In vivo turnover of different cholesterol esters in rat liver and plasma

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SUMMARY Turnover rates of different cholesterol esters in rat liver and plasma were determined after injecting fasting rats intravenously with mevalonate-2-C^{14}. Liver and plasma samples were collected at time intervals of 21 min to 24 hr. Gas–liquid chromatography and thin-layer chromatography on silica gel impregnated with silver nitrate were used to determine specific radioactivities of saturated, mono-, di-, and tetra-unsaturated cholesterol esters.

The liver samples showed considerable variability from animal to animal, whereas the plasma showed very little individual variability. In liver cholesterol esters, the rate of appearance of total radioactivity in the monounsaturated esters was greater than that in any of the other esters, and the fractional turnover rate of the mono- and diunsaturated esters was greater than that of the saturated esters and arachidonate. These findings are consistent with the results of previous in vitro studies on the hydrolysis and formation of cholesterol esters in liver. All the plasma samples showed a much more rapid fractional turnover of the monounsaturated, compared with the other, esters. The plasma findings can be explained by a more rapid turnover of the very low density lipoprotein cholesterol esters, which contain relatively more cholesteryl oleate, although other explanations are also possible.

RECENT STUDIES of the enzymatic hydrolysis (1) and synthesis (2) of cholesterol esters in rat liver have demonstrated considerable differences in the rates of hydrolysis and synthesis of different cholesterol esters. Unsaturated cholesterol esters were hydrolyzed more rapidly than saturated ones (1), and cholesteryl oleate was synthesized more rapidly than any of the other esters (2).

The experiments reported here were undertaken in an attempt to relate these in vitro findings to the intact animal, by studying the in vivo turnover of different cholesterol esters in rat liver. Previous studies by Klein and Martin (3) have demonstrated that the turnover rates of liver cholesterol esters are heterogeneous. The present experiments were designed to study the details of this heterogeneous turnover in individual animals. In addition, the turnover rates of different cholesterol esters in the plasma were compared with those in the liver in the same animals. A preliminary report of part of this work has been published (4).

METHODS

Mevalonic acid-C^{14} was purchased from the New England Nuclear Corporation (Boston, Mass.) as its lactone, and was converted to the sodium salt by warming at 37° for 1.5 hr in dilute $NaHCO_3$ solution (pH about 8.5). Twelve male Sprague-Dawley rats weighing approximately 175 g each and fasted overnight were each injected intravenously with a solution containing 16 μc/ml of DL-mevalonic acid-2-C^{14} in isotonic saline. Rats 1–4 were injected with 1.6 ml; rats 5–7 with 1.3 ml; and rats 8–12 with 1.1 ml. At specified time intervals, from 21 min to 24 hr, the animals were anesthetized with ether, and as much blood as possible (6–7 ml) was withdrawn from the abdominal aorta into syringes moistened with a solution of heparin. The livers were immediately excised, weighed, homogenized in a Potter-Elvehjem homogenizer with 10 ml of isotonic saline solution, and then extracted with 25 volumes of $CHCl_3$–CH_3OH, 2:1 (v/v). Plasma was obtained after centrifuging the blood samples at 2000 × g for 30 min at 4°, and was similarly extracted with $CHCl_3$–CH_3OH. The extraction mixtures were split into two phases by the addition of 5 volumes of 0.05% H_2SO_4, and the $CHCl_3$ phases collected and evaporated to dryness under a stream of nitrogen.

Portions (75 mg or less) of the total lipid so obtained were chromatographed on 5-g columns of silicic acid (Unisil, 100–200 mesh, Clarkson Chemical Co., Williamsport, Pa.), using a modification of the method of Horning, Williams, and Horning (5). Serial elutions were carried out with 40 ml of 10% benzene in hexane (v/v), 80 ml of 21% benzene in hexane, 70 ml of 72% benzene in hexane, and 25 ml of benzene plus 75 ml of $CHCl_3$,

to yield respectively, hydrocarbons, cholesterol esters, triglycerides, and free cholesterol [plus free fatty acids (FFA) and partial glycerides]. Frequent checks were made, using H^3-cholesterol esters and C^{14}-triglycerides (6) on similar columns, to ensure that cholesterol esters, uncontaminated by triglycerides, were being quantitatively recovered in the second fraction.

Portions of each cholesterol ester and free cholesterol fraction were subjected to the Sperry-Webb cholesterol determination (7); other portions of these fractions were dissolved in a solution of 0.5% diphenyloxazole in toluene and assayed for C^{14} by means of a Packard liquid scintillation counter. The specific radioactivity of the free cholesterol, and the average specific radioactivity of the esterified cholesterol, were calculated, as well as the concentrations of free and esterified cholesterol in each plasma and liver sample.

Gas–Liquid Chromatography (GLC)

Fatty acid methyl esters were prepared from each cholesterol ester sample by heating portions of less than 1 mg with 2 ml of absolute methanol containing 2% H_2SO_4 and 0.2% benzene under nitrogen at 60° overnight, using a screw-cap vial with a Teflon-lined cap. An equal volume of water was added and the methyl esters were extracted three times with hexane. Approximately 1 μl of a 0.2% solution of methyl esters was chromatographed at 170° on a helical glass column (8 ft × 4 mm i.d.) containing 12% diethylene glycol succinate polyester on Chromosorb W, using a Barber-Colman model 25 GLC apparatus (argon ionization detector, radium source). Methyl stearate was eluted from the column in 9.5 min. Each analysis was carried out for a period slightly greater than the retention time of methyl arachidonate; fatty acid compositions were determined by triangulation. Several analyses were also carried out for 3–4 times as long as usual; no significant peaks were observed after that of methyl arachidonate. The apparatus was calibrated with NIH methyl ester standards A to F, which were kindly provided by the Metabolism Study Section of the National Institutes of Health.

The fatty ester methods were tested by the use in several instances of a known amount of heptadecanoic acid as an internal standard in the methylation of plasma cholesterol esters. The total fatty acid mass determined by GLC (8) was compared with that calculated from the cholesterol mass analyses, using an average cholesterol ester fatty acid molecular weight (for rat plasma) of 290. The ratio of the results obtained with the two methods (GLC: cholesterol analysis and calculation) was 1.09 (range 1.07–1.11). This agreement is quite satisfactory although a small systematic error in one of the methods was apparently present. These results also finally excluded the possibility that significant amounts of cholesterol ester fatty acids with GLC retention time greater than that of methyl arachidonate had gone undetected.

Thin-Layer Chromatography (TLC)

The distribution of radioactivity among the different cholesterol esters in each sample was determined by TLC on Silica Gel G impregnated with $AgNO_3$, using benzene–hexane 1:1 (v/v) as ascending solvent. This method separates cholesterol esters differing in the number of double bonds in the fatty acid moiety, and is similar to the method employed by Morris, who used a different solvent system (9). Best results were obtained with thin layers prepared from a slurry of 25 g of silica gel plus 55 ml of 0.7% $AgNO_3$ in water. The developed plates were sprayed with Rhodamine 6G in methanol and examined under ultraviolet light.

Chromatography of mixtures of pure cholesteryl palmitate, stearate, oleate, linoleate, and arachidonate showed four widely separated bands of cholesteryl palmitate + stearate ($R_F \sim 0.8$), cholesteryl oleate ($R_F \sim 0.65$), linoleate ($R_F \sim 0.4$), and arachidonate ($R_F \sim 0.1$). TLC of portions of the liver and plasma cholesterol ester samples separated each sample into four corresponding zones. The identities of the zones were verified by TLC of unlabeled cholesterol esters from human and rat plasma; lipids from each zone were eluted with $CHCl_3$, methanolyzed, and analyzed by GLC. All the long-chain saturated esters were recovered from the first (saturated) zone, and all the monounsaturated esters (16:1, 18:1, and 20:1) from the second (Δ1) zone; linoleic acid was found in the third (Δ2) zone, and arachidonic acid in the fourth (Δ4) zone. Similar findings have been reported by Morris (9). In order to determine the distribution of radioactivity, each zone was separately scraped on to a filter funnel, eluted with 15 ml of $CHCl_3$, and assayed for C^{14}. Further elution with hot benzene–diethyl ether, 1:1, was necessary in order to completely recover cholesteryl arachidonate from the Δ4 zone; the other, more saturated esters were satisfactorily eluted with $CHCl_3$ alone.

The TLC method just described was extensively tested to determine the validity of the C^{14} distributions so obtained. The recovery of samples of pure cholesteryl-H^3 palmitate, oleate, and linoleate, after TLC singly or in mixtures and elution of each TLC zone with 15 ml of $CHCl_3$, was consistently 85–90%. In contrast, the recovery of cholesteryl-H^3 arachidonate, when eluted with 15 ml $CHCl_3$ alone, was only 50–60%, but could be increased to more than 90% by further elution with 40 ml of hot benzene–ethyl ether, 1:1. The analyses of known mixtures of the four labeled cholesterol esters shown in Table 1 demonstrate the close agreement between the expected and observed distributions of radioactivity. The total recovery of C^{14} was approximately 90% in both

TABLE 1 THIN-LAYER CHROMATOGRAPHY OF STANDARD MIXTURES OF CHOLESTEROL-H³ ESTERS

TLC zone	Solution A H³ % Distribution		Solution B H³ % Distribution	
	Expected	Observed	Expected	Observed
Saturated	19.9	19.0	29.9	29.2
Δ1	27.6	27.6	39.0	39.2
Δ2	26.8	26.1	24.5	24.2
Δ4	25.8	27.3	6.3	7.4

Solutions A and B represent two standard mixtures of pure labeled cholesterol esters.

analyses shown. The observed values for the saturated, mono-, and diunsaturated esters were within 5% of the expected values for each zone. Slightly more radioactivity than expected was consistently found in the arachidonate (Δ4) zone; this was probably due to a small amount of trailing from the other esters. The error in the Δ4 zone values was usually in the range of 5–10%; greater error (up to 25% of observed value) was observed in this zone when cholesteryl arachidonate represented less than 10% of the total radioactivity. This error was partly corrected, in the experimental analyses, by reducing the observed liver Δ4 values by 10%, since these Δ4 zones all contained less than 13% of the total radioactivity.

Cholesteryl-H³ palmitate, oleate, and linoleate were prepared as described by Deykin and Goodman (1). Cholesteryl-H³ arachidonate was prepared by the ester interchange method as described by Mahadevan and Lundberg (10), using cholesteryl-H³ acetate as prepared by Deykin and Goodman (1) and methyl arachidonate purchased from Applied Science Inc. (State College, Pa.).

In the early stages of these studies, some TLC analyses were conducted before it was discovered that cholesteryl arachidonate was incompletely recovered when eluted with 15 ml of $CHCl_3$ alone. The results so obtained were subsequently corrected for the expected lower recovery of cholesteryl arachidonate. After applying this correction, the total recovery of radioactivity, after TLC, was 85–90% in every case. Corrections were applied to the liver and plasma samples of rats 1, 2, 3, 6, 8, and 10. Because of the necessity for these corrections, the Δ4 values for these rats are less reliable than those of the others, and the data derived from these values should be considered only semiquantitative. The application of corrections to these Δ4 values does not affect the reliability of the data relating the saturated, mono-, and diunsaturated esters to each other. Only the corrected values have been tabulated in the results that follow.

TABLE 2 THE CONCENTRATION OF CHOLESTEROL IN RAT LIVER AND PLASMA

	Liver	Plasma
	mg/100 g	*mg/100 ml*
Free cholesterol	149 ± 9.0*	15.3 ± 0.9*
Esterified cholesterol	42.7 ± 6.7	32.1 ± 2.2
Total cholesterol	192 ± 13.5	47.4 ± 3.0
Ester %	21.6 ± 2.3	67.6 ± 0.6

* Mean ± standard error. The results for liver were determined from the 12 liver samples. The results for plasma were determined from 11 samples (plasma sample 3 was lost before it was extracted).

RESULTS

The average values for the concentration of free and esterified cholesterol in the whole series of plasma and liver samples are shown in Table 2. The concentrations were similar in all the rats. The percentage of cholesterol present as esterified cholesterol was fairly constant in the 11 plasma samples (range 64–70%); greater variation was seen in the ester percentages for the 12 liver samples (range 11–32%).

The values of the specific radioactivity of free and esterified cholesterol in each liver and plasma sample are

TABLE 3 CHOLESTEROL SPECIFIC RADIOACTIVITY IN LIVER AND PLASMA AFTER INTRAVENOUS MEVALONATE-2-C¹⁴

Rat No.	Time	Cholesterol Specific Radioactivity		Specific Radioactivity Relative to Plasma Free Cholesterol		Specific Radioactivity Relative to Liver Free Cholesterol	
		Free	Ester	Free	Ester	Free	Ester
	hr	*cpm/mg*					
Liver							
1	0.35	88,700	16,230	3.70	0.677	1.00	0.183
2	0.67	152,110	74,500	1.60	0.782	1.00	0.490
3	1.00	96,000	39,429	...*	...	1.00	0.411
4	1.43	100,625	36,182	1.24	0.447	1.00	0.360
5	2.10	69,615	34,200	1.21	0.594	1.00	0.491
6	2.62	62,245	46,882	1.06	0.800	1.00	0.753
7	3.17	79,565	43,400	1.34	0.730	1.00	0.545
8	4.00	46,576	30,083	1.16	0.750	1.00	0.646
9	6.08	35,607	24,417	1.13	0.771	1.00	0.686
10	7.58	37,091	32,815	1.17	1.03	1.00	0.885
11	17.25	32,033	26,143	1.23	1.01	1.00	0.816
12	24.0	29,740	29,239	1.14	1.12	1.00	0.983
Plasma							
1	0.35	23,980	3,026	1.00	0.126	0.270	0.034
2	0.67	95,315	23,385	1.00	0.245	0.627	0.154
4	1.43	80,960	32,200	1.00	0.398	0.805	0.320
5	2.10	57,565	34,458	1.00	0.599	0.827	0.495
6	2.62	58,583	34,040	1.00	0.581	0.941	0.547
7	3.17	59,459	45,944	1.00	0.773	0.747	0.577
8	4.00	40,107	33,833	1.00	0.844	0.861	0.726
9	6.08	31,650	27,409	1.00	0.866	0.889	0.770
10	7.58	31,712	34,913	1.00	1.10	0.855	0.941
11	17.25	25,946	27,192	1.00	1.05	0.810	0.849
12	24.0	26,184	27,028	1.00	1.03	0.880	0.909

* Plasma sample 3 lost.

TABLE 4 CHOLESTEROL ESTER FATTY ACID COMPOSITION OF RAT LIVER AND PLASMA

Fatty Acid*	Relative Retention Time‡	% of Total Fatty Acids			
		Liver		Plasma	
		Mean§	Observed Range	Mean§	Observed Range
14:0	0.31	0.2	0.1–0.4	0.2	0.0–0.4
unid.	0.42	0.2	0.1–0.4	0.3	0.1–0.5
unid.	0.48	0.1	0.0–0.1	0.4	0.2–1.0
16:0	0.56	16.1 ± 1.5	10.1–25.1	9.0 ± 0.25	7.8–10.0
16:1	0.68	7.7 ± 1.3	3.1–18.3	5.1 ± 1.0	1.7–11.4
unid.	0.90	0.9 ± 0.14	0.3–1.9	0.7 ± 0.15	0.0–1.4
18:0	1.00	4.5 ± 0.4	2.4–7.3	0.9 ± 0.11	0.4–1.6
18:1	1.18	40.8 ± 2.6	26.3–52.7	14.7 ± 1.5	9.8–23.7
unid.	1.34	0.2	0.0–0.7	0.1	0.0–0.1
18:2	1.53	19.2 ± 1.1	12.3–25.6	21.7 ± 1.0	17.7–27.5
20:0	1.87	0.1	0.0–0.3	0.8 ± 0.15	0.0–1.7
20:1	2.11	0.3	0.0–0.9	0.3	0.0–1.3
20:4	3.77	9.6 ± 1.0	5.5–15.7	45.0 ± 2.5	34.4–58.9

* Number of carbon atoms: number of double bonds. The abbreviation unid. means unidentified. Several minor peaks (less than 0.2% of total in both liver and plasma) have been omitted from this tabulation.

‡ Relative to methyl stearate (18:0) taken as 1.00.

§ Mean ± standard error (n = 12 for liver and 11 for plasma). Standard errors have not been calculated for fatty acids comprising less than 0.5% of the total.

listed in Table 3. Since the rats were injected with different amounts of mevalonate-C^{14} (see Methods) the specific radioactivity values have also been expressed in relative terms, namely relative to the specific radioactivity of the corresponding plasma and liver free cholesterol for each rat.

Radioactivity rapidly appeared in the liver free cholesterol, with the highest specific radioactivity observed in sample 2, 40 min after injection. Previous studies (11) have shown that the biosynthesis of cholesterol in rat liver from intravenously injected mevalonate is largely completed in 30 min. Radioactivity also appeared fairly rapidly in the liver esterified cholesterol. Full equilibration of the free and esterified cholesterol pools of liver was seen only with the longest time intervals studied. The extent of equilibration between the liver free and esterified cholesterol did not increase steadily with increasing time, but fluctuated a good deal from sample to sample. This fluctuation probably reflects differing degrees of inhomogeneity of liver cholesterol esters from animal to animal.

In contrast, the plasma samples showed a steady increase in the extent of equilibration between free and esterified cholesterol with increasing time, reaching full equilibration between 6 and 10 hr. The peak specific radioactivity for plasma free cholesterol occurred at approximately 40–60 min, and for cholesterol ester at between 3 and 5 hr.

Table 3 also demonstrates the rapidity of equilibration of the liver and plasma free cholesterol. Nearly full equilibration between these two fractions was achieved in approximately 2 hr. The liver free cholesterol specific radioactivity, however, remained consistently slightly greater than that of the plasma free cholesterol.

Table 4 summarizes the fatty acid composition of the cholesterol ester fractions from the 12 liver and 11 plasma samples. Similar GLC data have been published previously (12) and by others (13–15), but without statistical data on the variation of composition among individual rats. Linoleic acid (18:2) was the only identified diunsaturated cholesterol ester fatty acid in both liver and plasma, and arachidonic acid (20:4) the only Δ4 acid.

In order to compare the cholesterol ester mass distribution, as determined by GLC, with the C^{14} distribution as determined by TLC, it was first necessary to correct the GLC distributions for the different molecular weights of the different fatty acid methyl esters. This was done for each identified fatty acid listed in Table 4, and the distribution of cholesterol esters was then tabulated in molar terms for each sample. The saturated and monounsaturated ester values were then summed for each sample, and the distribution of esterified cholesterol mass expressed in terms of saturated, Δ1, Δ2, and Δ4 fractions.

Table 5 lists the distribution of cholesterol mass among the four ester fractions, together with the distribution of cholesterol-C^{14} as determined by TLC, for each liver and plasma cholesterol ester sample. The specific radioactivity of each ester fraction in each sample was then determined by multiplying the average specific radioactivity of the whole ester fraction (Table 3) by the ratio of per cent of total C^{14} to per cent of total mass, for each ester fraction. The specific radioactivity value of

TABLE 5 THE DISTRIBUTION OF CHOLESTEROL MASS AND RADIOACTIVITY AMONG THE FOUR CHOLESTEROL ESTER FRACTIONS IN LIVER AND PLASMA

Rat No.	Cholesterol Ester Fraction	Liver			Plasma			S.A. Ratio Plasma/Liver
		% Distribution of Cholesterol		Relative S.A.* (liver free = 1.0)	% Distribution of Cholesterol		Relative S.A.* (plasma free = 1.0)	
		Mass	C^{14}		Mass	C^{14}		
1	Sat'd	18.0	8.3	0.09	12.4	Lost	...	...
	Δ1	54.0	57.5	0.19	22.8	Lost	...	...
	Δ2	21.6	28.7	0.24	27.8	Lost	...	...
	Δ4	6.4	5.5	0.16	37.0	Lost	...	...
2	Sat'd	27.0	14.9	0.27	9.3	8.6	0.23	0.5
	Δ1	38.9	51.5	0.65	15.4	30.5	0.49	0.5
	Δ2	19.0	24.1	0.62	23.9	25.1	0.26	0.3
	Δ4	15.2	9.5	0.31	51.4	35.9	0.17	0.4
3	Sat'd	31.4	19.7	0.26				...
	Δ1	43.8	55.3	0.52		Sample lost		...
	Δ2	17.6	18.9	0.44				...
	Δ4	7.2	6.1	0.35				...
4	Sat'd	15.4	13.3	0.31	11.1	11.9	0.23	1.1
	Δ1	51.0	55.0	0.38	15.3	27.6	0.72	1.5
	Δ2	19.3	21.2	0.39	18.4	20.9	0.45	0.9
	Δ4	14.3	10.5	0.29	55.1	39.6	0.29	0.8
5	Sat'd	19.8	14.5	0.36	12.4	11.3	0.55	1.2
	Δ1	47.4	55.2	0.57	19.6	25.5	0.78	1.1
	Δ2	25.0	23.8	0.47	27.7	23.5	0.51	0.9
	Δ4	7.8	6.5	0.41	40.4	39.7	0.59	1.2
6	Sat'd	30.2	19.3	0.48	11.6	10.6	0.53	1.0
	Δ1	48.1	59.3	0.93	23.6	33.2	0.82	0.8
	Δ2	12.2	12.6	0.78	19.8	15.9	0.47	0.6
	Δ4	9.4	8.8	0.71	44.9	40.3	0.52	0.7
7	Sat'd	22.0	14.4	0.36	11.6	11.2	0.75	1.5
	Δ1	47.8	57.4	0.65	18.4	21.3	0.90	1.0
	Δ2	20.4	18.9	0.51	29.2	24.5	0.65	0.9
	Δ4	9.8	9.3	0.52	40.8	43.0	0.81	1.2
8	Sat'd	32.0	21.0	0.43	12.5	12.0	0.81	1.7
	Δ1	37.4	48.3	0.83	21.6	26.1	1.02	1.1
	Δ2	19.1	19.2	0.65	25.1	22.1	0.74	1.0
	Δ4	11.5	11.5	0.71	40.8	39.8	0.83	1.0
9	Sat'd	16.9	12.0	0.49	12.2	12.7	0.90	1.6
	Δ1	57.9	62.3	0.74	31.8	35.3	0.96	1.1
	Δ2	18.1	18.6	0.70	20.3	18.5	0.79	1.0
	Δ4	7.0	7.1	0.69	35.7	33.5	0.81	1.0
10	Sat'd	13.1	12.6	0.85	10.1	11.5	1.25	1.3
	Δ1	63.6	63.2	0.88	34.1	38.3	1.27	1.2
	Δ2	17.7	17.7	0.91	22.6	19.8	0.96	0.9
	Δ4	5.6	6.0	0.95	33.2	30.4	1.00	0.9
11	Sat'd	22.6	17.9	0.65	12.6	13.4	1.12	1.4
	Δ1	49.6	53.4	0.88	21.3	22.6	1.11	1.0
	Δ2	19.4	21.6	0.91	20.9	19.8	1.00	0.9
	Δ4	8.4	7.2	0.70	45.2	44.2	1.03	1.2
12	Sat'd	14.8	16.2	1.07	10.0	10.5	1.08	0.9
	Δ1	52.3	48.6	0.92	13.1	15.4	1.21	1.2
	Δ2	25.8	27.6	1.05	19.2	18.6	1.00	0.8
	Δ4	7.1	7.6	1.05	57.6	55.4	0.99	0.8

* S.A. = Specific radioactivity.

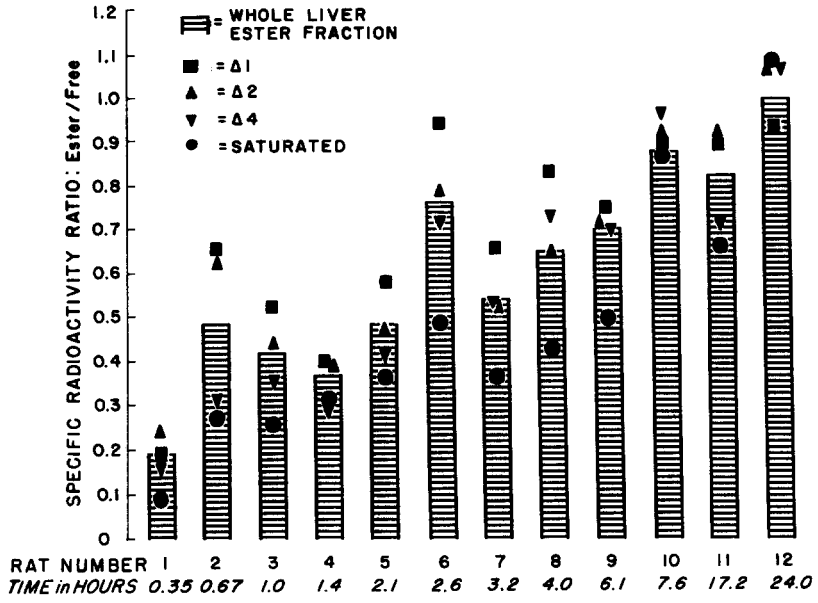

FIG. 1. The turnover of cholesterol esters in rat liver, after intravenous injection of mevalonate-2-C^{14}. Each vertical column shows the results obtained with one sample, from one rat. Each striped bar shows the ratio of the average specific radioactivity of the whole cholesterol ester fraction to that of the free cholesterol in the same sample. The relative specific radioactivities of the four individual cholesterol ester fractions are plotted as points in the same column.

each ester fraction, relative to the free cholesterol specific radioactivity from the same sample of liver or plasma, is shown in columns 5 and 8 of Table 5.

The relative specific radioactivity data from Tables 3 and 5 are also presented graphically in Figs. 1 and 2. For any one sample the height of the horizontally striped bar (obtained from Table 3) is really the average of the four points shown in the same vertical column (representing data from Table 5), appropriately weighted for relative mass in each ester fraction. These results demonstrate that substantial differences existed in the relative rates of appearance of label in the different cholesterol esters in both liver and plasma. In all the liver samples, the rate of appearance of total radioactivity in the monounsaturated esters was much greater than that in any of the other cholesterol esters. This was not surprising, since monounsaturated esters comprised roughly half the total cholesterol ester mass in most of the livers. In addition, the relative specific radioactivity of the mono- and diunsaturated esters was greater than that of the saturated and arachidonate esters. This was seen in all the early liver samples, despite considerable quantitative variation from animal to animal (Fig. 1).

In contrast, the plasma cholesterol ester samples showed a smooth and clear-cut pattern with very little individual variation (Fig. 2). In every animal the relative specific radioactivity of the monounsaturated plasma cholesterol esters was much greater than that of the other plasma cholesterol esters. At the earliest time intervals studied the specific radioactivity of the monounsaturated esters was almost twice as great as that of the other esters. With longer time intervals the differences between the esters became much smaller.

The ratio of the specific radioactivity (in counts per minute per milligram) of each plasma cholesterol ester fraction to the specific radioactivity of the corresponding liver cholesterol ester fraction, in the same rat, is shown in the last column of Table 5. The probable error in these values is estimated to be of the order of ±25%. In the earliest samples, the specific radioactivity of the plasma cholesterol esters was considerably less than that of the liver cholesterol esters. By 2.1 hr, however (rat 5) the specific radioactivity of each of the four ester fractions was virtually identical in plasma and liver. The specific radioactivities of the Δ1, Δ2, and Δ4 esters were also practically identical in plasma and liver in all later samples (samples 7–12). Only the saturated cholesterol esters showed significant differences between plasma and liver; the specific radioactivity of the plasma saturated esters was considerably greater than that of the liver saturated esters between 3 and 17 hr (rats 7–11). With but a single exception (the Δ1 fraction of rat 4) the specific radioactivity of the Δ1, Δ2, and Δ4 cholesterol esters of plasma was never significantly higher than the specific radioactivity of the corresponding cholesterol ester fraction in liver.

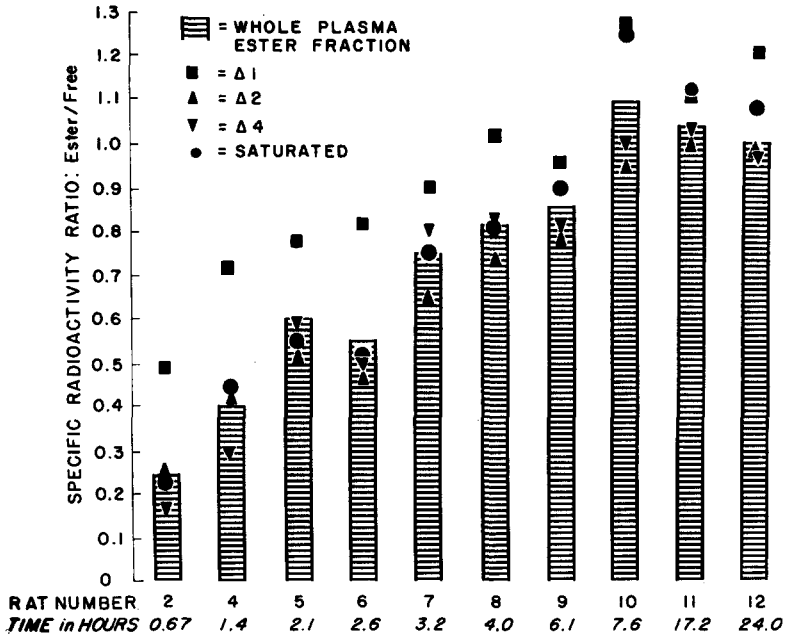

FIG. 2. The turnover of cholesterol esters in rat plasma, after intravenous injection of mevalonate-2-C^{14}. See legend of Fig. 1 for details.

DISCUSSION

The experiments reported here demonstrate considerable differences in the relative rates of appearance of radioactivity, after intravenous injection of mevalonate-C^{14}, in the different cholesterol esters of rat liver and of rat plasma. If one assumes that a steady state existed during the early intervals of this study, then these differences can be taken to indicate comparable differences in the turnover rates of the different cholesterol esters. Since the animals were postabsorptive, and the experimental periods were not prolonged, the assumption of the presence of a steady state is probably correct. Although the liver samples showed considerable variation from animal to animal, a more rapid fractional turnover rate of the mono- and diunsaturated esters than of the saturated and arachidonate esters was consistently observed. All the plasma samples showed a much more rapid fractional turnover rate of the monounsaturated, compared to the other, esters. In addition, the plasma samples showed markedly less individual variability than did the liver samples.

At any one time, the composition and concentration of cholesterol esters in liver are the net resultant of several metabolic processes. These include the uptake of cholesterol esters from chylomicrons originating in the intestinal tract (12), the hydrolysis and synthesis of cholesterol esters in the liver (1, 2), and transport from the liver of cholesterol esters incorporated into plasma lipoproteins (15). Recent studies have demonstrated a relative specificity for the formation of cholesteryl oleate during fat absorption and chylomicron formation (16). Other studies have shown an even more striking relative specificity for the synthesis of cholesteryl oleate by liver enzymes (2), and a somewhat lesser specificity for the hydrolysis of cholesteryl oleate and linoleate (1). The present findings are consistent with these in vitro observations. Clear-cut conclusions are, however, impossible, since the observed differences between the several esters might also reflect the presence of inhomogeneity between different cholesterol ester pools in liver. Such inhomogeneity is suggested by the fact that different subcellular fractions of liver, prepared by differential centrifugation, have different cholesterol ester compositions (2). It should also be noted that the enzymes involved in cholesterol ester turnover have different locations within the liver cell; most of the hydrolyzing activity is associated with a soluble enzyme (1), whereas the esterifying enzymes are exclusively particulate (2).

Klein and Dahl have reported that the relative order of labeling of liver cholesterol esters 30 min after *intraperitoneal* injection of *acetate*-C^{14} was influenced by the method of handling the rats (17). In the present study, the animals were handled in a manner similar to that employed in previous studies from this laboratory (1, 2, 12, 16). The results therefore provide in vivo data which should be comparable to the results of previous in vitro (1, 2) and in vivo (12, 16) studies on cholesterol ester metabolism. The possibility that different results might have been obtained had the animals been dif-

ferently handled cannot be excluded without further study.

Substantial evidence exists that the liver is the major source of both the protein and the lipid portions of the circulating plasma lipoproteins (15, 18–22). Nevertheless, it has been observed repeatedly that the composition of rat plasma cholesterol esters differs strikingly from that of rat liver cholesterol esters (12–15). This observation has raised the question of whether plasma and liver cholesterol esters might not be formed in different sites, and by different mechanisms. Experimental support for this suggestion has recently been derived from the work of Glomset, who has demonstrated a transesterification reaction in incubated plasma whereby free cholesterol becomes esterified with β-fatty acids from lecithin (23). Since the β-fatty acids of lecithin are largely polyunsaturated, it has been suggested (23) that the operation of this mechanism might explain the high proportion of polyunsaturated fatty acids in plasma cholesterol esters, although no data are available on the extent to which transesterification participates in the normal in vivo formation and turnover of plasma cholesterol esters.

The results presented here cannot be satisfactorily explained in terms of the plasma transesterification reaction. First of all, the monounsaturated plasma cholesterol esters turned over relatively much more rapidly than the polyunsaturated ones, whereas one would expect the polyunsaturated cholesterol esters to turn over at least as rapidly if the transesterification reaction contributed in a major way to their formation. In addition, it was observed that the specific radioactivity of the polyunsaturated plasma cholesterol esters never exceeded the specific radioactivity of the corresponding liver cholesterol esters, whereas one would expect it to do so if formation of polyunsaturated plasma cholesterol esters mainly proceeded by way of the plasma transesterification reaction. It is, of course, possible that two independent processes operated simultaneously in plasma. Thus the liver might have been delivering relatively high specific radioactivity cholesterol esters into the plasma compartment at a relatively high rate, perhaps primarily into one plasma lipoprotein (see below). Simultaneously, conversion of free to ester cholesterol might have been occurring more slowly in plasma. The data presented do not permit a quantitative evaluation of the extent to which plasma cholesterol esters are normally formed in liver or in plasma. The data do indicate that some of the plasma cholesterol esters, particularly of the monounsaturated esters, must originate in the liver.

Some uncertainty exists as to the processes involved in the cholesterol ester turnovers studied in these experiments. In liver, the observed turnover probably mainly reflected continuing hydrolysis and reesterification in situ. The mechanism involved in the observed plasma cholesterol ester turnover are, however, not clear. One possibility is that the observed plasma turnover depended upon continuing hydrolysis and formation of plasma cholesterol esters, within plasma lipoproteins. If this occurred, hydrolysis probably took place during lipoprotein circulation through the liver and involved liver enzymes, since there is no evidence for the existence of hydrolytic enzyme activity in rat plasma (24). Esterification might then have involved both the liver and plasma esterification mechanisms, as discussed above. Hydrolysis and/or reesterification of plasma esters might also occur at other, as yet undetermined, tissue sites. A second possibility is that intact cholesterol esters were removed from lipoproteins during their circulation through tissues and that these esters were replaced during circulation through the liver. This removal might selectively involve certain cholesterol esters, or might equally involve all the cholesterol esters within a given lipoprotein. An extreme variation of this process might involve turnover of entire lipoprotein molecules, or of the entire lipid portion of these molecules. Third, there is the possibility that the observed turnover of plasma cholesterol esters reflected equilibration of these esters, by exchange, with a turning-over pool of liver cholesterol esters. Since cholesterol esters normally do not exchange among the different lipoproteins in vitro (12, 15), in order for such equilibration to occur between liver and plasma esters it would be necessary to hypothesize the occurrence of some conformational change in the lipoprotein during its circulation through the liver, permitting exchange to occur. It is also possible that several mechanisms operate concurrently, and to variable extents, in plasma.

Two explanations based on experimental data are available for the finding that the monounsaturated esters were the most rapidly turning over cholesterol esters in plasma. The first involves the possibility that plasma cholesterol esters are in closest equilibrium with a pool of newly synthesized liver cholesterol esters. Since the liver enzymes responsible for cholesterol esterification display a strong relative specificity for cholesteryl oleate formation (2), this could explain the more rapid appearance of label in the monounsaturated esters.

The second explanation depends on the fact that the turnover of cholesterol esters in the different plasma lipoproteins in probably heterogeneous. Gas–liquid chromatographic studies in this laboratory (unpublished) have shown the presence of relatively more oleic acid in rat very low density ($d < 1.019$) lipoprotein, compared to rat whole plasma cholesterol esters, and differences between the cholesterol esters of rat very low density and other lipoproteins have been described

by Roheim et al. (15). In addition, evidence is available, from in vitro experiments with perfused rat livers, suggesting that the protein and lipid portions of the very low density lipoproteins turn over more rapidly than the corresponding portions of the other rat plasma lipoproteins (15, 18). More rapid turnover of the very low density lipoprotein cholesterol esters, consisting of relatively more cholesteryl oleate, could partly or even entirely account for the relatively more rapid turnover of the monounsaturated cholesterol esters observed in whole plasma. More complete understanding of the metabolism of plasma cholesterol esters will require detailed studies on the turnover of each different cholesterol ester in each plasma lipoprotein.

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