Turnover of cholesteryl esters of plasma lipoproteins in the rat

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ABSTRACT Turnover of individual classes of cholesteryl esters (classified on the basis of the degree of unsaturation of the fatty acid moiety) in rat plasma lipoproteins and liver was studied after the administration of mevalonic acid-5-^{3}H and mevalonic acid-2-^{14}C.

The relative turnover rate was greatest in the d < 1.019 lipoproteins, with monoenes > saturated = dienes > tetraenes. In the d > 1.063 lipoproteins, all cholesteryl esters had slower turnover rates, but tetraenes = pentaenes > dienes > monoenes = saturated.

Comparisons of specific activities of individual cholesteryl ester classes of liver subcellular fractions and lipoproteins suggest that the d < 1.019 lipoprotein cholesteryl esters are synthesized from newly synthesized cholesterol in the liver and are rapidly released into this lipoprotein. Tetraenoic cholesteryl esters, however, may originate from esterification of free cholesterol in plasma. Tetraenoic esters are formed from cholesterol in plasma during incubation or ultracentrifugation unless a thiol-reacting or alkylating agent is added. Failure to add such a reagent to plasma results in erroneous specific activities.

In the adrenal, relative rates of synthesis of cholesteryl esters are monoenes = dienes > tetraenes > trienes = pentaenes > saturated.

It is concluded that cholesteryl ester turnover in the rat, as opposed to man, is determined not only by the particular lipoprotein class but also by the fatty acid moiety of the ester.

KEY WORDS turnover · cholesteryl esters · plasma lipoproteins · degree of unsaturation · biosynthesis · liver subfractions · adrenals · rat · plasma acyltransferase

RECENT STUDIES of the cholesteryl esters of rat plasma lipoproteins have shown a marked heterogeneity of fatty acid composition among the lipoprotein fractions (1). The cholesteryl esters of lipoproteins of d < 1.019 and of the liver had a very similar composition, with very low proportions of arachidonic acid. On the other hand, the cholesteryl esters of d > 1.063 lipoproteins had a totally different composition, with a very high proportion of arachidonic acid. These differences in fatty acid composition could be due to differences in synthesis and turnover of the lipoprotein carrier and (or) to differences in the mechanism and rate of formation of cholesteryl esters in the various lipoproteins. Goodman and his colleagues have reported that there are differences in the rates of hydrolysis (2) and synthesis (3) of different cholesteryl esters. In a later publication, Goodman and Shiratori investigated the turnover of different cholesteryl esters in liver and plasma (4) and showed that there are indeed differences in the fractional turnover rates of saturated, mono-, di-, and tetraunsaturated cholesteryl esters. Sugano and Portman (5) also showed that there was heterogeneity of labeling of plasma cholesteryl esters soon after administration of mevalonate-2-^{14}C or cholesterol-4-^{14}C.

The present experiments were designed to study the fractional rate of turnover of different cholesteryl esters in lipoprotein fractions. Goodman has shown that in man the cholesteryl esters in various lipoproteins have the same fatty acid composition (6), and that within each lipoprotein class the individual cholesteryl esters have the same rate of turnover (7). Similar findings were also reported by Nestel, Couzens, and Hirsch (8). The heterogeneity of ester composition in rat plasma lipoproteins led us to suspect that in the rat there may be heterogeneity of turnover of different cholesteryl esters within each lipoprotein class.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Holtzman Company, Madison, Wis.), maintained on rat pellets (Rockland Farms,

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New City, N. Y.), and weighing between 410 and 530 g were used in these studies.

Mevalonic acid-2-^{14}C and mevalonic acid-5-^{3}H were obtained as the *N,N'*-dibenzylethylene diamine salts from New England Nuclear Corp., Boston, Mass. These salts were dissolved in water and 6 N NaOH was added until the pH was 12.6. The solution was extracted five times with 10-ml volumes of ether to remove the organic base. The aqueous solution was adjusted to pH 7.5 with N HCl, heated to remove traces of ether, and diluted with Ringer's solution. The specific activities were 2.88 mc/mmole and 196 mc/mmole for the ^{14}C and ^{3}H compounds, respectively.

Three experiments were performed. In one, only mevalonic acid-2-^{14}C was administered; in the other two, both ^{14}C- and ^{3}H-labeled mevalonate were administered. The isotopes in all experiments were administered by intraperitoneal injection (0.5–1.0 ml). After a specified time interval, the rats were anesthetized with ether and killed by exsanguination. The protocol of the experiments is outlined in Table 1. The administration of mevalonic acid labeled with both isotopes enabled us to study cholesterol synthesis at two different times. In experiment 2, for example, rats were given mevalonic acid-5-^{3}H at zero time and mevalonic acid-2-^{14}C 159 min later. The animals were sacrificed 42 min after the last injection.

Blood was obtained by heart puncture and pooled in heparinized tubes. In experiment 3, livers were removed and weighed; in experiments 2 and 3, adrenals were also removed, trimmed of surrounding fat and tissue, and weighed.

Isolation of Plasma Lipoproteins

Before ultracentrifugal separation of the plasma lipoproteins, a reagent reacting with thiol groups was added to the plasma. Glomset (9) has reported that a fatty acid transferase in rat plasma catalyzes the esterification of free cholesterol but that the action of this enzyme is inhibited by various sulfhydryl-blocking or alkylating agents. Such an esterification reaction could lead to erroneously high concentrations and incorrect specific activities of esterified cholesterol. Therefore, in experiment 1, 0.04 M sodium *p*-chloromercuriphenyl sulfonate was added to the plasma to a final concentration of 0.01 M. In experiments 2 and 3, 5,5'-dithio-bis(2-nitrobenzoic acid) was added to the plasma to a final concentration of 0.007 M.[1] The plasma in all the experiments was then adjusted to d 1.019. Three lipoprotein fractions were isolated according to the method of Havel, Eder, and Bragdon (10): very low density lipoproteins (d < 1.019), low density lipoproteins (d 1.019–1.063), and high density lipoproteins (d > 1.063).[2]

TABLE 1 SCHEDULE OF ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED MEVALONIC ACID

Expt.	No. of Rats	^{14}C/Rat	Mean time of Sacrifice after ^{14}C	^{3}H/Rat	Mean Time of Sacrifice after ^{3}H
		μc	*hr*	*μc*	*hr*
1	29	16.7	3.48 (3.31–3.57)*	—	—
2	21	19.4	0.70 (0.62–0.77)	95.2	3.35 (3.03–3.62)
3	16	12.2	1.77 (1.72–1.83)	62.7	7.52 (7.47–7.58)

* The numbers in parentheses are the ranges of times.

Chemical Analyses

Total cholesterol analyses (11) were carried out on plasma and on lipoprotein fractions in order to evaluate the recovery of cholesterol during ultracentrifugal separations. Analyses of free and total cholesterol (12) were performed on plasma to measure the degree of esterification of free cholesterol during the ultracentrifugation and to assess the effectiveness of the inhibitors.

Liver Fractionation

The livers of experiment 3 were pooled and homogenized in 0.25 M sucrose. The homogenate was then subfractionated into four major fractions: mitochondria, microsomes, supernatant solution, and free floating fat (13).

Extraction of Lipids

Plasma and lipoprotein fractions were extracted by the method of Folch, Ascoli, Lees, Meath, and Le Baron (14). Aqueous suspensions of liver subfractions (experiment 3), and homogenates of adrenals (experiments 2 and 3) were extracted by the method of Folch, Lees, and Sloane Stanley (15). All lipid extracts were washed three or more times with water equilibrated with mixtures of $CHCl_3$, CH_3OH, and $CaCl_2$. The extracts were taken to dryness in vacuo and redissolved in $CHCl_3$. These solutions were then stored at −20°C until used.

Isolation of Cholesterol and Cholesteryl Esters

The lipid extracts were concentrated in vacuo and applied to several thin-layer plates of Silica Gel G. The plates were developed in hexane–diethyl ether–acetic acid 83:16:1, and the lipid fractions were located by spraying the plate with 0.02% 2',7'-dichlorofluorescein in 95% ethanol. Bands corresponding to free cholesterol and cholesteryl esters were scraped off the plates and the

[1] This concentration was never actually achieved since some of the reagent did not dissolve and was removed by centrifugation.

[2] The high density lipoproteins have densities between 1.063 and 1.21. However, in these studies separation at the latter density was not performed since the d > 1.21 fraction contains virtually no cholesteryl esters (10).

respective lipids were eluted from the silica gel by three successive washes of chloroform. The cholesteryl esters were further fractionated on Silica Gel G thin-layer plates impregnated with $AgNO_3$ (16). Two different solvent systems were utilized: benzene–hexane 60:40, and hexane–diethyl ether–acetic acid 70:29:1. With benzene–hexane, saturated, monounsaturated, and diunsaturated cholesteryl esters were separated from one another while esters of tri-, tetra-, penta-, and hexaunsaturated esters remained at or near the origin. With the hexane–diethyl ether–acetic acid solvent, saturated, monounsaturated, and diunsaturated esters moved with the solvent front, hexaunsaturated esters remained at the origin, and penta-, tetra-, and triunsaturated[3] esters were separated from one another. In practice the plates were developed first with hexane–diethyl ether–acetic acid. The cholesteryl esters at the solvent front (made visible with dichlorofluorescein) were eluted from the gel and were applied to $AgNO_3$ plates which were then developed in benzene–hexane. The individual bands on the first plate (hexane–diethyl ether–acetic acid) were eluted with warm benzene–ether 1:1. The tetraene band was rechromatographed in hexane–diethyl ether–acetic acid. The second chromatography was particularly required for the cholesteryl esters of the adrenals and of high density lipoproteins, which contained relatively high proportions of polyenoic esters.

The relative purity of the various fractions was estimated by gas chromatographic analyses of the cholesteryl ester fatty acids (1, 17). There were some monoenoic and saturated acids in the saturated and monoenoic fractions, respectively, but the purity of each fraction was approximately 90–95%. Similar degrees of purity were observed for other fractions.

^{14}C and ^{3}H Assays

The specific activities of the free cholesterol and the various cholesteryl ester fractions were determined by the method of Kabara (18). The digitonides of free cholesterol or of esterified cholesterol (after hydrolysis) were dissolved in dioxane containing 1% H_3PO_4. Aliquots (usually 0.5 or 1.0 ml) were taken for cholesterol assay and for radioassay. The counting was carried out in a Model 314 EX Packard Liquid Scintillation Spectrometer. The voltage and channel settings were adjusted so that ^{14}C and ^{3}H were counted simultaneously. The ^{14}C and ^{3}H counts were calculated by the discrimination-ratio method (19).

[3] No trienoic cholesteryl esters were found in the present studies. However, the procedure used was developed for the separation of cholesteryl esters from adrenals of essential fatty acid-deficient rats. These esters had a high content of trienoic acids (17).

RESULTS

Effect of Reagents Reacting with Thiol Groups on Esterification of Free Cholesterol

Glomset (9) has demonstrated that a fatty acid transferase is present in plasma and is inhibited by alkylating or sulfhydryl-blocking agents. If the esterification reaction occurred in rat plasma during the ultracentrifugal separation of lipoproteins, free cholesterol with a high specific activity would be esterified. The resultant esters would then have specific activities greater than those of the esters already present if the plasma was obtained from rats before isotopic equilibration of plasma free and esterified cholesterol had occurred. Table 2 shows the results of an experiment in which 17.6 μc of mevalonic acid-2-^{14}C was administered to each of 14 rats. The rats were divided into two groups, A and B, and were killed after 1.5 hr. Part of the plasma was processed immediately (cholesterol analyses and lipid extraction), and the remainder was centrifuged at 105,000 *g* for 72 hr at 15°C and then analyzed. The data clearly indicate that the concentration of esterified cholesterol increased during ultracentrifugation, and that the specific activity of the esterified cholesterol was modified. The most striking and significant change was in the specific activity of the tetraene fraction (cholesteryl arachidonate) which increased approximately 43%, as compared to increases of 20–25% and 15–18% for the total esters and dienes, respectively.

It was believed that the most serious changes in esterified cholesterol specific activity would occur at those times when the ratio of free cholesterol specific activity/esterified cholesterol specific activity would be very high. Therefore, the effects of various thiol-reacting substances were tested in an experiment in which 15 animals were killed 29 min after administration of 12 μc of mevalonic acid-2-^{14}C. The plasma from these rats was pooled and

TABLE 2 EFFECT OF ULTRACENTRIFUGATION ON ESTERIFICATION OF FREE CHOLESTEROL

	Group A		Group B	
	Direct Analysis	Centrifuged	Direct Analysis	Centrifuged
Total cholesterol esterified, %	59.5	71.0	65.9	74.1
Specific activity		*dpm/mg cholesterol*		
Free cholesterol	6600	6480	7540	7360
Esterified cholesterol	2100	2620	2770	3290
0=*	1140	1650	1790	—
1=	3350	3130	3790	3460
2=	1720	2040	2310	2660
4=	1810	2570	2180	3160

Groups A and B consisted of 7 rats each.
* Number of double bonds in fatty acid moiety of cholesteryl ester.

then subdivided into four portions; to three of these was added a thiol-reacting substance. Each portion was then subdivided further into three smaller volumes. One aliquot was extracted immediately, one was incubated at room temperature for 24 hr and then extracted, and one was centrifuged for 72 hr at 105,000 *g* at 15°C and extracted. Table 3 shows the ratio of free cholesterol specific activity/esterified cholesterol specific activity. The ratio of activities of the plasma extracted immediately is taken as the norm. Any values lower than this value indicate an increase in esterified cholesterol specific activity. As can be seen, there was appreciable esterification of high specific activity free cholesterol as a result of incubation or ultracentrifugation. The thiol-reacting substances apparently inhibited the reaction, and this inhibition was most notable when the 5,5′-dithio-bis(2-nitrobenzoic acid) was used. A similar study using sodium *p*-chloromercuriphenyl sulfonate (experiment 1) gave similar results. The specific activity of esterified cholesterol of a small portion of plasma ultracentrifuged without added inhibitor was only 8% greater than the same plasma (used in the experiment) ultracentrifuged with the inhibitor. Thus, it is apparent that at the later time periods the esterification reaction is less serious with regard to changes in specific activity of cholesteryl esters.

Specific Activities of Cholesteryl Esters in the Lipoprotein Classes

In Fig. 1 are specific activity data of free cholesterol and total cholesteryl esters in three lipoprotein classes. Maximal activities for free cholesterol were reached in 1–2 hr, and declined thereafter. It is to be noted that two distinct curves were obtained for free cholesterol. At all time periods up to 7.5 hr the free cholesterol in the d > 1.063 fraction had lower specific activity than that of the cholesterol in the low density fractions.

There were three distinct and widely separated curves for the cholesteryl esters. The most rapid incorporation of mevalonate occurred in the very low density fraction, which showed a maximum at 3–4 hr. There was a slower rate of incorporation into the cholesteryl esters of the low density and high density lipoproteins and their specific activities rose slightly from 2 to 7.5 hr.

TABLE 3 RATIOS OF CHOLESTEROL SPECIFIC ACTIVITIES: FREE/ESTERIFIED

Inhibitor	Direct Analysis	Plasma Incubated, 24 hr	Plasma Centrifuged, 72 hr
None	12.1*	8.0*	7.7
Iodoacetamide (0.01 M)†	—	10.7	11.9
N-Ethylmaleimide (0.01 M)	—	10.2	10.8
5,5′-Dithio-bis(2-nitrobenzoic acid)(0.007 M)	12.1	12.0	13.0

* The specific activity of free cholesterol was not influenced by ultracentrifugation and (or) addition of inhibitor. The mean specific activity of the 10 free cholesterol samples in the table was 5967 ± 46 dpm/mg.

† Final concentration in plasma.

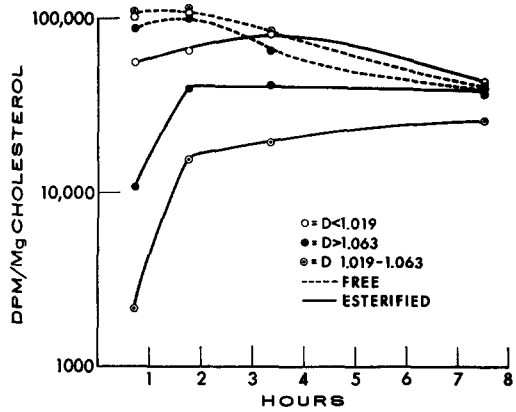

FIG. 1. Specific activities of free and esterified cholesterol of rat plasma lipoproteins.

The values in Fig. 1 represent specific activities of cholesterol normalized to a constant amount of radioactivity administered to each animal. In subsequent figures, the ratios esterified cholesterol activity/free cholesterol activity are plotted. These ratios are derived from the data and are therefore more directly comparable; the values are without regard to the isotope and amount of radioactivity administered.

Fig. 2 shows the incorporation of the mevalonate into the saturated cholesteryl esters. The most rapid incorporation occurred in the d < 1.019 fraction, and in general the pattern is similar to that for total esters seen in Fig. 1. The relative activities of the monoenoic esters are in Fig. 3. For these esters, there was an extremely rapid rate of incorporation into the d < 1.019 fraction, and, subsequent to 2 hr, the activity of the esters was greater than that of the free cholesterol. There were no differences between the d 1.019–1.063 and d > 1.063 lipoproteins. Curves for the dienoic esters are not shown since

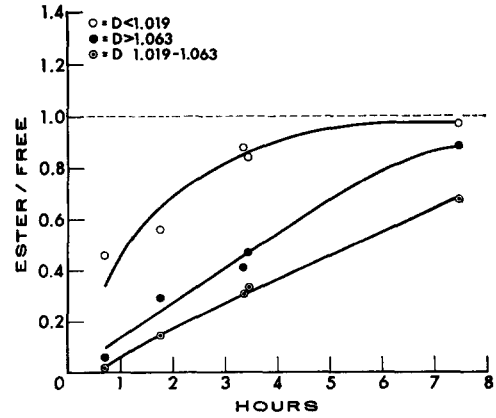

FIG. 2. Relative specific activities of saturated cholesteryl esters of rat plasma lipoproteins.

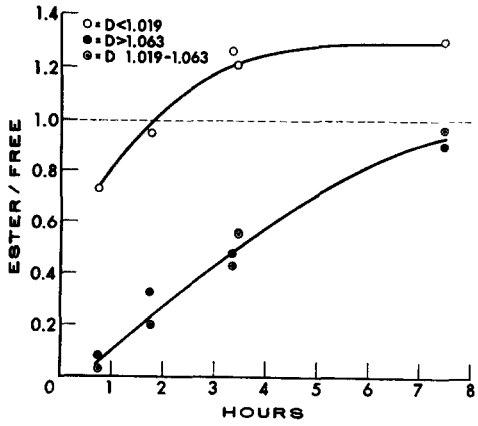

FIG. 3. Relative specific activities of monoenoic cholesteryl esters of rat plasma lipoprotein.

their pattern was virtually identical with that of the saturated esters. In the tetraenoic esters, the pattern of labeling was entirely different. For these esters (Fig. 4), the most rapid incorporation of mevalonate occurred in the d > 1.063 lipoproteins. The incorporation into the d 1.019–1.063 lipoproteins was the slowest.

Figs. 2–4 compare the rates of formation of the same ester class in three lipoprotein fractions. The observed differences are a function of the lipoprotein to which the cholesteryl esters are bound and therefore reflect differences in the metabolism of the lipoprotein per se. In Figs. 5 and 6 the rate of turnover of various esters in two lipoproteins is shown. Differences seen in these figures reflect differences in the rate of esterification and(or) combination of the esters with the lipoprotein.

Fig. 5 shows the rate of formation of four classes of cholesteryl esters in d < 1.019 lipoproteins. There are wide differences among the cholesteryl esters: very rapid formation for monoenoic esters and relatively slow formation of tetraenoic esters, with saturated and diunsaturated esters intermediate. Corresponding curves for d > 1.063 lipoprotein are shown in Fig. 6. There were only small

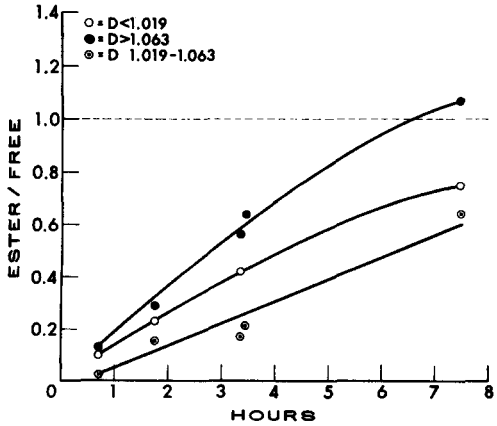

FIG. 4. Relative specific activities of tetraenoic cholesteryl esters of rat plasma lipoproteins.

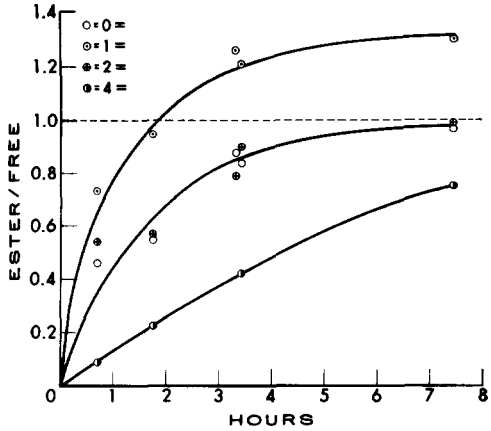

FIG. 5. Relative specific activities of cholesteryl esters of very low density lipoproteins (d < 1.019).

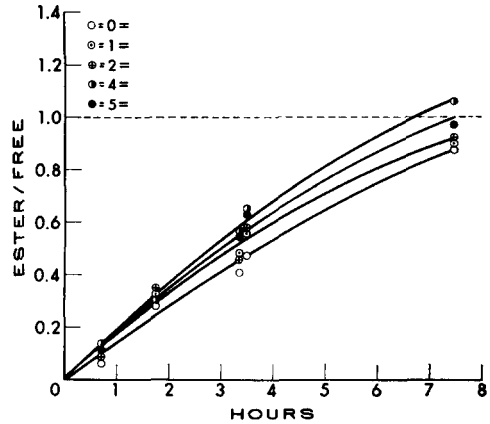

FIG. 6. Relative specific activities of cholesteryl esters of high density lipoproteins (d > 1.063).

differences between the esters, although tetraenoic and pentaenoic esters were synthesized at a slightly more rapid rate than the less saturated esters. The curves for the low density lipoproteins (d 1.019–1.063) had shapes similar to those of Fig. 6 except that the most rapid rate of incorporation of mevalonic acid was into the monoenoic esters, and the points for saturated, dienoic, and tetraenoic esters fell on the same curve.

Liver Cholesteryl Esters

Previous comparisons of cholesteryl ester composition of lipoproteins with those of liver have been made with whole liver (1). Table 4 shows the cholesteryl ester composition of various subcellular fractions of liver. The cholesteryl esters of the mitochondria have nearly the same composition as the microsomes, but the esters in the soluble fraction have considerably more arachidonate and somewhat less oleate. The esters in the fat layer contain a very low proportion of linoleate and only traces of arachidonate. In Table 5 are shown the specific activities of free and esterified cholesterol of two lipoprotein fractions and four subcellular fractions of liver. Data are for

TABLE 4 FATTY ACID COMPOSITION OF CHOLESTERYL ESTERS OF LIVER AND LIVER FRACTIONS

Fatty Acid	Liver	Microsomes	Mitochondria	Soluble Fraction	Fat Layer
		% of total fatty acids			
16:0	15.0(17.5)*	15.5	20.3	14.6	29.8
16:1	7.2(6.4)	7.1	8.5	6.4	9.7
18:0	2.2(2.7)	3.4	3.9	2.2	3.6
18:1	37.0(39.0)	40.8	40.0	26.6	47.0
18:2	24.9(25.0)	24.3	23.5	25.8	9.4
20:4	13.6(6.7)	8.9	3.9	24.1	tr.

Fatty acids are designated by number of carbon atoms: number of double bonds.

* The data in parentheses are from a previous study (1).

Incorporation of Mevalonic Acid into Adrenal Cholesteryl Esters

The adrenal gland is unique among tissues in that nearly all the cholesterol found in it is esterified. It has a high content of polyunsaturated cholesteryl esters (17). Therefore, it was of interest to determine whether the labeling of cholesteryl esters of the adrenal was heterogeneous as was the labeling of liver and lipoprotein esters. Fig. 7 is a block diagram showing the labeling pattern of the adrenal esters. For comparison, the normalized specific activities of free cholesterol at 0.7, 1.8, 3.4, and 7.5 hr were 21,800, 39,100, 31,200, and 22,300 dpm/mg, respectively. The corresponding specific activities of plasma free cholesterol were 95,900, 104,300, 77,200, and 39,000 dpm/mg, respectively (Fig. 1). As can be seen in the diagram, there were increases in relative specific activities over the 7.5 hr period although the activities at any time period were lower than those of corresponding esters in plasma lipoproteins. There were, however, differences in activities of different esters. In general the order of activities was monoene = diene > tetraene > triene = pentaene > saturated.

TABLE 5 SPECIFIC ACTIVITIES OF FREE AND ESTERIFIED CHOLESTEROL OF LIVER SUBCELLULAR FRACTIONS AND LIPOPROTEINS

	Lipoproteins					
	d < 1.019	d > 1.063	Microsomes	Mitochondria	Soluble Fraction	Free Fat
			dpm/mg cholesterol			
1.77 Hr						
Free cholesterol	108,800	101,900	114,400	103,800	104,500	85,000
Esterified "	66,300	39,200	95,200	63,700	40,500	39,200
0=	58,300	30,000	75,200	—	36,300	35,200
1=	98,700	34,600	100,900	72,700	64,200	50,600
2=	58,900	36,600	43,300	—	36,300	34,300
4=	24,300	30,000	37,800	—	22,700	13,600
7.52 Hr						
Free cholesterol	41,000	40,700	44,500	40,100	41,000	40,500
Esterified "	44,200	38,000	47,700	36,800	37,100	40,000
0=	39,300	35,600	39,600	—	37,100	36,100
1=	52,800	36,200	46,200	37,200	47,900	46,500
2=	40,200	36,700	26,600	—	35,700	38,900
4=	30,400	43,300	41,900	—	31,800	21,400

The ^{14}C specific activities have been adjusted by multiplying the experimentally determined values by 5.14 (62.7/12.2, see Table 1) so that they can be compared directly to ^{3}H specific activities.

two time periods—1.77 hr (^{14}C) and 7.52 hr (^{3}H) after administration of the mevalonic acid labeled with either ^{14}C or ^{3}H. At the earlier time there were slight differences in the specific activity of free cholesterol, with the highest activity in the microsomal fraction. The specific activities of free cholesterol in the lipoproteins were similar to those in all subcellular fractions except the free fat.

On the other hand, the specific activities of the cholesteryl esters in the various subcellular fractions and the lipoproteins varied greatly at 1.77 hr. The cholesteryl esters of the microsomes had the highest activities, with monoenes > saturated > dienes > tetraenes. In the other liver fractions and in the d < 1.019 lipoproteins the specific activity pattern was not very different: monoene > saturated = diene > tetraene. However, in the cholesteryl esters of the d > 1.063 lipoproteins the specific activities were all quite similar to one another. At 7.52 hr the specific activities of the cholesteryl esters more closely resembled that of free cholesterol and the difference between the various esters was less marked.

DISCUSSION

These studies have demonstrated that after the administration of labeled mevalonic acid to rats there are marked differences in the relative rates of appearance of radioactivity in the various cholesteryl esters of plasma and also within the various cholesteryl esters in individual lipoprotein fractions. These differences undoubtedly reflect differences in rates of turnover of the various cho-

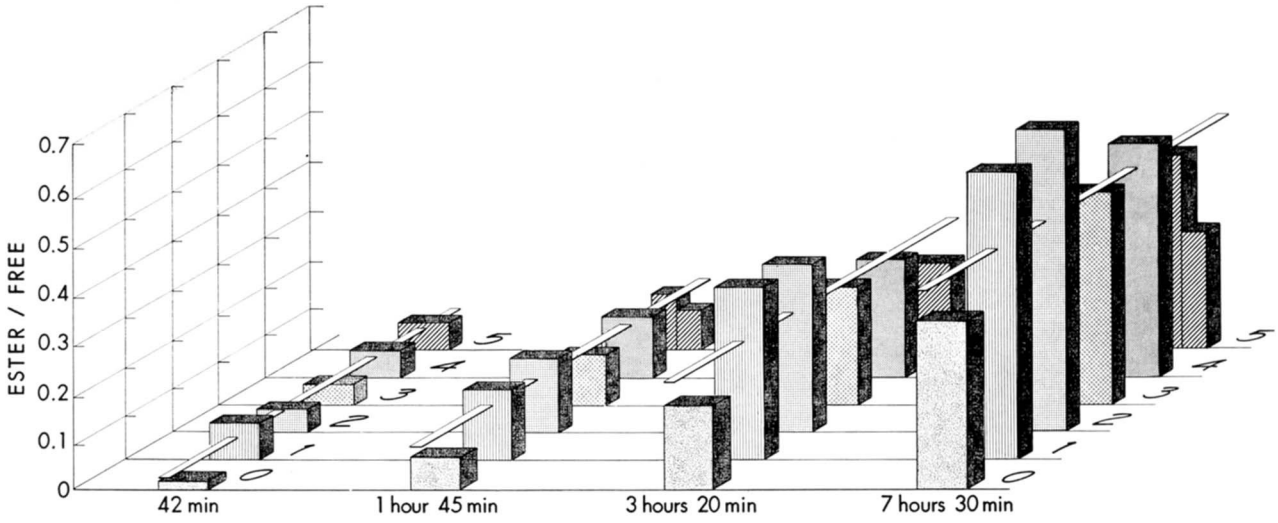

FIG. 7. Relative specific activities of adrenal cholesteryl esters. The numbers at the right (0–5) indicate the number of double bonds in the fatty acid moiety. The horizontal rod through each set of bars indicates the relative specific activity of the total cholesteryl esters.

lesteryl esters in the tissues or in each lipoprotein class since under the conditions of the study a steady state probably existed (4).

As with protein (20) the rate of turnover of the total cholesteryl esters in the $d < 1.019$ lipoproteins was the most rapid of any of the lipoproteins. In the individual classes of esters, the esters of the saturated, monoenoic, and dienoic acids turned over most rapidly in the $d < 1.019$ lipoproteins. This was not true for the tetraenoic cholesteryl esters, which turned over most rapidly in the $d > 1.063$ lipoprotein fraction. However, their turnover rate was considerably slower than for the other esters in the $d < 1.019$ fraction.

These differences in the rates of turnover of the cholesteryl esters of the various lipoproteins as well as the difference in the rate of turnover of cholesteryl esters within a given lipoprotein fraction could account for the differences in composition of the cholesteryl esters of the rat lipoproteins (1).

The data on the composition of cholesteryl ester of the subcellular fractions of the liver (Table 4) help to clarify certain discrepancies with regard to the similarity of composition of the cholesteryl esters of $d < 1.019$ lipoproteins and liver. In previous studies we had advanced the hypothesis that the cholesteryl esters of the $d < 1.019$ lipoproteins were derived by nonselective transfer from the liver on the basis of the similarity of the cholesteryl ester composition of the $d < 1.019$ lipoproteins and that of the liver (1). However, in studies with rats fed high fat diets there were discrepancies between the liver and $d < 1.019$ lipoproteins. The present studies show that there are considerable differences in the cholesteryl ester compositions of various subcellular fractions of the liver, with that of the fat layer differing markedly from that of the microsomes. Since the data of Table 4 suggest that cholesteryl esters of the $d < 1.019$ lipoprotein are derived from the microsomes, comparison must be made between the microsomes (rather than the whole liver) and the lipoproteins. When the fat layer is markedly increased in concentration, as it is in livers of rats fed a high fat diet, similarities between whole liver and lipoproteins might not be obtained even though the microsomes would have a composition identical with that of the lipoproteins. On the basis of the analyses it is evident that similarity of composition of cholesteryl esters of the whole liver and of the microsomes may be purely fortuitous.

That the cholesteryl esters of the $d < 1.019$ lipoproteins are derived directly and preferentially from the microsomes is further suggested by the data in Table 5. At the early time period the specific activities of the various cholesteryl esters are higher in the microsomes than in the other liver fractions. The monoenoic esters of the microsomes have a specific activity closely resembling that of free cholesterol. The monoenoic esters of the $d < 1.019$ lipoproteins have a specific activity almost identical with that of the microsomes, while the specific activities of the saturated and tetraenoic esters are lower than that of the microsomes but higher than that of the other liver fractions measured. These observations are consistent with the hypothesis that these esters are derived from cholesteryl esters newly synthesized in the microsomes and this hypothesis is consistent with the finding that cholesterol is synthesized (21) and esterified (3) in the microsomes. The differences in relative specific activities of the cholesteryl esters of the $d < 1.019$ lipoproteins and the microsomes are consistent with the findings of

Goodman, Deykin, and Shiratori (3), who showed that the relative rates of synthesis of esters from acyl CoA and free cholesterol were monoenoic > saturated > diene.

The slower rate of turnover of the cholesteryl esters of the d < 1.063 lipoproteins parallels the slower turnover of its protein portion (20). However, the apparently preferential incorporation of cholesteryl esters of polyunsaturated acids into these lipoproteins as well as the relatively greater rate of turnover of tetraenoic esters (compared with other d > 1.063 cholesteryl esters) suggests that the mechanism of formation of these esters may be different from that of the other esters of the lipoproteins. The specific activity data (microsomes, Table 5) suggest that cholesteryl arachidonate is synthesized in the liver, but the data do not permit any definitive statements regarding to what extent, if at all, this ester is transferred from the liver to the high density lipoproteins. If the liver were the major source of cholesteryl arachidonate there would have to be a rapid and selective transfer to the d > 1.063 lipoproteins, similar to the transfer of cholesteryl oleate into the d < 1.019 lipoproteins. This would be necessitated by the fact that the amount of cholesteryl arachidonate in liver is so low. An alternative site of origin of cholesteryl arachidonate is in the plasma. A number of investigators have demonstrated esterification of free cholesterol in plasma incubated at 37°C (22–25). Moreover, Glomset (9) has presented evidence that this esterification is mediated by an acyltransferase that catalyzes transfer of a fatty acid from the β-position of lecithin to free cholesterol. While there is no doubt about the existence of this type of reaction, there have been questions raised with regard to its importance in influencing the composition and concentration of plasma cholesteryl esters, notably cholesteryl arachidonate. Goodman and Shiratori in their study of turnover of different cholesteryl esters in liver and plasma (4) point out that if a transesterification did take place, one might expect to find that the specific activity of plasma cholesteryl arachidonate would exceed that of liver cholesteryl arachidonate. Goodman and Shiratori did not observe this, but they were examining total plasma arachidonate and total liver arachidonate. In Table 5, at both time periods, the specific activity of cholesteryl arachidonate in the d > 1.063 lipoprotein is greater than that of any of the liver fractions, except that of liver microsomes at 1.77 hr. However, since active esterification of cholesterol occurs in this liver fraction it is not surprising to find a relatively high activity of the cholesteryl arachidonate. Thus the data of Table 5 are not inconsistent with an extrahepatic synthesis of the cholesteryl arachidonate of the d > 1.063 lipoproteins. The relatively high specific activity of the dienoic esters of the d < 1.019 lipoproteins at both 1.77 and 7.52 hr may also be a reflection of extrahepatic synthesis.

Other evidence which strongly suggests that the plasma esterification reaction may be important is derived from the studies in which inhibitors of acyltransferase were added to the plasma. The data of Table 2 indicate that during the centrifugation of plasma, esterification of free cholesterol occurred and that cholesteryl arachidonate was formed at a greater rate than were the other esters. Furthermore, the data in Table 3 suggest that the esterification is an enzymatic process which can be partially or completely blocked by a variety of agents. If the esterification occurs during ultracentrifugation it may be inferred that it is also taking place in blood in vivo and that cholesteryl arachidonate as well as other cholesteryl esters is being formed. Sugano and Portman (5) also demonstrated that after intravenous administration of mevalonate-2-^{14}C or cholesterol-4-^{14}C to rats there were disproportionately high concentrations of radioactivity in plasma cholesteryl arachidonate during the first 6 hr. They attributed this to plasma esterification activity, and concluded that this reaction is of considerable importance in maintenance of the levels of plasma cholesteryl esters.

The present results on the turnover of the plasma lipoproteins differ significantly from results of similar studies in human subjects. In work with patients, Goodman (7) found that while there was heterogeneity of turnover of cholesteryl esters in different lipoproteins, within a given lipoprotein all the esters had the same fractional rate of turnover. Thus, in man the turnover of cholesteryl esters is governed primarily by the lipoprotein class in which the esters are found. On the other hand, in rats there is a greater degree of heterogeneity of cholesteryl ester turnover, which is determined not only by the particular lipoprotein class but also by the fatty acid moiety of the ester.

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