JCI The Journal of Clinical Investigation

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J Clin Invest. 1962;41(1):185-195. https://doi.org/10.1172/JCI104462.

Research Article

Journal of Clinical Investigation
Vol. 41, No. 1, 1962

TISSUE DISTRIBUTION OF C^{14} AFTER THE INTRAVENOUS INJECTION OF LABELED FREE FATTY ACIDS AND CHYLOMICRONS IN NEPHROTIC RATS *

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(Submitted for publication February 17, 1961; accepted August 24, 1961)

The etiology of the hyperlipemia observed in the nephrotic syndrome has been the subject of many papers in recent years. The results of these studies, although not mutually exclusive, have been interpreted in a variety of ways. The authors attribute this lipemia to an increase in the synthesis of lipids by the liver (1–4) joined to an increased hepatic protein synthesis (5), a deficiency of serum albumin (6), or the appearance of an antagonist to clearing factor in the circulating blood (7). A recent study by Saffran and Kalant (8) explained the hyperlipemia on the basis of "an increased hepatic synthesis and discharge of lipid" and "a decreased ability to remove chylomicron lipid from circulation."

The distribution of C^{14}, after intravenous injection into normal rats of sodium palmitate-1-C^{14} or of chylomicrons biosynthesized from palmitic acid-1-C^{14}, was studied by Bragdon and Gordon (9). The modifications in composition of plasma lipids and in liver function in nephrosis suggested a comparison of the distribution of C^{14}-palmitate and C^{14}-chylomicrons in nephrotic rats in order to see whether a difference of distribution of C^{14} could yield information that might help to explain the hyperlipemia of the syndrome.

METHODS

Preparation of rats. The experimental animals were male Osborne-Mendel rats weighing between 160 and 300 g. Nephrosis was induced by a single injection of a pool of rabbit antirat kidney serum prepared by the method described by Baxter and Goodman (10). At the time of injection, the weight of the rats was about 150 g. At least 4 days elapsed between injection and experiment. The criteria of nephrosis were proteinuria (more than 150 mg protein per 24 hours), hypoalbuminemia (lower than 3.0 g per cent, values under 1.0 g per cent being obtained in severe nephrosis), and at least slight edema or ascites.

Food was removed from the cages 20 hours before an experiment. Fasted animals were offered 0.45 per cent NaCl during the preceding night and received 2 ml of the same solution by stomach tube 1 hour before injection of the labeled material. Three normal, fasted rats received 2 ml of Lipomul (cottonseed oil emulsion) intravenously 5 minutes before the injection of C^{14}-palmitate. Two normal rats received 3 ml of a chylomicron suspension, containing 50 mg fat per ml, 5 minutes before the injection of C^{14}-chylomicrons, and 3 other normal rats received 3 ml of unlabeled chylomicron suspension, containing 15 mg fat per ml, immediately before the radioactive dose. Carbohydrate-fed rats were offered 10 per cent dextrose in 0.45 per cent NaCl during the night preceding the experiment. Each drank about 150 ml of the solution. They received an additional 2 ml of 50 per cent dextrose solution by stomach tube 1 hour before the injection of free fatty acids (FFA) or chylomicrons.

Each animal, normal or nephrotic, received a dose of 2 μc C^{14} as palmitate or 1 μc C^{14} as chylomicrons in a total volume of 1 ml by injection in the tail vein.

Preparation of the FFA solution. Approximately 0.32 mg of palmitic acid-1-C^{14}, containing about 25 μc radioactivity, was dissolved in 1 ml ethanol in a small flask. A moderate excess of 0.05 N NaOH was added and the mixture evaporated to dryness. The residue of sodium palmitate was dissolved in 1 ml distilled water by gentle heating, cooled, and 12.5 ml of normal rat serum at 4° C was added suddenly with vigorous agitation. No crystallization of sodium palmitate was observed. The solution was kept in the freezer.

Preparation of the chylomicron solution. The method used was a modification of the procedure of Bragdon (11). Donor rats, the cysternae chyli of which had been cannulated on the previous day, were given about 50 μc potassium palmitate-1-C^{14} in a few milliliters of water by stomach tube. These donor rats received saline and chow diet during the night. The chyle was collected in an ice-bath from 0.5 to 6 hours after the ingestion of potassium palmitate. On the following day 20 ml of chyle

* Presented in part at the meetings of the Federation of American Societies for Experimental Biology, April, 1960, Chicago, Ill., and at the Twelfth Annual Conference on the Nephrotic Syndrome, November, 1960, Princeton, N. J.

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was mixed with 1 ml of 2.6 M NaCl and 3.0 M KBr solution, layered under a 0.1 M citrate-phosphate buffer of pH 7.4 and centrifuged for 30 minutes at approximately 60,000 G. The chylomicrons were concentrated at the top of the centrifuge tube, where they were readily separated from the residual chyle proteins. They were washed twice more through saline or the same buffer solution by a second and a third centrifugation. Analysis of the preparation showed that more than 90 per cent of the radioactivity was contained in the triglyceride moiety and about 2 per cent in the free fatty acids. The lipid content of the chylomicron preparation ranged between 15 and 25 mg per ml. About 50 μc of radioiodinated human serum albumin (I^{131}-RISA) was added to 1 ml of normal rat serum, the mixture incubated at room temperature, and exhaustively dialyzed to remove unbound I^{131}. This material was added to 5 ml of washed chylomicrons in order to estimate the residual plasma content of tissues, following the procedure employed by Bragdon and Gordon (9). For determination of the I^{131} content, the protein precipitates of the original extracts of the tissues were counted in a well-type scintillation counter, directly or after digestion in ethanolic KOH.

Preparation of blood and organs. The animals were anesthetized intravenously with sodium pentobarbital, 3 or 10 minutes after the FFA injection and 10 minutes after the chylomicron injection. Five ml of blood was immediately withdrawn from the abdominal aorta into a heparinized syringe. The organs and tissues were removed, washed in saline, blotted dry, weighed, and cut up into ethanol-acetone (1:1) as rapidly as possible, in the following order: piece of liver, spleen, fat bodies (epididymal or perirenal), kidneys, heart, muscle (right adductor), and lungs. The remainder of the liver was weighed and discarded. The carcass and remaining viscera were put through a meat grinder and then extracted in 3 L of ethanol-acetone (1:1) in a large blender for 5 minutes. All tissues were in fixatives within 10 minutes after sacrifice. The organ and tissue samples were extracted in three successive amounts of 15 ml of ethanol-acetone in a smaller blender. These were then transferred with their extracts into graduated centrifuge tubes, where they were brought to a final volume of 50 ml. Chloroform-methanol (2:1) extracts of the injected palmitate or chylomicrons and of the plasma were made. On the following day, after frequent mixing, the extraction mixtures were centrifuged and aliquots of the ethanol-acetone extracts evaporated under air at 57° C. A drop of olive oil was added to the chloroform-methanol extracts of plasma and labeled palmitate to inhibit sublimation of free fatty acids due to an insufficient amount of carrier fat. The dried lipids were then taken up in a final volume of 15 ml of a 0.5 per cent solution of 2,5-diphenyloxazole (DPO) in toluene and counted in a Packard Tri-Carb scintillation counter.

Chemical determinations on tissues. Plasma, liver, heart, kidney and muscle total lipids were determined in chloroform-methanol extracts by the method of Bragdon (12); free and total cholesterol in alcohol-acetone extracts by the procedure of Sperry and Webb (13); and lipid phosphorus according to Stewart and Hendry (14). The triglycerides were estimated by difference. The same methods were used on aliquots of carcass extracts. Triglycerides and FFA in the blood were separated according to Borgström's procedure (15) and the extracts were counted separately. FFA were determined by the method of Dole (16) or Gordon and Cherkes (17). Liver, kidney, heart and skeletal muscle extracts were evaporated under air at 50° C and taken up in 5 ml of chloroform. These chloroform extracts were separated on silicic acid columns into neutral lipids (including glycerides, cholesterol esters and free fatty acids) and phospholipids, by using successively pure chloroform and pure methanol (18). Chloroform and methanol fractions were evaporated and 15 ml of DPO solution in toluene added for counting. The albumin determination in the plasma was determined according to a modification of the method of Rutstein, Ingenito and Reynolds (19). Statistical calculations were done according to Fisher and Yates (20, 21).

RESULTS

The fraction of the injected dose that can be recovered as lipid-soluble C^{14} is called total recovery. This is the sum of all lipid-soluble radioactivity found in the extracts of all the organs, tissues, blood and carcass. That this recovery is less than the dose given can be explained by the oxidation of the radioactive lipid to metabolic intermediates, bicarbonate and $C^{14}O_2$, which are no longer extracted as lipids. The activity in each organ is expressed as a percentage of the dose injected; it is derived by multiplying the activity per gram by the total weight of the organ or tissue. A correction for the plasma contained in the organs and tissues was applied by the use of I^{131}-albumin (RISA), as described above. After the injection of labeled palmitate this correction is negligible, since only about 1 per cent of the dose remains in the blood; but after the injection of labeled chylomicrons this correction factor is very important. This is particularly true in nephrotic and in fat-loaded normal rats, when an appreciable amount of the radioactivity remains in the circulating blood for up to 10 minutes.

Weight, lipid content and blood content of the organs and tissues. The amount of plasma remaining in the various organs and tissues, deduced from the I^{131}-albumin content, is recorded in Table I. The results are in good agreement with those of Dewey (22), with the exception of adipose tissue. For this tissue, the present results are approximately three times greater and agree well

TABLE I

Mean values with standard errors of measurement for the experimental data from normal and nephrotic rats, fasted or carbohydrate-fed

Tissue: / Nutiritonal state:		Liver		Muscle		Kidney		Heart		Fat		Lung		Spleen	
		Fast	CHO	Fast	CHO	Fast	CHO	Fast	CHO	Fast	CHO	Fast	CHO	Fast	CHO
% of organ weight in total body weight	Normal	3.48 ±0.14	4.08 ±0.13			0.79 ±0.02	0.72 ±0.04	0.35 ±0.02	0.33 ±0.02	6.2 ±0.46	7.5 ±0.50	0.59 ±0.03	0.62 ±0.03	0.44 ±0.02	0.48 ±0.03
	Nephrotic	5.62 ±0.30	6.06 ±0.15	45		1.24 ±0.07	1.27 ±0.06	0.33 ±0.02	0.35 ±0.02	4.0 ±0.42	4.3 ±0.43	0.57 ±0.02	0.60 ±0.03	0.42 ±0.03	0.50 ±0.04
ml of plasma/g tissue	Normal	0.09	0.11	0.009	0.01	0.09	0.10	0.07	0.08	0.025	0.03	0.11	0.11	0.09	0.10
	Nephrotic	0.10	0.10	0.009	0.01	0.27	0.22	0.09	0.08	0.035	0.030	0.11	0.10	0.09	0.09
mg triglycerides/g tissue	Normal	7.1 ±0.38	6.9 ±0.49	5.59* ±0.739		4.4 ±0.60	2.8 ±0.52	6.0 ±0.20	4.6 ±0.51						
	Nephrotic	6.7 ±1.31	12.2 ±1.40	2.08* ±0.218		4.8 ±0.61	8.2 ±1.86	6.8 ±0.69	6.6 ±0.30						
mg phospho-lipids/g tissue	Normal	40.4 ±0.69	26.1 ±0.89	10.9 ±0.50	11.5 ±0.45	32.6 ±0.80	32.9 ±0.27	25.2 ±0.40	26.2 ±0.73						
	Nephrotic	37.2 ±1.06	25.0 ±0.80	11.5 ±0.51	10.7 ±0.36	28.2 ±0.83	24.4 ±0.81	26.5 ±0.59	25.8 ±0.33						
mg total cholesterol/g tissue	Normal	2.63 ±0.05	2.00 ±0.04	0.66 ±0.020	0.64 ±0.033	4.6 ±0.15	4.7 ±0.10	1.36 ±0.025	1.29 ±0.025						
	Nephrotic	3.11 ±0.17	2.86 ±0.20	0.72 ±0.021	0.79 ±0.036	4.9 ±0.14	5.3 ±0.24	1.66 ±0.075	1.72 ±0.010						

* This value refers to milligrams of triglyceride of diaphragm muscle.

with those of Bragdon (23). The amount of plasma found in the kidneys of nephrotic rats is greater than normal. This finding may reflect the presence in the tubules of albumin which has been filtered and taken up by the tubules. Because of this factor the amount of I^{131} radioactivity cannot be considered as an accurate measure of plasma remaining in the nephrotic kidney but represents partially an abnormal tubular load of I^{131}; the plasma content was arbitrarily assumed to be equal to that found in the normal kidney.

Muscle mass was estimated as 45 per cent of the total body weight in the normal animal (24) and as 45 per cent of the body weight of the nephrotic rats after subtraction of the weight of ascitic fluid present. It was recognized that the presence of tissue edema in the nephrotic rat might cause the muscle mass to be overestimated. The weight of the liver in severely nephrotic rats was increased to a value approximately 50 per cent greater than normal. The nephrotic kidney showed an even greater weight increase, ranging from 57 to 76 per cent above normal.

Body fat was determined by chemical analysis of chloroform-methanol carcass extracts. No correction was made for water content of the sample of adipose tissue weighed, and the fat extracted from the carcass must have included small amounts of fat from tissues other than adipose tissue. The amount of adipose tissue, expressed in per cent of total body weight, was reduced in nephrosis to two-thirds or less of the normal value. The weight of the other organs did not differ appreciably from the normal value; this suggested that they were not edematous. These data are recorded in detail in Table I.

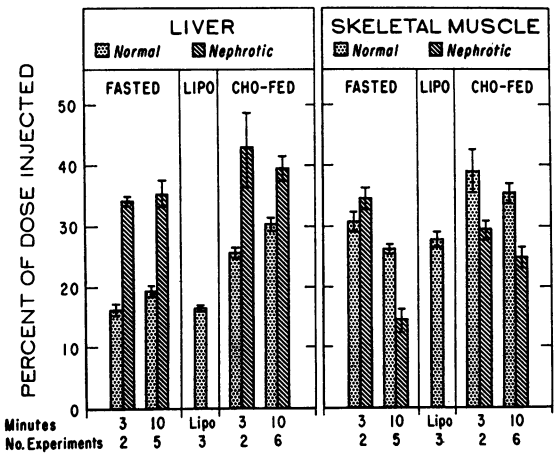

FIG. 1. RECOVERY OF C^{14} FOR LIVER AND SKELETAL MUSCLE, EXPRESSED AS PER CENT OF THE DOSE INJECTED, IN NORMAL AND NEPHROTIC RATS 3 AND 10 MINUTES AFTER THE INJECTION OF LABELED FREE FATTY ACIDS. I = standard deviation. LIPO = normal rats previously loaded with Lipomul.

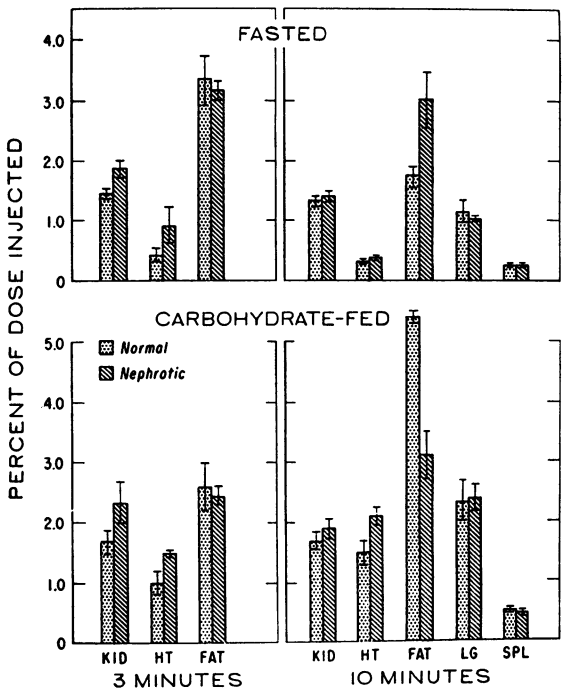

FIG. 2. ACTIVITY CLEARED BY KIDNEY, HEART, ADIPOSE TISSUE, LUNGS AND SPLEEN, EXPRESSED AS PER CENT OF THE DOSE INJECTED, IN NORMAL AND NEPHROTIC RATS SACRIFICED 3 AND 10 MINUTES AFTER THE INJECTION OF C^{14}-PALMITATE. I = standard deviation.

With the exception of skeletal muscle, the total lipid content of the various tissues did not differ significantly from normal. The phospholipid content per gram of muscle remained normal but there was a reduction in triglyceride content that was statistically significant ($p < 0.001$). The cholesterol content of liver, muscle, kidney and heart was increased in the nephrotic rats while the phospholipid content of the nephrotic kidney was decreased ($p < 0.05$).

Recovery and distribution of C^{14} after the injection of C^{14}-palmitate. After 3 minutes, the total recovery of C^{14} given as palmitate was 56 and 84 per cent of the dose in the fasted normal and nephrotic rats, respectively. In the carbohydrate-fed rats, the recovery was higher, 86 and 93 per

cent. The effect of carbohydrate feeding in sparing the oxidation of fatty acids has been previously demonstrated by Bragdon and Gordon (9) and by Lossow and Chaikoff (25). After 10 minutes the total recoveries in fasted normal and nephrotic rats decreased to 55 and 73 per cent of the dose, respectively. In carbohydrate-fed rats, the recoveries were 98 and 96 per cent. In fat-loaded rats (Lipomul), the total recovery was 55 per cent of the dose.

The per cent of the injected dose recovered in liver and skeletal muscle is shown in Figure 1. In the normal rat, whether fasted or fed carbohydrate, there was no significant change between 3 and 10 minutes. The livers of nephrotic animals uniformly contained more radioactivity than did those of normal animals ($p < 0.05$). The muscle was less radioactive in the nephrotic animals than in the normals ($p < 0.05$) sacrificed 10 minutes after the injection. When only 3 minutes elapsed between injection and sacrifice there was no significant difference between nephrotic and normal animals with respect to muscle radioactivity although, even after this short interval, the nephrotic livers contained more radioactivity than did the controls. Artificial hyperlipemia induced in the normal rat by the injection of Lipomul did not affect the recovery of radioactivity in liver and muscle.

The recovery of radioactivity in other tissues is shown in Figure 2. The results in Lipomul-injected rats are not presented in this figure, since no difference appeared between them and normal fasted rats. The only important difference between the normal and nephrotic groups occurred in the adipose tissue of rats sacrificed after 10 minutes. The fat of fasted nephrotic rats contained more radioactivity than did the fat of the control group. An opposite but even more significant ($p < 0.02$) change was observed among the carbohydrate-fed rats. In contrast to normal rats, the uptake by the adipose tissue of nephrotic rats was unaffected by the changes in the nutritional state. The amount of total adipose tissue was found to represent ± 4.0 per cent of the total body weight in both fasted and carbohydrate-fed nephrotic rats (see Table I).

The recovery of radioactivity per gram of each

TABLE II

*The activity of tissues * 3 and 10 minutes after the injection of C^{14}-labeled free fatty acids*

Tissue	Fasted					Lipomul-injected 10 min	Carbohydrate-fed				
	3 min†		10 min				3 min		10 min		
	NOR [2]‡	NEP [2]	NOR [5]	NEP [5]	p	NOR [3]	NOR [2]	NEP [2]	NOR [6]	NEP [6]	p
Liver§	28.3 ±0.45	43.0 ±10.0	30.7 ±2.95	37.0 ±3.28	NS‖	21.2 ±1.5	33.7 ±1.15	39.2 ±5.1	34.2 ±0.65	38.2 ±2.9	NS
Muscle	4.6 ±0.25	5.8 ± 1.3	2.9 ±0.14	1.7 ±0.20	<0.02	2.8 ±0.1	4.8 ±0.5	3.7 ±0.05	3.8 ±0.15	3.1 ±0.43	NS
Fat	4.1 ±0.25	4.3 ± 0.15	1.6 ±0.1	2.7 ±0.42	<0.01	1.55 ±0.15	2.6 ±0.4	2.5 ±0.15	3.9 ±0.05	3.0 ±0.46	NS
Kidney	10.9 ±0.05	9.0 ± 1.7	8.9 ±0.53	7.5 ±0.76	NS	8.2 ±0.3	10.6 ±1.3	8.3 ±0.7	12.8 ±0.9	8.6 ±1.07	<0.05
Heart	7.6 ±1.6	10.5 ± 2.2	4.6 ±0.44	5.3 ±0.74	NS	7.2 ±0.25	13.5 ±3.05	18.6 ±0.25	20.4 ±2.95	32.9 ±1.20	<0.02
Lung			9.7 ±1.11	8.9 ±0.70	NS	10.2 ±1.7			20.8 ±2.4	21.3 ±2.0	NS
Spleen			2.4 ±0.2	2.7 ±0.4	NS	2.2 ±0.2			5.7 ±1.3	4.3 ±0.6	NS

* Expressed as cpm × 10^{-3}/g wet weight, corrected for plasma content and dose.

† p Values for the experiments at 3 minutes were not statistically significant, due to the small number of experiments.

‡ Number of rats in each group is in brackets.

§ An analysis of variance for all the values of liver activity shows a significant difference between normal and nephrotic rats ($p = 0.02$). The two other factors (time and diet) have no significant effect on the variance.

‖ Not statistically significant.

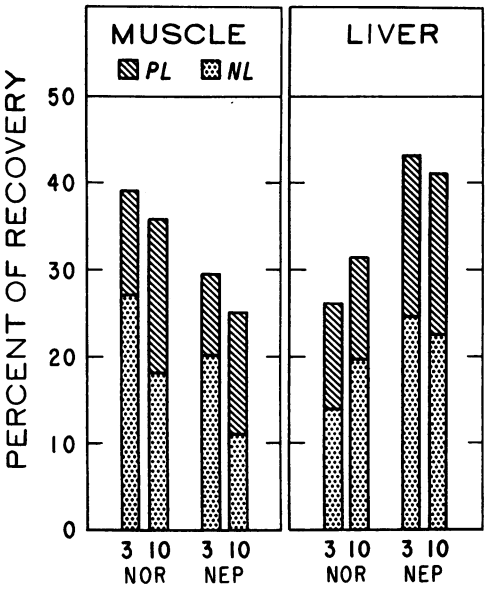

FIG. 3. CONTRIBUTIONS OF PHOSPHOLIPIDS (PL) AND NEUTRAL LIPIDS (NL) TO RECOVERY OF C^{14} IN LIVER AND MUSCLE OF NORMAL (NOR) AND NEPHROTIC (NEP) CARBOHYDRATE-FED RATS 3 AND 10 MINUTES AFTER INJECTION OF LABELED FREE FATTY ACIDS.

tissue is recorded in detail in Table II. The contribution of neutral lipids (essentially glycerides and cholesterides) and phospholipids to the recovery of C^{14} in liver and muscle is presented in Figure 3. Almost all the neutral lipid fraction of skeletal muscle is composed of triglycerides, since the cholesterol content of this tissue is very low (Table I). The decreased recovery of C^{14} in the muscle of nephrotic rats must be primarily attributable to the glyceride fraction.

The amount of radioactivity remaining in the plasma 3 minutes after the injection of C^{14}-palmitate varied from 0.4 to 1.6 per cent, with no significant difference between normal and nephrotic rats. After 10 minutes, however there was an important difference between nephrotic and normal rats; 0.4 to 0.7 per cent of the dose was found in the normal plasma, whereas in the nephrotic group from 0.8 to 4.2 per cent of the dose was in circulation. Separation of free fatty acids from triglycerides by the procedure of Borgström demonstrated that the increase in radioactivity was attributable to the triglyceride fraction. For 3 minutes the amount of radioactivity in triglyceride was negligibly small. The free fatty acid content of the plasma of the nephrotic rats did not differ significantly from normal values (0.2 to 0.5 μEq per ml). In agreement with the results of Forbes and Camlin (26) it was shown that storage of plasma for a few days at 4° C produced abnormally high FFA values. The specific radioactivity of the free fatty acids in the nephrotic rats was normal at 10 minutes (Table III). At 3 minutes the range of variation between nephrotics was very large and no valid change from normal values was noted.

Recovery of C^{14} after the injection of C^{14}-chylomicrons. The total recovery of lipid-soluble C^{14} for fasted normal and nephrotic rats was 65 and 77 per cent, respectively. After carbohydrate feeding the corresponding values were 98 and 90 per cent. The recovery of radioactivity in tissues 10 minutes after the injection is depicted in Figure 4. In the fasted nephrotic rats the recovery in the liver was greatly increased, the recovery in adipose tissue and muscle was decreased, and the amount remaining in the plasma was within the normal range. Normal fasted rats, made artificially hyperlipemic by an injection of chylomicrons, showed a decrease in recovery for liver, muscle and fat, while the fraction of the dose re-

TABLE III

Specific activity in counts per minute per milligram of triglyceride or per microequivalent of free fatty acid in plasma

Material injected	Nutrit. state	Triglycerides			Free fatty acids		
		Normal	Normal fat-loaded	Nephrotic	Normal	Normal fat-loaded	Nephrotic
C^{14}-palmitate 3 min	Fast	1.4 ± 0.05		0.4 ± 0.01	5,500 ± 1,600		10,600 ± 5,100
	CHO	0.7 ± 0.05		0.2 ± 0.03	9,500 ± 3,000		5,300 ± 1,000
C^{14}-palmitate 10 min	Fast	2.4 ± 0.87	0.1 ± 0.01	7.2 ± 2.6	2,900 ± 480	2,250 ± 450	3,000 ± 330
	CHO	2.0 ± 0.23		3.3 ± 1.2	1,140 ± 179		2,320 ± 176
C^{14}-chylomicrons 10 min	Fast	80 ± 5	41 ± 4	30 ± 6	10,600 ± 1,560	16,500 ± 750	3,300 ± 1,500
	CHO	51 ± 8		47 ± 9	4,600 ± 1,800		3,300 ± 705

TABLE IV

*Activity in tissues * and specific activity of plasma triglycerides (TG) 10 minutes after the injection of C^{14}-labeled chylomicrons*

	Fasting					Carbohydrate-fed					Fasting				
	Normal			Nephrotic		Normal		Nephrotic			Normal fat-loaded				
Liver	44.5	43.0	36.5	77.9	56.9	43.8	32.4	14.8	32.7	55.0	13.8†	8.0†	10.8†	0‡	0‡
Muscle	1.8	1.7	1.5	0.3	0.3	1.8	2.0	0.5	0	0.1	0.4	0.2	0.2	0	0
Fat	3.6	4.9	3.3	2.7	1.6	20.3	15.3	1.0	0	0	1.5	0.85	0.7	0	0
Kidney	5.5	4.2	3.0	1.5	0	4.7	4.4	0	0	0	0	0	0	0	0
Heart	8.0	14.0	12.5	4.8	3.7	8.6	11.5	6.3	1.0	1.0	4.7	2.8	2.7	0	0
Plasma TG (*cpm/mg TG*)	72	71	91	20	41	43	60	53	60	30	35	50	39	59	63
Plasma total lipids (*mg/100 ml*)	290	290	386	1,667	1,665	265	315	1,811	1,907	2,272	804	914	1,003	963	1,072

* Expressed as cpm $\times 10^{-3}$/g wet weight, corrected for plasma content and for dose.
† Normal rats receiving 45 mg of unlabeled chylomicrons immediately preceding the injection of labeled chylomicrons.
‡ Normal rats receiving 150 mg of unlabeled chylomicrons 5 minutes before the injection of labeled material.

maining in the plasma was increased. In muscle and adipose tissue, carbohydrate feeding exaggerated the discrepancy between the normal and nephrotic groups. Feeding carbohydrate to normal rats caused an increase in the amount of radioactivity recovered in muscle and adipose tissue, whereas in nephrotic rats these tissues showed barely detectable amounts of activity. The data on C^{14}-chylomicrons, expressed as radioactivity per gram of tissue, are recorded in detail in Table IV. "Normal fat-loaded" rats are those injected with unlabeled chylomicrons 5 minutes before injection of the radioactive material. In those rats receiving the largest load of chylomicrons (150 mg in 3 ml), 92 to 96 per cent of the radioactivity was found in the plasma, and the organs were not detectably radioactive; therefore, the results were not included in Figure 4.

After 10 minutes, about one-fourth of the radioactivity remained in the plasma in normal and nephrotic fasted rats (Figure 4 and Table IV). The specific activity of the triglycerides and free fatty acids was much lower in the nephrotic group (Table III). After carbohydrate feeding the nephrotic rats retained over 40 per cent of the dose in the plasma, whereas the normals had cleared all but 20 per cent. The normal and nephrotic carbohydrate-fed rats did not differ with respect to specific activity of FFA and triglycerides.

Recovery of radioactivity as a function of the degree of lipemia. Experimental data used for the first four tables and figures were obtained from severely nephrotic rats. Results from mildly nephrotic rats were not included. The arbitrary distinction between these two groups of nephrotic animals was based on the total lipid content of the plasma and the plasma albumin concentration. In mild nephrosis the lipemia does not exceed 900 mg per 100 ml and the plasma albumin level ranges between 1.0 and 4.0 g per 100 ml; severely nephrotic rats consistently showed lipemias above

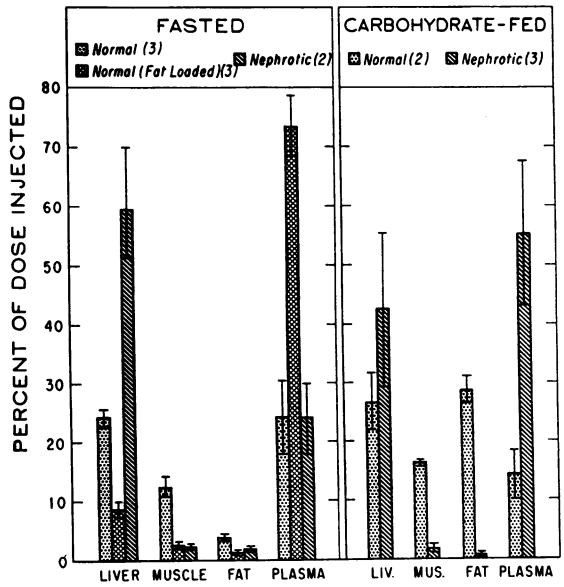

FIG. 4. RECOVERY OF C^{14} FOR LIVER, SKELETAL MUSCLE, ADIPOSE TISSUE AND PLASMA, EXPRESSED AS PER CENT OF THE INJECTED DOSE, IN NORMAL AND NEPHROTIC RATS AND IN NORMAL RATS PREVIOUSLY LOADED WITH UNLABELED CHYLOMICRONS, 10 MINUTES AFTER THE INJECTION OF LABELED CHYLOMICRONS. I = standard deviation. Number of rats in each group is in parentheses.

TABLE V

Percentage of injected dose of C^{14}-labeled palmitate or C^{14}-chylomicrons in various tissues of normal, normal fat-loaded, and nephrotic fasted rats as a function of plasma levels of total lipids (in mg/100 ml)

Tissue	C^{14}-palmitate				C^{14}-chylomicrons			
	Normal [5]*	Normal Lipomul-loaded [3]	Nephrotic [6]	Nephrotic [5]	Normal [3]	Normal fat-loaded [3]	Nephrotic [2]	Nephrotic [2]
Liver	19.6	16.5	19.5	35.5	24.3	8.7	21.3	59.6
Muscle	25.5	27.9	20.9	14.6	12.5	2.5	8.7	2.2
Fat	2.0	1.4	3.9	3.8	3.9	1.5	3.0	1.8
Kidney	1.3	1.5	1.4	1.4	0.6	0	0.8	0.15
Heart	0.3	0.5	0.3	0.4	0.7	0.25	0.9	0.25
Plasma TG (*cpm/mg TG*)	0.5	0.5	1.2	2.0	24	70	32	24
Plasma level of total lipids (*mg/100 ml*)	252	2,548	264 – 729	1,021 – 2,031	325	907	620 – 833	1,666

* Number of rats in each group is in brackets.

1,000 mg per 100 ml and albumin concentrations under 1.0 g per 100 ml.

Table V shows the recovery of radioactivity (expressed as percentage of injected dose) in relation to the degree of lipemia. Changes in the percentage of radioactivity in adipose tissue, muscle and plasma occurred even in mildly nephrotic rats whose liver radioactivity still remained normal. The difference in recovery of radioactivity in the liver between mildly and severely nephrotic rats is highly significant ($p < 0.01$). The change in liver recovery occurred when the lipemia exceeded about 900 mg per 100 ml; there was no linear relationship. Results were not different after administration of C^{14}-palmitate or C^{14}-chylomicrons.

In Table VI the activity of tissues as a function of plasma levels of total lipids is presented. The activity per gram of liver in mildly nephrotic rats is lower than that in normal and in severely nephrotic rats ($p < 0.01$). The activities per gram of muscle and adipose tissue were already changing to the values obtained in severe nephrosis. This finding occurred regardless of the form in which the radioactive material was injected.

DISCUSSION

Adipose tissue. Nephrotic rats have less total body fat than have normals of the same weight (Table I). This confirmed the diminished carcass-fat values obtained by others (1, 27).

In the normal fasted rat the recovery of radioactivity in the adipose tissue decreases between the third and tenth minutes after injection of C^{14}-

TABLE VI

*Activity of tissues * and specific activity of plasma triglycerides in relation to the plasma levels of total lipids (in mg/100 ml) 10 minutes after the injection of C^{14}-labeled palmitate or C^{14}-chylomicrons*

Tissue	C^{14}-palmitate				C^{14}-chylomicrons			
	Normal [5]†	Lipomul-loaded [3]	Nephrotic [6]	Nephrotic [5]	Normal [3]	Fat-loaded [3]	Nephrotic [2]	Nephrotic [2]
Liver	30.7	21.2	20.6	37.0	41.0	10.9	24.3	67.4
Muscle	2.9	2.8	2.0	1.7	1.75	0.25	1.1	0.3
Fat	1.6	1.6	2.1	2.7	4.0	1.0	2.8	2.1
Kidney	8.9	8.2	7.2	7.5	4.2	0	2.4	0.75
Heart	4.6	7.2	3.7	5.3	11.5	3.4	12.5	4.2
Plasma TG (*cpm/mg TG*)	2.4	0.1	3.0	7.2	78	41	55	30
Plasma level of total lipids (*mg/100 ml*)	252	2,548	264 – 729	1,021 – 2,031	325	907	620 – 833	1,666

* Expressed as cpm $\times 10^{-3}$/g wet weight, corrected for plasma content and for dose.
† Number of rats in each group is in brackets.

palmitate. This change suggests release and subsequent oxidation of free fatty acids in that interval. After carbohydrate feeding, however, the oxidation of fat is spared and C^{14} may accumulate in the adipose tissue. In nephrotic rats these changes failed to occur. No difference was observed between recovery at 3 and at 10 minutes in either the fasted or carbohydrate-fed rats (Figure 2).

After the administration of C^{14}-chylomicrons less radioactivity was recovered in the adipose tissue of nephrotic than of normal rats in the fasting state. Carbohydrate feeding failed to increase this recovery. The fraction of the dose found in the adipose tissue of nephrotic rats was similar to that found in normal fat-loaded rats.

Skeletal muscle. The production of artificial hyperlipemia with Lipomul did not alter the recovery of C^{14}, derived from palmitate, in the skeletal muscle of normal fasted rats, but it must be emphasized that this artificial hyperlipemia is not chemically identical with the hyperlipemia of nephrosis.

The radioactivity found in the muscle of normal rats did not change significantly between the third and the tenth minutes after palmitate injection in either the fasted or carbohydrate-fed state. In contrast, in nephrotic rats, there was a great decrease in C^{14} recovery in the skeletal muscle between the third and the tenth minutes after palmitate injection. It is not possible to explain this decrease solely on the basis of accelerated oxidation of fatty acids, since this would require the conversion of approximately 20 per cent of the dose of C^{14}-fatty acid into $C^{14}O_2$ or other nonlipid metabolites. In normal fasted rats, the excretion of $C^{14}O_2$ in the first 10 minutes after injection of C^{14}-palmitate accounts for approximately 15 per cent of the dose (28), and oxidation in skeletal muscle is, at most, a fraction of this total. Unless the fractional rate of oxidation of fatty acids by skeletal muscle is greatly increased in nephrosis [and preliminary experiments (29) indicate that it is in fact decreased], it is necessary to suppose that some radioactive lipid, originally taken up in the muscle, has subsequently been transported. The triglyceride content of skeletal muscle in nephrotic rats has been found to be significantly less than normal. One possible explanation is that there is some defect in the synthesis of triglycerides within nephrotic muscle. In this case, free fatty acids which have entered the muscle within 3 minutes after injection would be released into plasma, thus explaining the lower radioactivity in nephrotic muscle after 10 minutes. Preliminary *in vitro* experiments suggest that this explanation is indeed correct. In addition, if the quantity of fatty acids stored in the muscle as triglyceride is significantly less than normal, its fractional rate of conversion to CO_2 may be increased. A further factor that may contribute to the deficiency of fat in nephrotic muscle was the uptake of chylomicron fat by this tissue in nephrotic rats, which was shown to be much less than normal, and this uptake failed to increase after carbohydrate feeding.

Liver. Liver of severely nephrotic rats always contained more total radioactivity than normal, regardless of the nutritional state or the form in which the labeled fatty acid was injected. The difference was most striking among the fasted rats. The liver of nephrotic rats weighed, on the average, 60 per cent more than did that of the corresponding normals. This figure is in agreement with values given by Heymann and Hackel (1) and a little higher than those reported by Marsh and Drabkin (5). Radioactivity per gram of tissue was generally increased, except in the group of mildly nephrotic rats. In the rats whose lipemia did not exceed 900 mg per 100 ml, the liver was also increased in size, but the recovery of radioactivity was below normal.

Blood. Within 3 minutes of the injection of C^{14}-palmitate, 99 per cent of the radioactivity had been cleared from the blood. Only a small fraction of the 1 per cent remaining was present in the triglyceride fraction, and no difference was observed between normal and nephrotic rats. After 10 minutes the amount of radioactivity incorporated into the triglycerides of nephrotic plasma was significantly increased. The specific activity of this triglyceride fraction was also higher than normal, in spite of a much larger pool of plasma triglycerides. When C^{14}-chylomicrons were administered to carbohydrate-fed animals, the fraction remaining in the plasma after 10 minutes was higher in the nephrotic group. Specific activity of plasma triglycerides, however, remained identical in normal and nephrotic animals.

The data with injected C^{14}-palmitate as FFA

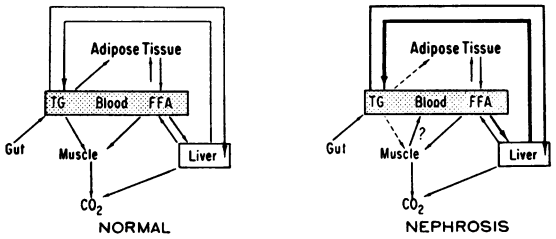

FIG. 5. POSSIBLE MECHANISMS FOR TRIGLYCERIDE (TG) AND FREE FATTY ACID (FFA) METABOLISM IN NORMAL AND NEPHROTIC RATS.

suggest that the nephrotic liver releases newly synthesized triglycerides into the circulation more rapidly than does the normal liver, or synthetizes more lipids than the normal liver, or both. An increased synthesis of lipoproteins of low density by the nephrotic liver was demonstrated by liver perfusion experiments (5). Our results show also that the uptake of triglycerides by the muscle of nephrotic rats is decreased. One might attempt to explain the smaller recovery of C^{14} given as chylomicrons in muscle and adipose tissue of nephrotic animals by dilution of the injected dose into a larger pool of plasma lipids. This assumption is unlikely because normal and nephrotic rats tend to show identical specific activities of plasma triglycerides 10 minutes after injection of chylomicrons, and an increased retention of radioactivity in the nephrotic liver is seen. It seems more likely that the primary difficulty is a decreased ability of the peripheral tissues to extract triglycerides from the blood. A distinct decrease in the rate of removal of chylomicron lipid from the serum of nephrotic rats was also reported by Saffran and Kalant (8).

Total recovery. In the fasted nephrotic rat the total recovery of lipid-soluble C^{14} is greater than in normal rats when either C^{14}-palmitate or C^{14}-chylomicrons is administered. This difference implies an impairment of the oxidation of C^{14}-fatty acids in nephrosis. Studies on the excretion of $C^{14}O_2$ after the intravenous injection of C^{14}-palmitate to nephrotic rats have shown a net reduction in excretion compared with that of normal rats. After injection of C^{14}-chylomicrons there was an important delay in the oxidation of this substrate in both the fasting and carbohydrate-fed state (29).

Conclusions. The present findings are summarized in Figure 5. It appears that in nephrosis an enlarged liver takes up triglycerides from the blood and releases them again at an abnormally increased rate. Free fatty acids enter the liver more rapidly than normal, are converted to triglycerides, and enter this cycle. The uptake of triglycerides in the periphery (primarily muscle and adipose tissue) is impaired.

In mildly nephrotic rats with a moderate degree of lipemia, the changes in the liver do not occur, whereas the decreased uptake in the peripheral tissues is apparent. These findings suggest that the peripheral changes are primary, and that the alterations in hepatic lipid metabolism could be their consequence, since they appear later when a certain increase of the lipid plasma pool is already present. Since skeletal muscle comprises 45 per cent of the body weight and adipose tissue 6 per cent or more, it is apparent that a small defect in the metabolism of these tissues could cause large changes in the plasma lipid concentration.

SUMMARY

Normal and nephrotic rats were injected intravenously with palmitate-1-C^{14} or C^{14}-chylomicrons. Some rats were fasted and some were fed carbohydrate. They were killed 3 or 10 minutes after the injection. Their blood and tissues were analyzed for C^{14}-lipid-soluble content and for lipid chemical composition.

The nephrotic rats differed from normal in the following ways:

1. The liver in nephrosis always contained more total radioactivity than normal. The activity per gram of tissue was not increased, and the difference was due to an increase in the size of the organ.

2. Except in animals sacrificed 3 minutes after administration of C^{14}-palmitate, the skeletal muscle in nephrosis was less active than normal. The triglyceride content of muscle was reduced, and after carbohydrate feeding the uptake of labeled triglyceride by muscle was negligible.

3. The adipose tissue of nephrotic animals did not respond in the normal way to changes in the nutritional state. The sparing effect of carbohydrate feeding on fat oxidation was not seen after the administration of C^{14}-chylomicrons to nephrotic rats.

4. The elevated triglyceride fraction in the plasma of nephrotic rats retained more radioactivity than normal 10 minutes after the injection of labeled palmitate or labeled chylomicrons.

The relative roles of changes in lipid metabolism in liver and at the periphery as primarily responsible for the etiology of nephrotic hyperlipemia are discussed.

ACKNOWLEDGMENTS

The author is grateful to Dr. Robert S. Gordon, Jr. for his suggestions, to Dr. James H. Baxter for making the nephrotoxic serum available, and to Mr. Carlos Schultz for instruction in the method of cannulation of the chyle duct in the rat.

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