J. Lipid Research, October 1963
Volume 4, Number 4

Effect of heparin on disappearance of the cholesterol moiety of an injected cholesterol-C^{14}-labeled, very low-density chyle lipoprotein fraction from the circulation of the rat*

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[Manuscript received March 22, 1963; accepted June 11, 1963.]

SUMMARY

A cholesterol-4-C^{14}-labeled, very low-density, lipoprotein fraction of chyle was injected into heparin-treated and control rats. The disappearance of the C^{14} from the whole blood was followed at intervals up to 25 min after the injection. Heparin increased the rate at which the injected cholesterol-C^{14} left the circulation during the first 10 min. Determination of the distribution of isotope among ultracentrifugally separated $S_f > 20$, S_f 0–20, and high-density plasma lipoproteins revealed that, at 10 min after the administration of the cholesterol-labeled chyle preparation, a much greater proportion of the plasma lipid-C^{14} was present in the S_f 0–20 lipoproteins isolated from the heparin-treated than from the control rats. The in vitro mixing of the cholesterol-labeled, very low-density chyle lipoproteins with blood obtained from heparin-treated and control rats also resulted in recovery of a disproportionately high percentage of the isotope in the S_f 0–20 lipoproteins of the heparin-treated rats. Such a distribution of isotope among the plasma lipoproteins in heparin-treated rats is compatible with the scheme of Lindgren and co-workers for the action of clearing factor lipase in which chylomicrons are degraded through a series of lipoprotein complexes to lipoproteins of the S_f 0–20 class, the cholesterol and phospholipid components of the original chylomicrons becoming part of the final end products. A very rapid removal of any of the lipoproteins resulting from the heparin-induced lipolysis could account for the effect of heparin on the disappearance of the injected cholesterol-C^{14} of the chyle lipoproteins from the circulation.

It has been amply demonstrated that the heparin-induced, lipemia-clearing reaction of plasma is brought about by an enzymatic lipolysis, restricted to the glyceride moiety of the low-density lipoproteins, in the course of which lipoprotein complexes are formed, higher in density and richer in both protein and non-triglyceride lipid components (1-5). In 1955, Lindgren, Nichols, and Freeman (6) reported that the end products resulting from heparin-induced lipolysis of human S_f 20-400 serum lipoproteins are lipoproteins of the S_f 0-20 class; more recently, Shore and Shore (7) have further restricted the products to the S_f 11-20 class. The fatty acids liberated by heparin action become bound to plasma albumin (8, 9) and, in this form, are rapidly removed from the circulation (10-15). It has been shown that heparin treatment increases the rate at which the triglyceride fatty acids of chyle lipoproteins leave the blood stream (16). But virtually nothing is known of the fate of the lipid components of the lipoproteins formed during the heparin-clearing reaction. The rate at which they disappear from the circulation could affect the rate at which the nontriglyceride components of the very low-density lipoproteins are removed from the circulation. For that reason, we have studied the influence of heparin treatment on the disappearance from the circulation of the cholesterol moiety of an intravenously-injected, very low-density lipoprotein fraction obtained from the chyle of rats.

* Aided by grants from the U.S. Public Health Service and the Life Insurance Medical Research Fund.

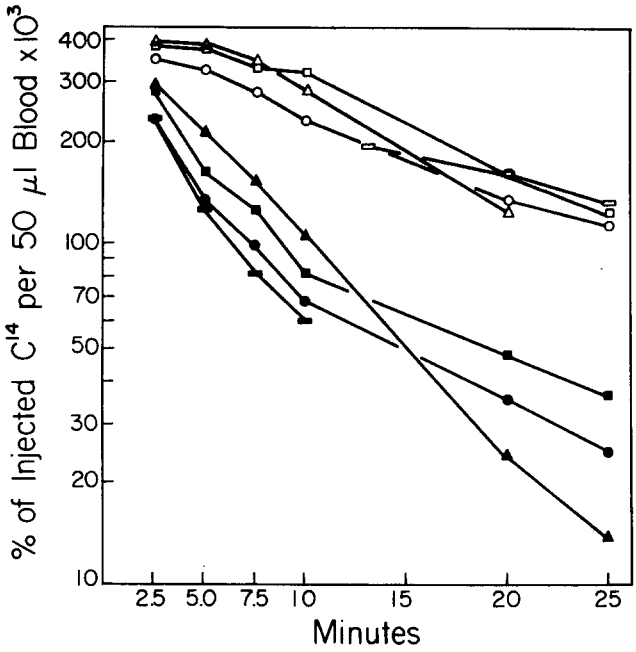

FIG. 1. Disappearance of lipid-C^{14} from the circulation of rats injected intravenously with cholesterol-labeled, very low-density chyle lipoproteins. Closed symbols, heparin-treated; open symbols, untreated.

METHODS

Preparation of Cholesterol-C^{14}-labeled Lipoprotein Fraction of Chyle. Cholesterol-4-C^{14}, purchased from the Nuclear-Chicago Corp., was purified on silicic acid columns (17). The thoracic ducts of donor rats were cannulated as described in (18). The preparation of the cholesterol-labeled, very low-density fraction of thoracic duct chyle (S_f classes > 20) collected from donor rats fed cholesterol-4-C^{14} has been described (19).

Treatment of Rats from which Serial Blood Samples were Obtained from the Tail Vein. Male, Long-Evans rats weighing 220–240 g were fasted overnight. Each rat designated *heparin-treated* received intravenously 20 U.S.P. units of heparin (Upjohn) in 0.5 ml of a 0.9% NaCl solution, and each designated *control* received, by the same route, 0.5 ml of the NaCl solution. Ten minutes later, each rat received intravenously 1 ml of the labeled chyle preparation. Samples of blood (100 µl) for duplicate analyses were then removed from the tail vein of each rat, in most cases at 2.5, 7.5, 10, 20, and 25 min after injection of the chyle preparation.

Treatment of Rats from which Blood Samples were Obtained from Arterial Cannulae for Ultracentrifugal Separation of Lipoprotein Classes. Rats of the same weight, sex, and nutritional status as those described above were used. Rats to be treated with heparin were lightly anesthetized with ether, and a polyethylene catheter filled with 0.9% NaCl solution was inserted into a femoral artery and secured there. Each of three rats so treated received, by way of a leg vein, 20 units of heparin in 0.5 ml of 0.9% NaCl solution and, 10 min later, 1 ml of the labeled chyle preparation. Blood samples (1 ml) were obtained from these rats at 10 and 20 min after injection of the labeled cholesterol by allowing the blood to drip from the arterial cannula into graduated, 15-ml centrifuge tubes. The cannula was refilled with 0.9% NaCl after each collection. Twenty-five minutes after injection of the labeled cholesterol, 5-8 ml of blood was withdrawn from the heart.

Two of the control rats were treated in exactly the same way as described above except that Teflon cannulae (obtained from R. S. Hughes Co., Palo Alto, Calif.) were used in place of polyethylene cannulae, and each rat received 0.5 ml of the 0.9% NaCl solution instead of the heparin-containing solution. The centrifuge tubes in which blood from these control rats was collected contained a drop of heparin solution, and the syringe used to withdraw blood from the heart was rinsed with heparin. The Teflon cannulae made it possible to collect blood without anticoagulant. Blood samples were obtained from a third control rat through a polyethylene cannula filled with a 10% solution of ethylenediamine tetra-acetic acid disodium salt (EDTA).

Ultracentrifugal Separation of Plasma Lipoprotein Classes. A 400-µl aliquot of the plasma obtained from each arterial blood sample and 400-µl and 2-ml aliquots of the plasma obtained from each heart blood sample were transferred to Spinco ultracentrifuge tubes and separated into three lipoprotein fractions: S_f > 20; S_f 0-20; and high-density lipoproteins (HDL). The S_f > 20 lipoproteins were those that floated (top 1.5 ml) in a NaCl solution of density 1.006, after centrifugation at 79,420 × *g* for 20 hr at 4° in a 30.2 rotor. The S_f 0-20 class contained the lipoproteins that floated (top 1.5 ml) after the density of the infranatant fraction was raised to 1.063 with NaCl, and the fraction was recentrifuged exactly as described above. High-density lipoproteins refers to those lipoproteins recovered in the infranatant fraction after removal of the S_f 0-20 class and includes the ultracentrifugal residue.

Extraction of Lipids and Determination of C^{14}. Total lipids of whole blood samples and of plasma fractions were extracted and assayed for C^{14} as described earlier (20). In a few of the terminal samples, it was determined by silicic acid chromatography (17) that all of the lipid-C^{14} was recovered in the sterol fractions. We have assumed, therefore, that the determination of plasma lipid-C^{14} is a measure of plasma cholesterol-C^{14}.

RESULTS

Disappearance of Cholesterol-C^{14} from Whole Blood. The disappearance curves for four heparin-treated and four control rats are shown in Fig. 1. The injection of heparin increased the rate at which the cholesterol-C^{14} of the injected chyle preparation was removed from the circulation during the first 10 min.

Recovery of the Cholesterol-C^{14} in Ultracentrifugally-Separated Plasma Lipoprotein Classes. The percentages of the injected C^{14} recovered in the various lipoprotein fractions, per milliliter of plasma, are shown in Table 1. At 10 min, much less of the injected C^{14} was recovered in the $S_f > 20$ plasma lipoproteins of the heparin-treated rats than in those of the control rats. At that time, the S_f 0-20 fraction in the heparin-treated rats contained more C^{14} than did that in the control rats, but this higher C^{14} recovery did not account for the difference between normal and heparin-treated rats in the C^{14} contents of the $S_f > 20$ fraction. At subsequent time intervals, differences between the heparin-treated and control rats were not found in the percentages of C^{14} recovered in the S_f 0-20 lipoproteins.

Extent of In Vitro Transfer of C^{14} from Chyle Lipoproteins to Higher-Density Plasma Lipoproteins during Handling and Centrifugation of Blood Samples. Since the exchange of cholesterol between plasma lipoprotein classes takes place in vitro (21–23), it was necessary to determine to what extent transfer of labeled sterol during centrifugation accounted for the results reported in the preceding section. This was done by adding 0.1 ml of the cholesterol-4-C^{14}-labeled chyle lipoprotein preparation to each of four samples (approximately 7 ml) of whole blood. Two of the samples were obtained from normal rats and two from rats that had been injected with 20 U.S.P. units of heparin in 0.5 ml of a 0.9% NaCl solution 10 min before withdrawal of blood. These blood samples were then treated in exactly the same manner as were those of the in vivo disappearance study. Table 2 shows the distribution of C^{14} among the three lipoprotein classes. An average of 4.1% of the lipoprotein-C^{14} was found in the two classes (S_f 0-20 and HDL) with densities higher than that of the original chyle preparation. The corresponding value (calculated from the data in Table 1) for normal rats 10 min after injection of the labeled cholesterol was 13.1%. The corresponding values for the in vitro and in vivo heparin experiments were 14.8 and 51.6%, respectively.

In the case of the blood obtained from normal rats, in both the in vitro and in vivo experiments, the C^{14} was either about equally distributed between the S_f 0-20 and HDL classes, or somewhat more was recovered in the latter. In blood obtained from heparin-treated rats, in the in vitro as well as the in vivo experiments, about three times as much C^{14} was recovered in the S_f 0-20 lipoprotein class as in the HDL.

TABLE 1. PERCENTAGES OF C^{14} OF INJECTED CHOLESTEROL-4-C^{14} RECOVERED PER MILLILITER OF PLASMA, IN ULTRACENTRIFUGALLY-SEPARATED LIPOPROTEIN FRACTIONS

Interval After Injection	Lipoprotein Classes: $S_f > 20$ Normal	$S_f > 20$ Heparin-Injected	S_f 0–20 Normal	S_f 0–20 Heparin-Injected	HDL plus Residue Normal	HDL plus Residue Heparin-Injected
min						
	(Rat 1)	(Rat 2)	(Rat 1)	(Rat 2)	(Rat 1)	(Rat 2)
10	3.78	0.74	0.23	0.74	0.30	0.22
20	0.83	0.24	0.14	0.17	0.26	0.17
25	0.73	0.04	0.13	0.19	0.29	0.11
	(Rat 3)	(Rat 4)	(Rat 3)	(Rat 4)	(Rat 3)	(Rat 4)
10	3.14	0.81	0.20	0.66	0.25	0.21
20	1.68	0.13	0.15	0.16	0.15	0.16
25	0.58	0.06	0.16	0.13	0.12	0.12
	(Rat 5)	(Rat 6)	(Rat 5)	(Rat 6)	(Rat 5)	(Rat 6)
10	2.88	0.89	0.25	0.58	0.25	0.18
20	0.66		0.17		0.17	
25	0.23	0.15	0.16	0.16	0.23	0.11

The HDL fraction of rats of the same weight, sex, and strain used in this study contains approximately three times as much cholesterol (about 35 mg/100 ml plasma) as does the S_f 0-20 class (about 12 mg/100 ml plasma). If the movement of C^{14} from the chyle to these two classes of lipoprotein is brought about exclusively by exchange of lipoprotein cholesterol, with both classes becoming labeled simultaneously in the process, and if the S_f 0-20 and HDL cholesterol equilibrate completely, than at no time should the HDL contain less C^{14} than the S_f 0-20 lipoproteins and, when equilibrium is reached, the HDL's would be expected to contain about three times as much C^{14} per milliliter of plasma as would the S_f 0-20 class. Cholesterol exchange could account for the movement of C^{14} in the in vivo experiment with normal rats, at 10 min, and in the in vitro experiment with normal blood, although other processes cannot be ruled out. But in the heparin experiments, both in vivo and in vitro, the

TABLE 2. DISTRIBUTION OF C^{14} AMONG ULTRACENTRIFUGALLY-SEPARATED PLASMA LIPOPROTEIN FRACTIONS AFTER ADDITION OF CHOLESTEROL-LABELED CHYLE LIPOPROTEINS TO WHOLE BLOOD*

Blood Obtained from	Lipoprotein Classes: $S_f > 20$	S_f 0–20	HDL plus Residue
Normal rats	94.5	1.9	3.6
	97.4	1.3	1.3
Heparin-injected rats	87.3	9.6	3.2
	83.1	12.5	4.4

* Values refer to percentages of total C^{14} recovered in all 3 fractions.

disproportionately high values for the C^{14} recovered in the S_f 0-20 class suggest that a process other than exchange was involved. Since the S_f 0-20 lipoproteins have been shown to contain the principal lipoprotein end products of lipolysis of human serum very low-density lipoproteins by post heparin plasma (6, 7), it seems probable that the high proportion of C^{14} found in the S_f 0-20 lipoproteins of our heparin-treated rats resulted from an enzymatic lipolysis of the cholesterol-labeled chyle lipoproteins.

DISCUSSION

Lindgren et al. (24) have presented a scheme whereby, under the influence of the clearing factor lipase, chylomicrons are degraded, through an innumerable series of lipoprotein complexes, to lipoproteins of the S_f 0-20 class, the cholesterol and phospholipid components of the original very low-density lipoproteins becoming part of the final end products. Consistent with such a scheme is our observation that, 10 min after the injection of cholesterol-labeled, very low-density chyle lipoproteins, a much greater proportion of the plasma-C^{14} was recovered in the S_f 0-20 lipoproteins in rats pretreated with heparin than in those not so treated.

It is shown here that heparin treatment speeds up the removal from the circulation, during the first 10 min, of the cholesterol-C^{14} injected in the form of the very low-density chyle lipoproteins. This can be accounted for by removal of the cholesterol-C^{14} of any of the lipoprotein complexes formed during the heparin-induced degradation more rapidly than either the cholesterol-C^{14} of the injected chyle lipoproteins or that transferred from the chyle to higher density lipoproteins by exchange reactions. The possibility cannot be ruled out, however, that heparin, in some manner, directly affects the rate of removal of the very low-density chyle lipoproteins before their degradation.

Whether the rate at which cholesterol of the S_f 0-20 lipoprotein end products of the lipolysis left the circulation played a part in the observed effect of heparin on the rate at which chyle cholesterol disappeared from the blood cannot be determined from our data. Labeled cholesterol of the chyle lipoproteins taken up by the liver was undoubtedly returned to the circulating S_f 0-20 lipoproteins to some extent (19, 20) during the period plasma samples were obtained for ultracentrifugal separation, and the amount so recirculated in the heparin-treated and normal rats might not have been the same. Hence, the rates of removal of labeled cholesterol of the S_f 0-20 lipoproteins should not be compared under the two conditions. Since heparin and a lipolytic enzyme with properties of clearing factor lipase can be detected in plasma of humans and animals, particularly after fat feeding (25), the lipoprotein end products of the lipolysis may be naturally-occurring constituents of plasma. Even so, it still remains to be established whether or not the lipoprotein end products are identical with plasma lipoproteins of the same flotation rate, not formed lipolytically. Shore and Shore (7) have reported that the S_f 11-20 lipoproteins produced in vitro by heparin lipolysis of S_f 20-400 human serum lipoproteins and lipoproteins of the same flotation rate isolated from normal individuals not treated with heparin have almost identical cholesterol, phospholipid, and triglyceride concentrations, the same electrophoretic mobility, and common NH_2-terminal amino acids. But these workers found that the concentration of three of the four NH_2-terminal amino acids was higher in the lipolytically produced S_f 11-20 lipoproteins than in lipoproteins of the same flotation rates obtained from individuals not treated with heparin. This latter observation raises the possibility that lipoproteins formed by enzymatic lipolysis of the very low-density lipoproteins may not be identical with lipoproteins of the same S_f class not formed in this manner. Experiments are now in progress to determine whether the S_f 0-20 lipoprotein end products of heparin lipolysis can be regarded as physiologically identical with other S_f 0-20 lipoproteins.

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