decreased rate of chylomicron removal from blood of nephrotic rats is not due to dilution of administered chylomicrons. 2. Nephrotic rats were given intravenous injections of 90 mg of normal rat serum albumin. This did not alter rate of removal of labeled chylomicrons from the circulation. 3. When nephrotic rat plasma was added to *in vitro* clearing system, consisting of normal rat plasma, normal rat chylomicrons and normal rat post-heparin plasma, it significantly decreased rate of clearing. Chylomicrons obtained from normal and nephrotic rats were cleared equally well by serum lipoprotein lipase. 4. Injection of nephrotic rat plasma into normal rats did not alter rate of removal of labeled chylomicrons.

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Rapid Adsorption of Vaccinia Virus on Tissue Culture Cells by Centrifugal Force.* (25767)

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Because of their large size, vaccinia virus particles diffuse slowly. The several hours generally allotted to adsorption of a virus inoculum to cells in monolayer can be reduced if adsorption takes place in suspensions in which the cells are quite concentrated(1). However, difficulty is still encountered in attaining adsorption of a high percentage of the virus in a short time. If accurate measurements of the time between adsorption of a

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