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Disposition of the cholesterol moiety of a chylomicron-containing lipoprotein fraction of chyle in the rat*

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SUMMARY

Cholesterol-27-C^{14} in the form of a chylomicron-containing lipoprotein fraction of chyle was injected intravenously into rats. About 75% of the injected sterol-C^{14} was in the ester and 25% in the free form. Ten minutes after the injection, about 25% of plasma lipid C^{14} was recovered in higher-density α- and β-lipoproteins. The transfer of labeled sterol to the higher-density plasma lipoproteins involved both esterified and free forms, but the proportion of free sterol transferred was greater than that in the injected preparation. In 10 minutes, C^{14} was recovered in the lipid fractions of the nine tissues studied. Liver, adipose tissue, and muscle accounted for about 70% of the injected C^{14}, and liver alone accounted for about 50%. Adipose tissue of glucose-fed rats incorporated about twice as much of the injected sterol-C^{14} as did the same tissue of fasted rats at this time. During the first 10 minutes, the proportion of sterol-C^{14} recovered in the free form increased in four of the tissues: adipose, bone, adrenals, and small intestine. At 24 hours, about 90% of the sterol-C^{14} was present in the free form in all tissues except the adrenal gland. In this gland, the initial increase in the proportion of sterol-C^{14} recovered in the free form was followed by a decrease, and, after 24 hours, about 90% of the sterol-C^{14} was in the esterified form. In plasma, an increase in the proportion of labeled sterol recovered in the free form was observed only at an interval between 10 minutes and 2 hours. Among the conclusions drawn are (1) hydrolysis is the principal fate of the injected cholesterol esters within a few hours after injection, and (2) release of labeled free sterol into plasma by liver or exchange of free sterol between liver and plasma occurs at a more rapid rate sometime between 10 minutes and 2 hours after injection than at any other time. To account for the unique observations in the adrenal gland, the suggestion is made that this gland stores specific sterol esters and hydrolyzes chylomicron esters to resynthesize the cholesterol esters characteristic of the gland.

The conversion of cholesterol to bile acids in the liver has received considerable study (1, 2). Little information is available, however, on the early fate of the cholesterol moiety of low-density lipoproteins of chyle, the form in which this sterol is transported from the intestinal tract to the circulation. The present report deals with this phase of cholesterol metabolism. A chylomicron-containing lipoprotein fraction,[1] prepared from thoracic-duct chyle that was collected from rats fed cholesterol-27-C^{14}, was injected intravenously into rats. The uptake and the relative distribution of the labeled cholesterol between free and esterified forms were studied in a variety of tissues of recipient rats

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[1] The preparation injected included lipoprotein classes of S_f 20 and higher. Because of lack of agreement on what constitutes chylomicrons, this fraction of chyle lipoproteins is described here as chylomicron-containing. Chylomicrons have, for example, been designated as composed of lipoprotein classes of S_f 20 and higher (3), S_f 400 and higher (4), and S_f 10,000 and higher (5). The very low-density plasma lipoproteins have been divided by Lindgren (6) into those of S_f 400 and higher (lipomicrons and chylomicrons) and those of S_f 20–400. Albrink (7) has divided lipoprotein classes of S_f 20 and higher into "heavy" and "light" chylomicrons.

during the period from 10 minutes to 24 hours after the injection.

MATERIALS

Cholesterol-27-C^{14}. Part of the cholesterol-27-C^{14} was synthesized by Dauben and Bradlow (8). The remainder was purchased from the New England Nuclear Corp., Boston, Massachusetts. Immediately before use, the labeled cholesterol was purified on a silicic acid column by the procedure of Barron and Hanahan (9).

Preparation of a Chylomicron-containing, Cholesterol-27-C^{14} Lipoprotein Fraction of Chyle. Chyle from the thoracic ducts of rats was collected for 12 hours in ice-cold tubes. The rats had received by stomach tube 0.5 ml of olive oil in which 15 to 20 mg of the labeled cholesterol had been dissolved. The procedure for cannulation of the thoracic duct has been previously described (10). The cannulation was performed the day before the labeled cholesterol was fed, and thereafter the rats had access to a solution of 0.9% NaCl for drinking purposes. The clot in the collected chyle was removed, and the defibrinated chyle was diluted ninefold with 1.1% NaCl (D = 1.006 g per ml) and centrifuged at 4° for 16 hours, at 30,000 rpm, in a model L Spinco ultracentrifuge with a 30.2 head (79,420 × g). The top turbid layer containing the chylomicrons was removed by mild suction.

EXPERIMENTAL METHODS

Treatment of Recipient Rats. Long-Evans male rats, raised and maintained on Diablo Labration and weighing 240 to 300 g, served as recipient rats. Those designated *fasted* received no food for 16 to 20 hours before the experiment. Those referred to as *carbohydrate-fed* were first fasted for 6 hours and then fed, by stomach tube, 2.5 ml of a 35% glucose solution per 100 g body weight 1 hour before the administration of the labeled chylomicrons. The rats were lightly anesthetized with ether, and 1 ml of the labeled chylomicrons was injected into a leg or tail vein. At the end of the experiment, the rats were again lightly anesthetized and immediately thereafter blood was removed from the heart by means of a heparinized syringe. The tissues and organs listed in the tables were then excised. The muscle taken was the gastrocnemius, the bone taken was the tibia. Subcutaneous fat was obtained from the abdominal region. Sections 8 mm in length were excised from the proximal, central, and distal parts of the small intestine. Their contents were washed out with saline, and the mucosa was scraped off with a blunt spatula.

Extraction of Lipids and Preparation of Lipid Fractions.

(*a*) *Liver, Kidney, Spleen, and Bone.* These tissues were homogenized in a Waring blendor, and lipids were extracted at room temperature — once overnight and twice for 2 hours — with 20 volumes of a 3:1 ethyl alcohol—ether mixture. The solvents of the combined extracts were evaporated under reduced pressure in a Rinco rotary evaporator, and the residue was dissolved in hexane.

In experiment 4 (rat 14), bone marrow, rather than the whole tibia, was analyzed. The marrow was removed by a procedure developed by Mazur and Carleton[2] of Cornell University Medical College, New York. The extremities of the tibia were cut open, and the marrow was forced out of the shaft with compressed air. The marrow was then extracted by the method used for adipose tissue.

(*b*) *Adipose Tissue, Muscle, Small Intestine, and Adrenal Glands.* These tissues were placed in the 3:1 ethyl alcohol—ether mixture and disintegrated by pounding with a stirring rod. Adipose tissue was extracted three times with 200 volumes of the solvent mixture; the other tissues were extracted three times with 30 volumes. The subsequent treatment of all four tissues was the same as that described in (*a*).

(*c*) *Plasma.* Plasma samples were placed in 20 volumes of a 3:1 ethyl alcohol—ether mixture and extracted as described in (*a*).

Plasma obtained from rats 5 and 14, which were killed at the 10-minute interval, was separated into supernatant and infranatant fractions as described below.

Two milliliters of plasma was diluted with 7 ml of 1.1% NaCl (D = 1.006 g per ml) and centrifuged at 4° for 16 to 20 hours at 30,000 rpm in a model L Spinco ultracentrifuge with a 30.2 head (79,420 × g). Each tube was sliced just below the upper turbid layer (1.8 cm from the top of the liquid meniscus in rat 5; 1.5 cm from the top in rat 14) with a tube slicer (11).[3] The supernatant, chylomicron-containing, lipoprotein fraction (S_f classes 20 and higher) was removed with suction. The remaining higher-density lipoproteins were concentrated at the bottom of the tube. The low-density β-lipoproteins, which in the rat constitute about 20% of the total α- and β-lipoproteins of the plasma (12), could be seen in a darkroom as a light-scattering ring less than 1 cm from the bottom by means of a pen light shining down through the liquid after the

[2] Personal communication.
[3] William H. Goldwater modification, Microchemical Specialties Co., Berkeley, California.

top layer was removed.[4] To assure a clear-cut separation of the upper, chylomicron-containing layer from the bottom layer containing the α- and β-lipoproteins, a middle layer (infranatant A) was removed after slicing the tube 1.5 cm from the bottom. This middle layer was slightly contaminated in its upper portion with lipoproteins of the chylomicron-containing layer and in its lower portion with β-lipoproteins. The bottom layer was designated infranatant B. The pellet residue (rat 14 only) was washed twice with saline, and the washings were added to the infranatant B fraction. These washings undoubtedly did not free all of the high-density α-lipoproteins from the other plasma proteins in the pellet.

In the case of rats 1–4, only the supernatant fractions were obtained. The lipids were extracted from these fractions by the method described for plasma.

The blood cells of rat 14 were also analyzed. They were washed three times with saline, lysed, and then extracted by the procedure used for plasma.

Silicic Acid Chromatography. Esterified and free sterol fractions were obtained by chromatographing aliquots of the lipid extracts on silicic acid columns by the procedure of Barron and Hanahan (9). Each column consisted of 15 g of a mixture of two parts silicic acid and one part Hyflo Supercel (Johns-Manville Corp., New York, N.Y.) The maximum loading was 60 mg of lipid per column, and each fraction was eluted with 250 ml of solvent.

C^{14}-Assays. The lipid samples obtained in experiments 1 and 2 were mounted on aluminum planchets and assayed for C^{14} with a thin-window Geiger-Müller counter (Nuclear-Chicago Corp., Chicago, Illinois). In experiments 3 and 4, the dried lipid samples were dissolved in 15 ml of toluene containing 45 mg of 2,5-diphenyloxazole, and they were assayed for C^{14} in a Tricarb liquid scintillation spectrometer (Packard Instrument Co., LaGrange, Ill.).

RESULTS

Recovery of the Intravenously Injected, Labeled Cholesterol in Tissues of Carbohydrate-Fed Rats. The earliest interval at which plasma and tissues were examined after the injection of the labeled cholesterol was 10 minutes (Tables 1 and 2). At that time, the lipid fraction of the liver contained the largest percentage of the labeled cholesterol — about 50%. If it is assumed that the lipid-C^{14} was uniformly distributed in adipose tissue and that this tissue constitutes 10% of the body weight for rats of the weight range used in this study (13), it can be calculated that, at the 10-minute interval, total body adipose tissue contained from 6% to 18% of the C^{14} in the form of lipids. It can also be calculated that total muscle, which constitutes 47% of the body weight (14), accounted for 6% to 12% of the cholesterol-C^{14}.

The other tissues—spleen, mucosa, and serosa of the small intestine, kidney, adrenal glands, and even bone—participated in the removal of the isotopic cholesterol from the blood stream 10 minutes after injection. During the interval between 10 minutes and 24 hours after the injection, the percentage of C^{14} found as lipid per gram of tissue showed no consistent decline in any tissue except liver (Table 2). In the liver, there was little or no change between 10 minutes and 2 hours after the injection, but, between 2 and 24 hours, the percentage of C^{14} recovered per gram of tissue fell to about one-fourth that found at 10 minutes. In the case of the intestine, an increase in the percentage of C^{14} recovered per gram of lipid was observed between 10 minutes and 5 hours after the injection. Excretion of labeled bile acids could account for the observed decrease in the liver. The increase in the intestines could be explained by an accumulation of labeled sterols absorbed into the intestine after their excretion through the bile duct or directly through the gut wall.

In plasma, the percentage of C^{14} per milliliter recovered at 2 hours was about one-sixth that recovered at 10 minutes. Between 2 and 16 hours, no further decline was observed. A small decrease was observed between 16 and 24 hours after injection.

Comparison of the Recovery of Labeled Cholesterol in Tissues of Fasted and Carbohydrate-Fed Rats 10 Minutes after the Injection. A distinct difference in the uptake of the injected cholesterol-C^{14} by adipose tissue was observed between fasted and carbohydrate-fed rats (Table 1). In each of two experiments the C^{14} recovered as lipid per gram of adipose tissue in the fed rat was about twice that in the fasted animal. No such difference was observed in liver and spleen in either experiment, but a similar difference was found in the kidney in one of the experiments.

Distribution of the C^{14} between Free and Esterified Sterol in Various Tissues. The percentages of the sterol-C^{14} found in the esterified form are recorded in Tables 1 and 3. Since, in most cases, the proportions of plasma sterol-C^{14} that were esterified differed from those in the tissues, it became important to estimate to what extent the sterol-C^{14} in the vascular compartments of the tissues could have affected the values for free and esterified sterol-C^{14} found in those tissues. To do

[4] This simple procedure for visualizing the β-lipoproteins was suggested to us by Dr. F. T. Lindgren of the Donner Laboratory, University of California, Berkeley.

TABLE 1. DISPOSITION OF THE CHOLESTEROL OF CHYLOMICRONS CONTAINING CHOLESTEROL-27-C^{14} 10 MINUTES AFTER THEIR INTRAVENOUS INJECTION INTO FASTED AND CARBOHYDRATE-FED RATS

Injected Chylomicron Cholesterol-C^{14}	Expt. 1 (Rats 1 and 2)	Expt. 2 (Rats 3 and 4)
% Esterified	75.1	85.7
% Free	24.9	14.3

Rat No.	Rat Nutritional State	Tissue	Injected C^{14} Recovered in Lipid Fraction: Per g tissue	Injected C^{14} Recovered in Lipid Fraction: Per whole organ*	Recovered Sterol-C^{14} Present in Esterified Form†
			%	%	%
1	CHO-fed	Adipose	0.49		52.1 (−0.7)
2	Fasted	"	0.24		55.1 (−0.9)
3	CHO-fed	"	0.92		59.6 (−0.2)
4	Fasted	"	0.41		34.6 (−1.4)
1	CHO-fed	Liver	4.9	52.8 (−2.1)	73.2 (−0.1)
2	Fasted	"	5.5	42.8 (−1.5)	70.6 (−0.1)
3	CHO-fed	"	7.5	59.3 (−1.6)	81.0 (−0.2)
4	Fasted	"	7.6	56.9 (−1.5)	81.2 (−0.2)
1	CHO-fed	Whole plasma	3.0		76.1
2	Fasted	" "	3.0		73.2
3	CHO-fasted	" "	3.0		84.7
4	Fasted	" "	3.1		84.8
1	CHO-fed	Plasma supernatant‡			83.8
2	Fasted	" "			82.5
3	CHO-fed	" "			90.2
4	Fasted	" "			86.4
1	CHO-fed	Kidney	0.66	1.10 (−0.23)	73.0 (−1.3)
2	Fasted	"	0.32	0.50 (−0.21)	71.1 (−1.6)
3	CHO-fed	"	0.35	0.58 (−0.23)	87.0 (+0.4)
4	Fasted	"	0.34	0.64 (−0.23)	82.0 (−2.0)
1	CHO-fed	Spleen	2.3	2.1 (−0.3)	70.6 (−2.7)
2	Fasted	"	1.7	2.8 (−0.6)	69.5 (−1.0)
3	CHO-fed	"	1.8	2.5 (−0.5)	80.2 (−1.2)
4	Fasted	"	1.8	2.6 (−0.6)	83.4 (−0.5)

* Numbers in parentheses are corrections for C^{14} in the tissue vascular compartment as estimated from the values given by Dewey (15) for tissue plasma content.

† $\frac{C^{14} \text{ recovered in esterified sterol fraction}}{C^{14} \text{ recovered in esterified and free sterol fraction}} \times 100.$

‡ Chylomicron-containing fraction. See analytical procedures.

this, we used Dewey's figures (15) for gram of vascular plasma per gram of tissue, and the calculated corrections are shown in parentheses in Table 3. These corrections are probably high because it is likely that our rats were more completely exsanguinated than were Dewey's. We exsanguinated by withdrawing blood from the heart, whereas Dewey exsanguinated by decapitation. An attempt was also made to correct for the content of sterol-C^{14} in red blood cells of the tissues. The blood cells of rat 14 contained about 0.1% per ml of the injected C^{14}, and over 90% of the sterol-C^{14} was in the free form. On the basis of published data on cholesterol exchange between plasma and red blood cells (16, 17), it was assumed that equilibrium for this exchange in our rats had been reached by 2 hours. It was also assumed that 90% of the blood cell sterol-C^{14} was in the free form and that, at equilibrium, the plasma and blood cells had equal amounts of C^{14}. Such calculations introduced little or no further change in the values for the percentages of free and esterified sterol-C^{14} in the tissues.

In experiments 1, 3, and 4, about 75% of the cholesterol-C^{14} in the chylomicron-containing fraction prepared from chyle was in the esterified form (Tables 1

TABLE 2. TISSUE LIPID C^{14} CONTENTS AFTER INJECTION OF CHOLESTEROL-C^{14}-LABELED CHYLOMICRONS INTO CARBOHYDRATE-FED RATS

		% of Injected C^{14} Recovered in Total Lipid Fraction†									
							Per g tissue				
		Whole organ		Adipose tissue							
Rat* No.	Time Killed	Liver	Adrenal gland	Liver	Peri-renal	Subcu-taneous	Muscle	Bone‡	Kidney	Spleen	Per ml plasma
5	10 min	50.5	0.27 (−0.05)	5.2	0.28	0.24	0.09 (−0.01)	0.10 (−0.05)	0.34 (−0.14)	1.8 (−0.4)	3.1
14§	10 min	79.5	0.61 (−0.12)	9.9	0.37	0.26	0.04 (−0.01)		0.29 (−0.14)		
6	2 hr	49.8	0.59	5.4	0.09	0.09	0.07		0.23 (−0.02)	2.1	0.51
7	2 hr	52.6	0.39	5.0	0.13		0.05	0.13 (−0.007)	0.20 (−0.02)	2.0	0.41
8	5 hr	33.6	0.57	3.7	0.11	0.17	0.07	0.35	0.36 (−0.03)	2.4	0.67
9	6 hr	30.2	0.32	3.6	0.12		0.09	0.24	0.86	2.3	0.40
10	16 hr	18.4	0.63		0.23	0.24		0.29	0.41	1.9	0.43
11	24 hr	12.3	0.65	1.3	0.13	0.13	0.10	0.39	0.30	2.0	0.26
12	24 hr	13.4	0.49	1.2	0.21	0.04	0.11	0.39	0.41	1.8	0.29

* Rats weighed 270 ± 30 g.

† In almost all cases the esterified and free sterol fractions accounted for 95% or more of the total lipid C^{14}. Corrections for plasma C^{14} content of tissue were estimated from Dewey's values for tissue plasma content (15) and are given in parentheses when the correction was 5% or more of the tabulated figure.

‡ Rats 5–12, whole tibia; rat 14, bone marrow only (0.02% per g was recovered in the marrow of one tibia).

§ In this rat the percentages of the injected C^{14} recovered per gram in the total lipid fraction were 0.04 for mucosa and 0.1 for serosa of the small intestine. The total lipid-C^{14} recovered in the samples of intestine increased between 2 and 5 hours after injection; as judged by the percentage of lipid-C^{14} recovered per gram intestinal fat, the increase between 10 minutes and 5 hours was about sevenfold for the mucosa and threefold for the serosa. The values were about the same between 5 and 24 hours.

and 3); in experiment 2, about 85% was esterified (Table 1). Ten minutes after the intravenous injection of the labeled cholesterol, no change (or a small one) was observed in the distribution of C^{14} between free and esterified sterol in the liver, spleen, and kidney. By the time 2 hours had elapsed, however, about 30% or less of the sterol-C^{14} in those tissues was esterified, and, at all later intervals (5, 6, 12, and 24 hours), 80% or more of the labeled sterol in those tissues was in the free form.

In adipose tissue, serosa and mucosa of the small intestine, bone or bone marrow, and adrenal gland, an appreciable increase in the proportion of sterol-C^{14} recovered in the free form was observed as early as 10

TABLE 3. TISSUE STEROL-C^{14} DISTRIBUTION AFTER INJECTION OF CHOLESTEROL-C^{14}-LABELED CHYLOMICRONS INTO CARBOHYDRATE FED RATS

Injected Chylomicron Cholesterol-C^{14}	Expt. 3 (rats 5–12)	Expt. 4 (rat 14)
% Esterified	74.7	71.2
% Free	25.3	28.8

		% of Sterol-C^{14} Present in the Esterified Form*										
						Adipose Tissue				Small Intestine‡		
Rat No.	Time Killed	Plasma	Liver	Kidney	Spleen	Peri-renal	Subcu-taneous	Muscle	Bone†	Mucosa	Serosa	Adrenal gland
5	10 min	79.5	73.6 (−0.3)	76.8 (−0.8)	67.8 (−3.1)	52.1 (−1.8)	22.2 (−3.2)	67.9 (−1.4)	61.5 (−23.7)	49.4	51.8	45.4 (−8.4)
14	10 min	79.9	72.6 (−0.2)	67.8 (−11.6)		51.8 (−0)	40.3 (−1.8)	38.8 (−20.1)	59.2	18.9	44.2	42.8 (−14.1)
6	2 hr	44.6	18.2 (−0.2)	29.8 (−1.6)	6.5 (−1.2)	25.7 (−0.7)	11.2 (−0.9)	6.0 (−2.5)		5.7	7.7	38.2 (−0.8)
7	2 hr	54.4	19.2 (−0.3)	37.3 (−1.8)	6.7 (−1.3)	25.6 (−0.4)		18.0 (−1.2)	18.7 (−2.7)	14.0	11.5	30.9 (−0.8)
8	5 hr	69.7	16.3 (−1.0)	20.6 (−4.8)	5.7 (−2.3)	12.2 (−1.7)	12.9 (−1.0)	8.0 (−2.8)	7.7 (−2.2)	10.9	14.9	68.1 (−0)
9	6 hr	72.2	10.9 (−0.4)	19.6 (−1.3)	4.1 (−0.9)	8.8 (−0.9)		6.1 (−1.2)	6.7 (−1.8)	9.2	9.6	68.9 (−0.9)
10	16 hr	73.6	15.9 (−0)	14.5 (−3.0)	2.6 (−2.1)	4.0 (−0.5)	8.4 (−0.4)	7.0	4.9 (−1.9)	6.9	5.9	87.6 (+0.4)
11	24 hr	71.7	10.8 (−0.9)	15.2 (−2.4)	2.9 (−1.1)	4.8 (−0.7)	5.4 (−0.5)	3.5 (−0.6)	3.9 (−0.8)	4.5	5.2	90.1 (+0.3)
12	24 hr	69.1	11.2 (−1.0)	9.4 (−2.0)	3.3 (−1.4)	5.0 (−0.3)	5.7 (−1.6)	3.3 (−0.6)	4.2 (−0.9)	6.9	3.9	89.8 (+0.4)

* $\frac{\text{C}^{14} \text{ recovered in esterified sterol fraction}}{\text{C}^{14} \text{ recovered in esterified and free sterol fraction}} \times 100$. Numbers in parentheses are corrections for C^{14} in the tissue vascular compartments estimated from the values given by Dewey (15) for gram vascular plasma per gram tissue.

† Rats 5–12, whole tibia; rat 14, bone marrow only. Dewey's values for vascular content are for whole bone only; therefore, no correction could be made for bone marrow.

‡ Dewey's values for vascular contents are for whole intestines only. Correcting the average values for the mucosa and serosa by the figure given by Dewey for the whole intestines reduced the tabulated percentages of sterol-C^{14} in the esterified form to 5% or less at all intervals.

minutes after the injection of the labeled cholesterol. In muscle, the results were not consistent; in one rat, a large increase in the percentage of free sterol-C^{14} was observed, while in the other the change was small. In the intestines, in view of the corrections made for the sterol-C^{14} in the vascular compartment, it is possible that over 90% of the sterol-C^{14} was present in the free form at all intervals. In all of these tissues, *except the adrenal gland*, the proportion of free sterol-C^{14} increased with time; at 24 hours, about 95% or more of the labeled sterol in these tissues was in the free form.

In the adrenal gland, the initial increase in the percentage of sterol-C^{14} recovered in the free form was followed by a decrease. At 16 and 24 hours, about 90% of the labeled sterol was in the esterified form. It should be noted that this latter degree of esterification is greater than that of the cholesterol in the injected chylomicron-containing fraction of chyle.

Carbohydrate feeding had no effect on the proportions of labeled free and esterified sterol recovered in the tissues (Table 1).

Since, in almost all cases, less than 2% of the total lipid-C^{14} was recovered in the triglyceride fraction of the various tissues, little of the C^{14} derived from the side chain of the injected cholesterol-27-C^{14} could have been recycled into lipids during the time involved in our experiments. Experiments with intravenously administered cholesterol in humans indicate that labeled carbon from the side chain is not reincorporated to a measurable extent into cholesterol even after several days (18). We therefore regard the labeled tissue sterol as having been derived almost entirely from the intact cholesterol molecule that was injected.

TABLE 4. DISTRIBUTION OF LIPID-C^{14} BETWEEN CHYLOMICRON-CONTAINING AND NONCHYLOMICRON-CONTAINING FRACTIONS OF PLASMA AND IN BLOOD CELLS 10 MINUTES AFTER INTRAVENOUS INJECTION OF CHOLESTEROL-LABELED CHYLOMICRONS

Injected Chylomicron Cholesterol-C^{14}	Rat 5	Rat 14
% Esterified	74.7	71.2
% Free	25.3	28.8

Rat No.	Fraction	Distribution of Lipid-C^{14} Among Fractions Recovered	Recovered Sterol-C^{14} Present as: Esterified	Free
		%	%	%
5	Supernatant*	73.0	83.2	16.8
	Infranatant A	4.1	75.6	24.4
	Infranatant B†	22.9	58.6	41.4
14	Supernatant	55.6	84.6	15.4
	Infranatant A	6.7	83.1	16.9
	Infranatant B	25.0	62.2	37.8
	Pellet	7.2	67.8	32.2
	Blood cells	5.5	7.0	93.0

* Chylomicron-containing fraction.

† Contained the bulk of the higher-density α- and β-lipoproteins.

Distribution of Isotopic Sterol (a) Among Plasma Fractions Separated by Ultracentrifugation and (b) in Blood Cells 10 Minutes after the Intravenous Injection of the Chylomicron-Containing Lipoprotein Fraction of Chyle. The plasma of rats 5 and 14, which were killed 10 minutes after injection of the labeled cholesterol, was separated into a chylomicron-containing turbid layer (supernatant fraction) and a layer at the bottom (infranatant fraction B) that contained the bulk of the higher-density plasma α- and β-lipoproteins. Infranatant A, the layer between the above two fractions, is a salt solution slightly contaminated in its upper portion with lipoproteins of the chylomicron-containing layer and in its lower portion with low-density β-lipoproteins. The pellet isolated from plasma of rat 14 undoubtedly contained some of the high-density α-lipoproteins that were not removed by the washing procedure.

Table 4 shows that the higher-density plasma lipoproteins (infranatant fraction B) contained about 25% of the total lipid-C^{14} in the plasma fractions. In the transfer of labeled cholesterol to the higher-density plasma lipoproteins, isotope appeared in both esterified and free forms. The proportion of sterol-C^{14} found in the free form in the lipoprotein fraction, however, increased from about 25% (the proportion observed in the injected chyle) to about 40%. At the same time, the proportion of sterol-C^{14} recovered in the free form in the supernatant fraction (chylomicron-containing) decreased to about 16%.

The supernatant plasma fractions of rats 1–4 (Table 1) were also isolated and analyzed. In three of four cases, a decrease was observed in the proportion of sterol-C^{14} recovered in the free form.

It is apparent that, as early as 10 minutes after the injection, a small amount of the labeled cholesterol appeared in blood cells (Table 4, rat 14). The transfer was confined almost entirely to free cholesterol. The transfer of free cholesterol from plasma to blood cells has been reported by others (16, 17).

DISCUSSION

All nine tissues selected for study participated in the early disposition of the labeled cholesterol, injected in

the form of a chylomicron-containing lipoprotein fraction of chyle. At the 10-minute interval, about 50% of the injected C^{14} was recovered in the lipid fraction of the liver; at that time, liver, adipose tissue, and muscle accounted for about 70% of the injected cholesterol. These same three tissues were found by Bragdon and Gordon (19) to be the chief recovery sites of lipid-C^{14} 10 minutes after the injection of chylomicrons that had been labeled with triglyceride-C^{14}. The maximum incorporation of triglyceride-C^{14} by liver, however, did not exceed 21%. Biggs (20) also observed a much higher recovery in liver of labeled cholesterol than of labeled fatty acid 30 minutes after the intravenous administration of chyle containing either cholesterol-H^3 or triglycerides labeled with H^3-stearic acid. Although this difference could have resulted from a greater uptake of the cholesterol than of the fatty acid by the liver, it is conceivable that the initial uptakes of the two labeled lipids were the same and that the difference in their recoveries resulted either from a more rapid return to the circulation of the fatty acid than of the sterol or from a more rapid oxidation of the fatty acid.

Bragdon and Gordon (19) have reported that adipose tissue of rats fasted for 20 hours incorporated considerably less of the injected triglyceride-labeled chylomicrons than did adipose tissue of glucose-fed rats. We also found in two pairs of animals that previous glucose feeding augmented the incorporation—in this case twofold—of injected chylomicron cholesterol by adipose tissue at the 10-minute interval.

Some of the factors that might have influenced the disposition of the cholesterol-C^{14} shortly after its injection are (a) exchange in plasma of the labeled cholesterol of the chylomicron-containing fraction with unlabeled, higher-density plasma lipoprotein cholesterol, (b) intravascular disruption of the chylomicrons and incorporation of the isotopic cholesterol into higher-density lipoproteins of plasma, and (c) disruption of the chylomicrons in tissues, followed by return of the labeled cholesterol to the circulation in the higher-density lipoproteins. Apparently one or more of these processes occurred quite early; at 10 minutes after injection, about 25% of the plasma lipid-C^{14} was recovered in the higher-density plasma α- and β-lipoproteins. Although both labeled esterified and free sterol were transferred to higher-density lipoproteins, it is clear, from the data recorded in Table 4, that the free sterol was present in the nonchylomicron-containing plasma fractions and blood cells in a proportion greater than that in which it existed in the injected form, and it was also present in the chylomicron-containing fraction in a proportion smaller than that in which it existed in the injected material.

The transfer of labeled free sterol from the chylomicron-containing to the nonchylomicron-containing fractions of plasma in a proportion greater than that in which it existed in the injected material might be accounted for by an arrangement of the sterol in lipoproteins whereby the free sterol is either more accessible for intravascular exchange reactions than is the esterified or is more readily split off in the event of intravascular disruption of chylomicrons. The low-density lipoproteins obtained from the thoracic duct lymph of rats fed labeled cholesterol may contain several classes of lipoproteins, each with a different ratio of labeled free to esterified cholesterol. If this is the case, our findings of a disproportionate transfer of the labeled free sterol could also be explained by either intravascular exchange reactions, intravascular disruption, or removal and disruption in tissues followed by return of the labeled sterol to plasma involving only select classes of the chyle lipoproteins.

Our observation that both labeled esterified and free sterol are transferred from chylomicron-containing to the nonchylomicron-containing plasma fractions during the first 10 minutes after injection appears to differ from the results reported by Fredrickson *et al.* (21) for the intact dog. After the injection of cholesterol-C^{14} labeled chylomicrons, these investigators found considerable radioactivity in the free cholesterol of the high-density plasma lipoproteins in the early intervals but no C^{14} in the esterified form for 2 hours. It should be noted, however, that although our studies and those on the dog (21) were concerned with the transfer of isotopic cholesterol from chyle lipoproteins to higher-density plasma lipoproteins, the classes of lipoproteins isolated from chyle and plasma were not identical[5] in the two investigations. In our experiments, the chyle lipoproteins included higher-density classes, and the plasma lipoproteins contained lower-density classes than did those isolated in the dog experiments.

In the intact rat, hydrolysis appears to be the principal fate of the esterified cholesterol moiety of the chylomicron-containing lipoprotein fraction of chyle. It is not possible to state whether or not all tissues participated in the hydrolysis of the esterified cholesterol. An increase in the proportion of free sterol-C^{14} in a given tissue might have resulted from a disproportionate uptake of free and/or removal of esterified cholesterol. It is possible, too, that hydrolysis of esterified cholesterol occurred in one tissue with subsequent transport of the labeled free cholesterol to another tissue in which no hydrolysis took place.

[5] Personal communications from Drs. D. S. Fredrickson and R. Havel.

In four extrahepatic tissues—adipose, mucosa and serosa of the small intestine, bone or bone marrow, and adrenal gland—the fraction of labeled sterol present in the free form increased at a time (10 minutes) when the fraction of labeled sterol in free form in the liver was essentially the same as that in the injected material. This observation and our demonstration that hydrolysis of the cholesterol esters of a chylomicron-containing lipoprotein fraction of chyle can be brought about *in vitro* by these four tissues in addition to liver[6] suggest that hydrolysis of the injected cholesterol esters occurred in extrahepatic tissues as well as in the liver.

In plasma, an increase in the proportion of labeled free sterol was observed only between 10 minutes and 2 hours. This increase during this time interval could have been brought about either by the almost complete removal from the circulation of chylomicrons (high in labeled esters) with little or no removal of high-density lipoproteins or by recirculation of labeled free cholesterol (release of free cholesterol from the tissues into the plasma or the exchange of free cholesterol between tissues and plasma). Evidence for a relation between the latter mechanism and the increase has been provided by disappearance curves for cholesterol-C^{14}, injected in the same form as that used in this study, which show an almost threefold increase in the percentage of C^{14} in the circulation between 40 minutes and 2 hours after injection.[6] Although any tissue might have contributed to the relative increase in plasma labeled free sterol, it is probable that the liver was chiefly responsible for it because (a) free cholesterol in liver and plasma has been reported to exchange rapidly (17) and (b) a pronounced increase in the proportion of labeled sterol recovered in the free form was also observed in the liver between 10 minutes and 2 hours after injection. Further evidence for this role of the liver is our finding that, in the eviscerated rat, no increase in the proportion of labeled free sterol in the plasma occurred after injection of a cholesterol-labeled, chylomicron-containing fraction prepared in the same manner as the one used in this study.[6] The subsequent restoration of the plasma ratio of labeled esterified to free sterol to its initial value was undoubtedly brought about by plasma esterase. Esterification of free cholesterol *in vitro* by rat and human sera has been reported (22–26). Elimination of plasma free sterol-C^{14} via bile following its exchange with liver free sterol and uptake of the free sterol by other tissues and blood cells could also have contributed to restoration of the ratio of esterified to free sterol-C^{14}. The inability of serum esterase to maintain constant the ratio of labeled esterified to free sterol between 10 minutes and 2 hours after the cholesterol injection may be explained by release of labeled free sterol by liver or exchange of free sterol between liver and plasma at a more rapid rate during that interval than at any other time.

The situation in the adrenal gland was unique. The proportion of the sterol-C^{14} recovered in this gland in the free form in the earliest intervals was higher than that in the injected preparation, but subsequently the proportion of free sterol-C^{14} decreased and at 24 hours about 90% of the sterol-C^{14} was esterified. If we assume that the adrenal gland removed the labeled chyle preparation from the circulation, it is apparent that the cholesterol esters were hydrolyzed and new esters were subsequently synthesized. It is known that the adrenals store cholesterol chiefly in the esterified form (27, 28), and it has been recently reported that the fatty acid composition of sterol esters of the adrenal gland and plasma of the rat differs (29, 30). Hydrolysis of chylomicron cholesterol esters would permit the gland to resynthesize its characteristic esters.

[6] Unpublished observation.

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