In vitro uptake and hydrolysis, by rat tissues, of cholesterol esters of a very low density, chyle lipoprotein fraction

NATHAN BROT,* W. J. LOSSOW, and I. L. CHAIKOFF

Department of Physiology, University of California, Berkeley, California

SUMMARY A very low density, lipoprotein fraction of chyle containing cholesterol-C^{14} (of which about 70% or more was in the esterified form) was incubated with various rat tissues. The tissues and their incubation media were then analyzed for the C^{14} contained in the esterified and free sterol fractions. Adipose tissue, adrenal gland, mucosa of the small intestine, liver, kidney, and muscle incorporated the cholesterol-C^{14} of the chylomicrons and hydrolyzed the esters. Adipose tissue, adrenal gland, and mucosa of the small intestine showed the greatest hydrolytic activity. On the basis of these and other, in vivo, observations, the conclusion is drawn that, while the liver removes the bulk of the cholesterol esters of very low density chyle lipoproteins from the circulation, all tissues that incorporate them hydrolyze them.

A PREVIOUS report from this laboratory (1) dealt with the disposition in the rat of the cholesterol moiety of an intravenously injected, cholesterol-C^{14}-labeled, very low density chyle lipoprotein fraction. The preparation included lipoprotein classes of S_f 20 and higher, and about 75% of the cholesterol-C^{14} in it was esterified. In that study with intact rats, it was concluded that hydrolysis was the principal fate of the cholesterol esters, but it was not possible to state whether all of the tissues studied participated in the hydrolysis. It is shown here that six isolated tissues are capable of hydrolyzing the cholesterol esters of the chyle lipoproteins. Adipose tissue, adrenal gland, and mucosa of the small intestine were the most active of the tissues studied.

METHODS

Cholesterol-4-C^{14} and -27-C^{14} were purchased from the New England Nuclear Corporation, Boston. Immediately before use, the labeled cholesterols were purified on a silicic acid column by the procedure of Barron and Hanahan (2). The preparation of the very low density, cholesterol-labeled, lipoprotein fraction of chyle used as substrate was described in reference 1; it contained 270–290 μg cholesterol/ml.

Treatment of Animals and Incubation Procedures. Male, Long-Evans rats weighing between 240 and 320 g, which had been raised and maintained on an adequate stock diet (Diablo Labration), were lightly anesthetized with ether and exsanguinated by cardiac puncture. The tissues and organs listed in the tables were then excised and placed immediately in an ice-cold Krebs-Ringer phosphate buffer (3) of pH 7.4. Adipose tissue was taken from the perirenal area. The small intestine was excised and its lumen washed with a 0.9% solution of NaCl. The mucosa was removed with the aid of a blunt spatula and incubated without further treatment. Strips from the sternomastoid muscle were prepared as described by Richardson, Shorr, and Loebel (4). Slices of liver, adipose tissue, and kidney were prepared with the McIlwain-Buddle tissue chopper (5). The adrenal glands were quartered.

The tissues were incubated with the chyle lipoprotein fraction in the phosphate buffer. The incubations were carried out with shaking, at 37°. The contents of each flask were then filtered through glass wool and the tissues thoroughly washed with distilled water. Lipids were extracted separately from the tissues and the filtrate (medium plus washings).

Analytical Procedures. The tissues were placed in ethanol–ether 3:1 (v/v) 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 combined extracts in the case of each tissue were evap-

* Predoctoral Fellow of the National Heart Institute, U. S. Public Health Service.

orated under reduced pressure in a Rinco rotary evaporator, and the residue was dissolved in hexane.

The medium-plus-washings was extracted with about 30 volumes of ethanol–ether, the solvent was evaporated, and the residue dissolved in hexane.

Esterified and free sterol fractions were separated by chromatographing aliquots of the lipid extract on silicic acid columns (2). Each column consisted of 15 g of a mixture of 2 parts silicic acid and 1 part Hyflo Super-Cel (Johns-Manville Corp.) The maximum loading was 60 mg lipid/column, and each fraction was eluted with 250 ml of solvent.

Dried lipids were dissolved in 15 ml of toluene containing 45 mg of 2,5-diphenyloxazole, and their C^{14} contents were determined in a Packard Tri-Carb liquid scintillation counter.

RESULTS

At the end of the incubation period, 33–77% of the C^{14} that had been added to the medium was recovered in the washed adipose tissue slices, and somewhat less (20–54%) was recovered in the liver slices (Table I). The C^{14} uptake by adrenal gland slices amounted to 25–40%; that by small intestine mucosa, 13–29%; by kidney slices, 13–29%; and by the sternomastoid muscle preparations, 23–42%. The 10-fold dilution of the chylomicron preparation did not change the percentage of C^{14} taken up by adipose, adrenal, and liver tissue, indicating that the rate of uptake of the labeled cholesterol was directly proportional to the concentration of chyle lipoprotein. Such a proportionality might be explained by the binding of lipoproteins at the cell surface, possibly as a first step in pinocytosis, under conditions in which the number of binding sites far exceeded the number of lipoprotein particles at the higher concentration. If a specific enzyme had participated in the uptake of cholesterol by the tissue slices, the concentration effect suggests than enzyme saturation was not approached at the higher cholesterol level with the undiluted chyle lipoprotein preparation (0.1 ml of the undiluted preparation contained about 28 μg of cholesterol).

The percentages of sterol-C^{14} in the esterified form in the control flasks of each experiment shown in Table 1 were either the same as, or differed only slightly from, those of the chyle preparations. The proportion of the cholesterol-C^{14} recovered in the esterified form was reduced in all experiments with adipose tissue, liver, kidney, adrenal gland, and mucosa, both for the tissue and medium. The reduction of the proportion of labeled esters was more pronounced in the experiments with chyle that was diluted 10-fold. In the experiments with muscle, a distinct reduction was observed only when the chylomicron added to the medium had been diluted 50-fold.

Since the percentage of the cholesterol-C^{14} recovered in the ester form was reduced in both tissue and medium, the reduction cannot be accounted for by an exchange of free cholesterol-C^{14} between tissue and buffer, but must have resulted from the action of hydrolytic enzymes in the tissues.

DISCUSSION

Although hydrolysis of cholesterol esters has been observed in homogenates and saline extracts of a number of tissues of various animals (6–13), direct uptake of cholesterol esters by isolated tissues had not hitherto been demonstrated. Stern and Shapiro (14) concluded that adipose tissue did not incorporate serum cholesterol esters in vitro. That conclusion was based on the observation that no decrease in the concentration of cholesterol esters occurred in serum that was incubated with adipose tissue. However, since serum contains an enzyme that esterifies free cholesterol (first shown by Sperry (15) and recently confirmed by Glomset et al. (16, 17)), uptake of the cholesterol esters could have been masked by the esterification of the free cholesterol. Such esterification probably did occur in Stern and Shapiro's experiments, for in some cases an increase in the concentration of cholesterol esters in the serum was observed at the end of the incubation period. Our findings demonstrate that adipose tissue did incorporate C^{14}-labeled cholesterol esters of chyle lipoproteins in vitro, at least from Krebs-Ringer phosphate buffer medium.

In one experiment with adipose tissue and liver (Table 2), plasma was used instead of phosphate buffer as the incubation medium. A decrease in the proportion of esterified sterol-C^{14} (compared with that in the medium originally) was again observed in both tissues, but the proportion of labeled esterified sterol *increased* in the plasma (from 75.3% to over 80%) during the incubation, both in the control flasks and in those containing the tissue slices. With respect to the changes in the proportion of the cholesterol esters in plasma, then, our results resemble those of Stern and Shapiro, but the labeling reveals that uptake and hydrolysis of the esters by adipose tissue and liver did occur in both media. It should be noted that the percentages of cholesterol-C^{14} recovered in the tissues were considerably lower when plasma was substituted for the phosphate buffer. The following factors might be responsible: (*a*) The labeled $S_f>20$ chyle lipoproteins were diluted with unlabeled $S_f>20$ plasma lipoproteins. (*b*) The labeled cholesterol might have moved from the chyle lipoproteins to a large pool of unlabeled, higher density plasma lipoproteins, from which the rate of cholesterol uptake by the tissues could

TABLE 1 UPTAKE AND HYDROLYSIS OF C^{14}-LABELED CHOLESTEROL ESTERS OF VERY LOW DENSITY CHYLE LIPOPROTEINS BY VARIOUS RAT TISSUES INCUBATED IN PHOSPHATE BUFFER

Each flask contained 5 ml of Krebs-Ringer phosphate buffer, pH 7.4, 0.1 ml of cholesterol-27-C^{14}-labeled chyle lipoproteins (Experiment 1), or 0.1 ml of cholesterol-4-C^{14}-labeled chyle lipoproteins (Experiments 2–4), and the amounts of tissue recorded below. The gas phase was 100% O_2 and the incubations were for 3 hr (Experiment 1) or 2 hr (Experiments 2–4) at 37°.

Expt. No.	Tissue	Amount of Tissue	Dilution of Chyle Lipoproteins Added to Medium	At End of Incubation: % of C^{14} Recovered in Tissue Lipids	% of Sterol-C^{14} Esterified in*		% Decrease† of Sterol-C^{14} Present in the Esterified Form	
					Tissue	Medium	Tissue	Medium
		mg						
1	Control‡					73.2		
	Adipose tissue	409	Undiluted	59.3	42.5	10.3	41.9	85.9
	Adipose tissue	410	"	58.1	46.9	18.7	35.9	74.4
	Liver	402	"	53.6	66.2	57.1	9.6	22.0
	Liver	408	"	32.2	71.8	56.7	1.9	22.5
2	Control‡					78.7		
	Adipose tissue	288	Undiluted	69.3	42.0	37.7	46.6	52.2
	Adipose tissue	270	"	77.1	42.0	41.5	46.6	47.3
	Adipose tissue	293	10-fold	66.2	14.0	15.8	82.2	79.9
	Adipose tissue	301	"	74.0	11.6	8.5	85.3	89.2
	Liver	350	Undiluted	22.3	67.4	73.1	14.4	7.1
	Liver	379	"	19.9	66.0	72.9	16.1	7.4
	Liver	348	10-fold	24.3	59.3	69.6	24.6	11.6
	Liver	361	"	21.4	58.6	62.7	25.6	20.4
	Adrenal gland	48	Undiluted		61.6	62.8	21.7	20.2
	Adrenal gland	52	10-fold	41.8	45.7	45.0	41.9	42.8
	Musoca of small intestine	259	"	29.3	28.8	30.2	63.4	61.6
	Mucosa of small intestine	270	"	24.0	54.4	54.9	30.9	30.2
	Kidney	297	"	13.2	50.3	68.6	36.2	25.5
	Kidney	294	"	18.3	63.2	30.4	19.7	61.4
	Muscle	303	"	39.6	73.2	77.7	7.0	1.3
	Muscle	290	"	38.2	76.6	77.3	2.6	1.8
3	Control‡		10-fold			73.5		
	Adipose tissue	50	"	37.9	37.0	23.8	49.7	67.6
	Adipose tissue	43	"	33.3	37.1	25.6	49.5	65.2
	Adipose tissue	52	"	47.6	47.1	35.9	35.9	51.2
	Adrenal gland	49	"	25.2	30.7	28.5	58.2	61.2
	Adrenal gland	44	"	27.8	38.5	26.2	47.6	64.4
	Adrenal gland	40	"	29.8	37.3	29.9	49.3	59.3
	Mucosa of small intestine	43	"	13.3	29.1	28.9	60.4	60.7
	Mucosa of small intestine	41	"	12.9	31.9	30.8	56.6	58.1
	Kidney	309	"	29.1	55.6	56.1	24.4	23.7
	Kidney	305	"	26.9	61.6	64.4	16.2	12.1
	Muscle	278	"	31.4	68.4	69.3	6.9	5.7
	Muscle	298	"	24.3	66.4	71.1	9.9	3.3
4	Control‡		50-fold			67.9		
	Muscle	312	"	42.4	56.0	62.0	17.5	8.7
	Muscle	301	"	35.0	49.8	56.6	26.7	16.6
	Muscle	299	"	23.8	57.8	67.8	14.9	0.1

* $\frac{C^{14} \text{ recovered in esterified sterol fraction}}{C^{14} \text{ recovered in total sterol fraction}} \times 100.$

† Compared with control medium. Given by $\left(1 - \frac{\text{Per cent } C^{14} \text{ in esterified form in incubated tissue (or medium)}}{\text{Per cent } C^{14} \text{ in esterified form in incubated control medium}}\right) \times 100.$

‡ Control flasks contained medium and labeled chyle lipoproteins, but no tissue.

be lower. (*c*) Plasma lipoprotein might have served as a carrier for the removal of cholesterol from the tissues, following its uptake, in the same manner that albumin serves as a vehicle for the release of free fatty acids from adipose tissue in vitro (18).

Adrenal gland, mucosa of the small intestine, kidney, and muscle also incorporated and hydrolyzed the labeled cholesterol esters of the very low density chyle lipoproteins. Adipose tissue, adrenal gland, and mucosa incorporated at least as much labeled cholesterol per

TABLE 2 UPTAKE AND HYDROLYSIS OF C^{14}-LABELED CHOLESTEROL ESTERS OF VERY LOW DENSITY CHYLE LIPOPROTEINS BY VARIOUS TISSUES INCUBATED IN RAT PLASMA

Each flask contained 5 ml of heparinized rat plasma, 0.1 ml of cholesterol-27-C^{14}-labeled chyle lipoproteins, and the amounts of adipose tissue and liver recorded below. Incubations were for 3 hr at 37°; gas phase was 95% O_2-5% CO_2. The chylomicrons added to the incubation medium contained 75.3% of its cholesterol-C^{14} in the esterified form.

Sample	Tissue Wt	% of Incubated C^{14} Recovered in Tissue Lipids	% of Sterol-C^{14} Esterified in:*		% Decrease† of Sterol-C^{14} Present in the Esterified Form	
			Tissue	Medium	Tissue	Medium
	mg					
Control‡				87.8		
Adipose tissue	404	9.3	44.4	83.7	49.4	4.7
Adipose tissue	415	11.8	57.6	81.2	34.4	7.5
Liver	418	5.3	28.9	84.4	67.0	3.9
Liver	434	6.3	21.1	87.5	75.9	0.3

* See footnote, Table 1.
† Compared with incubated control medium. See footnote, Table 1.
‡ Control flasks contained plasma and labeled chyle lipoproteins, but no tissue.

milligram as did the liver, and showed the greatest hydrolytic activity.

In our earlier study (1) with carbohydrate-fed rats that received intravenously 1 ml of the same chyle preparation as that used in the present study, about 25% of the C^{14} was recovered in the circulation 10 min after the injection, about 50% in the liver and about 20% in 8 extrahepatic tissues. During that interval the proportion of sterol-C^{14} in the free form increased from the initial value (about 30%) to 50% or more in adipose tissue, intestines, and adrenal gland. At 2 hr, about 70% or more of the sterol-C^{14} was in the free form in all nine tissues examined. Somewhat similar findings were recently reported by Goodman (19). This investigator equilibrated cholesterol-labeled chyle lipoproteins with whole blood before the injection, thereby increasing the proportion of sterol-C^{14} in the esterified form to almost 90%. Twenty minutes after injection of 2 ml of this preparation into fasted rats—at which time only about 3% of the C^{14} remained in the circulation—the liver contained about 88% of the C^{14}, and all other tissues about 10%. Between 20 min and 1 hr after the injection, an abrupt increase in the proportion of free sterol-C^{14} was observed in eight tissues. In most of the extrahepatic tissues, 60% or more of the sterol-C^{14} was in the free form; in the liver, about 40% was in this form. During that same time interval, no increase in total C^{14} was observed in any of the extrahepatic tissues.

In the intact animal, the liver is chiefly responsible for the removal of the cholesterol of very low density lipoproteins from the circulation. The high values observed for the in vitro uptake of labeled cholesterol by extrahepatic tissues might be explained by the fact that in vitro, in contrast to in vivo, chyle lipoproteins come into contact with those tissues without passing through a capillary membrane. The conclusion to be drawn from these studies with the cholesterol-labeled, very low density chyle lipoproteins, in vivo and in vitro, is that although the liver removes the bulk of the chyle cholesterol esters from the circulation, all tissues that incorporate them hydrolyze them. This conclusion is supported by our observation (unpublished) that when eviscerated rats were injected with the same cholesterol-labeled, chyle lipoprotein preparation as that used in this and in our earlier study (1), the proportion of sterol-C^{14} recovered in the free form increased in all tissues studied, namely, adipose tissue, adrenal gland, kidney, and muscle, during a period when the proportion of free sterol-C^{14} in plasma did not decrease.

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