Journal of Clinical Investigation
Vol. 45, No. 7, 1966

Turnover of Individual Cholesterol Esters in Human Liver and Plasma*

P. J. NESTEL † AND E. A. COUZENS

(From the University of Melbourne Department of Medicine, the Royal Melbourne Hospital, Victoria, Australia)

There are several lines of evidence which suggest that the liver is a major source of plasma cholesterol esters. First, eviscerated rats cannot maintain a normal concentration of cholesterol esters in the plasma (1); second, when isotopically labeled mevalonic acid is administered to rats, the specific radioactivity of the hepatic cholesterol esters initially exceeds that of the plasma cholesterol esters (2); and third, the perfusion of livers from cholesterol-fed rats leads to an increased secretion of cholesterol esters from the liver into the perfusate (3).

Despite this, the composition of cholesterol esters in the liver and plasma is dissimilar, both in the rat (2, 3) and in man (4, 5). This has raised the question of whether an additional source of plasma cholesterol esters exists outside the liver. A transesterification reaction between free cholesterol and the beta fatty acid of lecithin has been demonstrated in plasma and could account for a portion of the plasma cholesterol esters (6, 7). Simultaneous esterification of cholesterol in liver and plasma could lead to the formation of cholesterol esters of differing composition.

Some of the factors that govern the formation and composition of cholesterol esters have been determined in the rat. The turnover of cholesterol esters in rat liver and plasma is heterogeneous. The fractional turnover rate of plasma cholesteryl oleate is greater than that of the other esters (2) and may be related to the high degree of specificity for cholesteryl oleate formation by liver enzymes (8). When rats are fed cholesterol with different fatty acids, cholesteryl oleate accumulates in the liver irrespective of the fatty acid fed, yet cholesteryl arachidonate remains the predominant ester in the plasma and is the major cholesterol ester secreted by the liver in the fasting rat (9).

The heterogeneity of plasma cholesterol ester turnover found in the rat does not apply in man. Goodman (10) has demonstrated a similarity in the fractional turnover rates of individual cholesterol esters within a given class of plasma lipoproteins. This finding has been confirmed by Nestel, Couzens, and Hirsch (5), who have shown this to apply equally in men with low and high plasma cholesterol concentrations.

This paper reports further studies in man in which the incorporation of radioactivity into the individual cholesterol esters of liver and plasma has been measured. We have compared the specific radioactivities of individual esters in liver and plasma in order to determine, first, whether the turnover of cholesterol esters is homogeneous in the liver as it is in the plasma, and second, whether the plasma esters can be shown to be derived from those in the liver.

Methods

The incorporation of either cholesterol-4-^{14}C [1] or *dl*-mevalonic acid-2-^{3}H [1] into the cholesterol esters of liver and plasma was studied in five subjects in whom abdominal surgery was carried out. No pathology was demonstrated in subject M, a 48-year-old female; a small acute gastric ulcer was found in subject C, a 63-year-old male; a gastric ulcer that required partial gastrectomy was found in subject L, a 44-year-old female; and an inoperable gastric cancer was found in subjects H and Mo, each of whom was a 47-year-old male. Each subject had suffered from dyspepsia and had been on a restricted diet for some weeks: subjects M, C, H, and Mo had been on low fat diets and subject L on a diet containing vegetable oils. Subjects C and H had lost weight. None of the subjects had abnormal liver function tests, although small secondary deposits were found in the liver of sub-

* Submitted for publication November 1, 1965; accepted April 18, 1966.

Supported by a grant from the National Heart Foundation of Australia.

† Address requests for reprints to Dr. P. J. Nestel, Dept. of Medicine, University of Melbourne, Royal Melbourne Hospital Post Office, Victoria, Australia.

[1] Radiochemical Centre, Amersham, England.

ject H. Blood transfusion was not required before or during surgery.

Approximately 50 μc of cholesterol-4-^{14}C was given to subjects M, C, L, and H. The cholesterol was dissolved in 0.5 ml ethanol, diluted with 0.9% sodium chloride solution, and slowly added to about 15 ml of the patient's own plasma that had been collected the same day. The plasma was agitated in a water bath at 37° C for 1 hour and then injected intravenously. Less than 1% of the total injected radioactivity was found in esterified cholesterol. About 400 μc of tritiated mevalonic acid was injected intravenously into subject Mo after the lactone ring had been opened by incubating with $NaHCO_3$ solution (pH 8.5) for 1 hour at 37° C.

The patients were fasted for about 16 hours before surgery. A sample of blood was collected at the time of liver biopsy soon after the abdomen had been opened.

A sample of liver weighing at least 4 g [2] was obtained 18 hours after the injection of radioactivity in subjects M and H, after 23 hours in subject C, and after 43 hours in subject L. The liver was weighed, washed in 0.9% NaCl, cut into small pieces with scissors, rinsed several times in 0.9% NaCl to remove blood, and then homogenized in 0.9% NaCl. The homogenate was then extracted by the procedure of Folch, Lees, and Sloane-Stanley (11).

A sample of liver weighing about 13 g was obtained from subject Mo after 30 hours. After homogenization about 3 g was extracted (11) and the remainder separated into subcellular fractions. The cellular debris was removed by centrifuging at about $1,000 \times g$ for 10 minutes, and the mitochondria, microsomes, and supernatant fractions were prepared by centrifuging at $10,000 \times g$ for 30 minutes and $104,000 \times g$ for 60 minutes, respectively, in a model L Spinco ultracentrifuge.

Further samples of blood were obtained from the subjects over the next 2 days in order to establish the time of equilibration between the specific radioactivities of plasma free and esterified cholesterol. Plasma was extracted by the procedure of Folch and associates (11).

Samples of liver weighing about 1.5 g were obtained from two additional subjects, A and B. These samples were separated into subcellular fractions as described above. Radioactive compounds were not given to these subjects, and only the fatty acid composition of the cholesterol esters in each fraction was measured.

Analytical methods. Samples of the lipid-containing phases were evaporated under nitrogen and separated into fractions containing esterified cholesterol and free cholesterol as described elsewhere (5, 12). Separation of cholesterol esters from other lipids was greater than 99% and was checked with labeled standards and by chromatographing the column fractions with thin layer chromatography (13).

Samples of the cholesterol-containing fractions were assayed for radioactivity and chemical concentration (14).

[2] The amount of liver obtained was determined by the surgeon and depended on the ease and safety with which the procedure could be carried out.

Further samples of the cholesterol esters were separated into individual esters, and their radioactivity and mass were measured by thin layer and gas chromatography. These procedures have been described in detail in a previous publication (5).

Briefly, the cholesterol esters were separated by a modification of the method of Morris (15) on thin layers of silver nitrate-impregnated Kieselgel G.[3] The cholesterol esters were applied in isooctane and separated in a solvent system of benzene-hexane (40:60, vol:vol). The plates were dried and the cholesterol esters made visible in ultraviolet light with 0.1% rhodamine G. The esters were scraped off the plates and eluted with ether, and their radioactivity was counted. Duplicate runs showed that the over-all reproducibility of the method was at least 90%. Elution of the cholesterol esters resulted in at least 95% recovery of cholesteryl linoleate and 90% of cholesteryl arachidonate. Standard mixtures of chemically pure cholesterol esters [4] were run repeatedly.

Samples of the cholesterol esters were transmethylated by refluxing with 2% sulfuric acid in methanol for 2 hours at 70° C. The methyl esters were extracted into isooctane and separated on a Packard gas chromatogram on a 13% diethyleneglycol succinate Chromosorb W column.[4] An argon ionization detector was used; the flow of argon was 60 ml per minute, and the column temperature was 185° C. Known mixtures of methyl esters of fatty acids [5] were run repeatedly, and the reproducibility for the major fractions was of the order of 90% and for the minor fractions 85%.

The distribution of labeled cholesterol in the individual cholesterol esters was determined after separation by thin layer chromatography. The fatty acid composition of the total cholesterol ester fraction was obtained from the gas chromatographic analysis. The specific activity of each cholesterol ester could then be calculated after correcting for the molecular weights of the individual methyl esters. Specific activities could not be calculated for the cholesterol esters in the subcellular fractions be-

TABLE I

Concentrations of esterified and free cholesterol in liver and plasma in the five patients studied

Subject	Age	Sex	Liver		Plasma	
			Esterified cholesterol	Free cholesterol	Esterified cholesterol	Free cholesterol
	years		*mg/g*		*mg/100 ml*	
M	48	F	0.60	2.45	116	53
H	47	M	0.33	2.85	68	48
C	63	M	0.50	1.21	166	67
L	44	F	0.34	0.80	98	50
Mo	47	M	0.40	1.21	128	60

[3] Fluka A.-G., Switzerland.

[4] Applied Science Laboratories, State College, Pa.

[5] Obtained from the National Institutes of Health, Bethesda, Md.

TABLE II

Specific activities of total esterified cholesterol and free cholesterol in the liver and plasma

Subject	Time of sampling	Dose of cholesterol-^{14}C	Esterified cholesterol			Free cholesterol		
			Liver	Plasma	Liver/ Plasma	Liver	Plasma	Liver/ Plasma
	hours	*cpm*		*cpm/mg*			*cpm/mg*	
M	18	52.5 × 10^6	824	1,320	0.62	4,080	3,470	1.2
H	18	106 × 10^6	1,000	4,120	0.24	8,290	7,120	1.2
C	23	53 × 10^6	541	1,350	0.40	2,960	2,680	1.1
L	43	48 × 10^6	730	1,750	0.42	2,680	2,150	1.2

cause there was insufficient material for the measurement of chemical concentration.

Results

The concentrations of esterified and free cholesterol in liver and plasma in the five subjects are shown in Table I. Esterified cholesterol in the liver accounted for about one-quarter of total cholesterol.

The specific radioactivities of free and total esterified cholesterol in liver and plasma in the four subjects given cholesterol-4-^{14}C are shown in Table II. The specific activities of free cholesterol in liver and plasma were almost the same, but that in the liver consistently exceeded that in the plasma by a small amount. By contrast, the specific activities of esterified cholesterol were always considerably higher in plasma than in liver. The specific activity of free cholesterol was higher than that of esterified cholesterol in both liver and plasma in subjects M, H, and C, in whom samples were obtained 18 and 23 hours after the injection of radioactivity. In subject L, who was studied at 43 hours, the specific activities of free and esterified cholesterol were approaching equilibrium in the plasma, but in the liver, the specific activity of free cholesterol was still much higher than that of esterified cholesterol. Despite the intervention of surgery, measurements of cholesterol specific activity in plasma were continued for 3 days; it was found that equilibration between plasma free and esterified cholesterol was reached in 52 hours after the injection of radioactivity in subject L.

The fatty acid composition of the cholesterol esters in liver and plasma is shown in Table III. The proportion of saturated esters (palmitate and stearate) was strikingly higher in liver than in plasma, whereas that of linoleate was higher in plasma than in liver. Stearate comprised about 12% of the total in liver.

The distribution of radioactivity among individual cholesterol esters is also shown in Table III. In general, in plasma, the distribution of radioactivity was similar to the chemical composition. By contrast, in liver, the distribution of radioactivity differed substantially from the fatty acid composition, especially with respect to linoleate and the saturated esters, but resembled to

TABLE III

Distribution of radioactivity within hepatic and plasma cholesterol esters and their fatty acid composition after the injection of cholesterol-4-^{14}C-labeled lipoproteins*

Subject	Tissue	Fatty acid composition				Distribution of radioactivity			
		Sat.	Δ1	Δ2	Δ4	Sat.	Δ1	Δ2	Δ4
M	Liver	32	31	24	13	16	38	35	11
	Plasma	14	35	40	11	14	37	39	10
H	Liver	25	46	19	10	19	46	28	7
	Plasma	13	42	37	8	16	41	33	9
C	Liver	33	34	21	12	16	41	30	13
	Plasma	13	41	35	11	15	41	32	12
L	Liver	31	27	31	11	18	24	47	11
	Plasma	13	17	59	11	16	17	57	10

* Sat. = saturated esters; Δ1 = monounsaturated esters; Δ2 = diunsaturated esters; Δ4 = tetraunsaturated esters.

TABLE IV

Specific activities of individual cholesterol esters in the liver and plasma

Subject	Liver esters					Plasma esters				
	Total	Sat.	Δ1	Δ2	Δ4	Total	Sat.	Δ1	Δ2	Δ4
M	824	412	1,010	1,200	697	1,320	1,320	1,390	1,290	1,200
H	1,000	760	1,000	1,473	700	4,120	5,060	4,021	3,670	4,614
C	540	262	651	771	585	1,350	1,557	1,350	1,234	1,473
L	730	423	649	1,107	730	1,750	2,154	1,750	1,690	1,590

some extent the distribution of radioactivity among the plasma esters.

The specific radioactivities of each cholesterol ester in liver and plasma, after correction for the different molecular weights, are shown in Table IV. In plasma, the specific activities of the individual esters were similar, indicating that their fractional turnover rates were of the same order. In liver, on the other hand, the specific activities of the saturated esters were much less than those of the others, whereas the specific activity of linoleate was greater than that of any other ester. The specific activities of the monounsaturated esters and of arachidonate were sometimes less and sometimes greater than the average value for all the esters; in only one subject (M) did the specific activity for monounsaturated esters clearly exceed the mean total specific activity.

The distribution of radioactivity and the fatty acid composition of cholesterol esters in the liver, plasma, and subcellular fractions of liver, 30 hours after the injection of tritiated mevalonic acid into subject Mo, are shown in Table V. (For technical reasons, recoveries of the arachidonate fraction after thin layer chromatography were unsatisfactory in this study; therefore, it seemed preferable to exclude arachidonate from the calculations.)

TABLE V

Distribution of radioactivity and fatty acid composition in cholesterol esters of whole liver, subcellular fractions of liver, and plasma in subject Mo

Tissue	Fatty acid composition*			Distribution of radioactivity*		
	Sat.	Δ1	Δ2	Sat.	Δ1	Δ2
		%			%	
Whole liver	35	43	21	30	41	29
Mitochondria	54	25	20	26	37	37
Microsomes	49	26	20	42	19	37
Supernatant	19	36	45	19	35	46
Plasma	18	33	49	15	37	48

* Calculations have been limited to three classes of esters for reasons given in text.

Table V shows that the results for the other three classes of cholesterol esters were similar to those found in the other studies. In plasma, distribution for radioactivity resembled the fatty acid composition, whereas in whole liver there was proportionately more radioactivity in linoleate and less in the saturated esters, although this difference was not so great as when cholesterol-^{14}C was given. This heterogeneity was, however, more marked in the mitochondria and microsomes, which were relatively rich in saturated esters and yet contained proportionately less radioactivity within that group of esters. On the other hand, a disproportionately large amount of radioactivity was incorporated into linoleate in mitochondria and microsomes. The findings with respect to the monounsaturated esters were variable. The supernatant, or liver cell sap, on the other hand, resembled plasma in that the distribution of radioactivity was almost identical to the fatty acid composition, and in both these respects the values for plasma and liver supernatant were similar.

The fatty acid composition of the cholesterol esters in the plasma and different liver fractions in subject Mo is shown in greater detail in Table VI, which also contains similar data for subjects A and B. The mitochondrial and microsomal fractions were especially rich in saturated esters.

Discussion

These studies demonstrate a probable heterogeneity in turnover rates among cholesterol esters in liver but not in plasma. The similar specific radioactivities of individual plasma cholesterol esters (Table IV) are consistent with previous observations that the fractional turnover rates of individual cholesterol esters within a specific class of lipoproteins are similar (5, 10). Within the

TABLE VI

Fatty acid composition of cholesterol esters in whole liver, subcellular fractions of liver, and plasma in subjects A, Mo, and B

Fatty acid*	Whole liver			Mitochondria			Microsomes			Supernatant			Plasma		
	A	Mo	B	A	Mo	B	A	Mo	B	A†	Mo	B	A	Mo	B
14:0	4	2	2	3	1	2	2	3	3		1	1	2	2	1
Unidentified	3			4			3	3	2						
16:0	20	21	29	21	22	27	16	14	22		13	18	11	12	13
16:1	6	6	4	7	3	4	7	7	4		5	4	6	4	3
Unidentified	3		2	2	1	2	4	8	2			1			
18:0	10	10	11	12	28	20	16	27	24		4	7	2	2	2
18:1	22	34	27	19	20	20	20	16	24		28	28	27	26	28
18:2	26	20	20	22	19	16	23	14	14		42	34	44	44	46
20:4	6	7	5	10	6	9	9	8	5		7	7	8	10	7

* Major fatty acids between myristate (14:0) and arachidonate (20:4) that comprised at least 1% of the total. The two unidentified peaks had retention times relative to stearate of 0.44 and 0.82, respectively.

† Specimen lost.

liver, on the other hand, the specific radioactivity of cholesteryl linoleate was always greater than that of any other group of esters, whereas that of the saturated esters was consistently the least (Table IV).

The heterogeneity in the rate of appearance of radioactivity in the different cholesterol esters was equally striking in the mitochondria and microsomes of the liver (Table V). Although this conclusion is based on only one study, the proportionately greater incorporation of radioactivity into linoleate than into the saturated esters suggests that the turnover of linoleate may be greater than that of the other esters throughout most of the liver. The cholesterol esters in the supernatant appear to be an exception in this respect, provided the finding in this fraction does not represent significant contamination with plasma cholesterol esters. The composition and metabolism of the esters within the supernatant were strikingly similar to those in the plasma, and although this does not imply that the cholesterol esters in the plasma are necessarily derived from those in the supernatant, it does suggest that the cholesterol esters in both the supernatant and the plasma may have a common site of origin within the liver.

Differences in composition among the cholesterol esters in homogenates of whole liver and the various subcellular fractions were demonstrated. The major differences were in the proportions of stearate and oleate, the mitochondria and microsomes having a particularly high content of stearate.

The cholesterol esters in the two samples of liver supernatant closely resembled those in plasma.

These findings, taken together, are consistent with the existence of several pools of cholesterol esters within the liver differing both in composition and metabolism. The relationship between the metabolism of these pools of hepatic cholesterol esters and those in plasma has not been resolved by our studies. The similarity in the distribution of radioactivity among the cholesterol esters of whole liver and plasma is in contrast to the marked differences in their composition. The distributions in radioactivity in liver and plasma were certainly not identical but were sufficiently close in most of the studies to suggest an intimate metabolic relationship. However, the heterogeneity of cholesterol ester composition and metabolism found in the subcellular fractions indicates the complexity of the problem. This is also reflected in the differences in the specific radioactivities of the individual cholesterol esters in whole liver and plasma (Table IV). The differences in the fractional turnover rates of cholesterol esters in different lipoproteins (5, 10) may also reflect the presence of more than one precursor pool within the liver.

Findings derived from similar studies in the rat have been recently reviewed by Goodman (16). It is probable that both in man and in the rat there are heterogeneity and compartmentalization of cholesterol esters in the different subcellular fractions of liver. Our findings in man may resemble those reported in the rat in several respects. A heterogeneity in fractional turnover rates has been previously reported for the cholesterol esters of plasma and liver in the rat. Goodman and Shiratori (2)

have demonstrated a greater fractional turnover rate for mono- and diunsaturated esters than for saturated esters and arachidonate in rat liver and a greater fractional turnover rate for monounsaturated than for all other esters in rat plasma. This appears to be consistent with the known specificity for cholesteryl oleate formation (8) and hydrolysis (17) by rat liver enzymes. Cholesteryl oleate has also been shown to be the predominant ester in rat liver under normal circumstances and when cholesterol (18) and fats are fed (9). Heterogeneity in turnover rates among hepatic cholesterol esters has also been described in the rat by other authors (19, 20), although the ester with the highest specific radioactivity in the liver was sometimes cholesteryl oleate (2, 20) and sometimes cholesteryl arachidonate (9).

As in the rat, in man the rate of appearance of radioactivity in hepatic cholesterol esters differs among the different esters. Unlike rat liver, human liver accumulated radioactivity most rapidly in cholesteryl linoleate. This appears to be a major difference between the metabolism of cholesterol esters in the two species. The apparently greater turnover of linoleate is consistent with our previous observation that the consumption of fat is associated in man with an increase in the proportion of cholesteryl linoleate in plasma lipoproteins (21).

The different location within the liver cell of the enzymes involved in hydrolysis and esterification may also contribute to the inhomogeneity of cholesterol ester metabolism (8, 17). It would be of interest to discover whether the specificities of the liver enzymes subserving the formation and hydrolysis of cholesterol esters differ in man and in the rat and, in particular, whether in man there is a preference for the formation of cholesteryl linoleate.

In the present studies in man, the free cholesterol in the liver and plasma appeared to equilibrate rapidly. This has been demonstrated previously in man (22). Nevertheless, the specific radioactivity of free cholesterol was always slightly higher in the liver than in plasma (Table II); this finding has also been reported in the rat (2). However, in all our studies, the specific radioactivities of the free cholesterol fractions were higher than those of the esterified fractions. Equilibration between free and esterified cholesterol was almost reached in the plasma at 43 hours in subject L. Equilibration between hepatic free and esterified cholesterol was not apparent at this time in this subject (Table II), another finding that suggests the existence of several pools in the liver. In the rat equilibration between the cholesterol fractions appears to occur sooner in liver than in plasma (2).

Our findings could also be interpreted to support the importance of the plasma cholesterol esterifying system (6, 7). The higher specific radioactivity of the cholesterol esters in plasma than in liver and the similarity in the distribution of radioactivity among hepatic and plasma esters may have reflected the primary esterification of free cholesterol within plasma followed by an exchange of intact cholesterol esters between those in plasma lipoproteins and liver (16). The plasma esterifying system involves a transferase reaction in which free cholesterol is esterified with fatty acids derived from the beta position of lecithin (7). Since this position is occupied to a large extent by linoleic acid, the apparently greater turnover of hepatic cholesteryl linoleate would also be consistent with the transfer of cholesterol esters from plasma lipoproteins to the liver.

Summary

The incorporation of radioactivity into the individual cholesterol esters of liver and plasma was studied in five subjects requiring abdominal surgery. After the injection of either cholesterol-4-^{14}C-labeled lipoproteins or tritiated mevalonic acid, the distribution of radioactivity and the fatty acid composition were measured in the separated cholesterol esters. Similar studies were carried out in the subcellular fractions of liver in one of the subjects. In the livers of two additional subjects, only the fatty acid composition was determined in the cholesterol esters of the subcellular fractions. The following results were obtained.

1. The composition of cholesterol esters differed in liver and plasma in that the proportion of saturated esters (palmitate and stearate) was considerably higher in liver, whereas that of linoleate was higher in plasma. There were major differences in the cholesterol esters of the different subcellular fractions. The mitochondria and microsomes were particularly rich in stearate.

2. In the liver, the distribution of radioactivity differed substantially from the fatty acid composition, especially with respect to linoleate and the saturated esters. Proportionately more radioactivity was incorporated into linoleate than into the other esters and proportionately less into the saturated esters. This heterogeneity among the esters was also found in the mitochondria and microsomes, but not in the supernatant.

3. The distribution of radioactivity among the cholesterol esters of plasma resembled the fatty acid composition, indicating that their turnover rates were of the same order.

4. The distribution of radioactivity among the hepatic cholesterol esters resembled to some extent that in the plasma esters, indicating a degree of relationship between them.

5. The results are consistent with the existence of several pools of cholesterol esters in the liver differing both in composition and metabolism.

Acknowledgment

We wish to thank Dr. N. Johnson who obtained the samples of liver.

References

1. Friedman, M., and S. O. Byers. Observations concerning the production and excretion of cholesterol in mammals. XVI. The relationship of the liver to the content and control of plasma cholesterol ester. J. clin. Invest. 1955, **34**, 1369.
2. Goodman, D. S., and T. Shiratori. In vivo turnover of different cholesterol esters in rat liver and plasma. J. Lipid Res. 1964, **5**, 578.
3. Roheim, P. S., D. E. Haft, L. I. Gidez, A. White, and H. A. Eder. Plasma lipoprotein metabolism in perfused rat livers. II. Transfer of free and esterified cholesterol into the plasma. J. clin. Invest. 1963, **42**, 1277.
4. Swell, L., H. Field, Jr., P. E. Schools, Jr., and C. R. Treadwell. Fatty acid composition of tissue cholesterol esters in elderly humans with atherosclerosis. Proc. Soc. exp. Biol. (N. Y.) 1960, **103**, 651.
5. Nestel, P. J., E. Couzens, and E. Z. Hirsch. Comparison of turnover of individual cholesterol esters in subjects with low and high plasma cholesterol concentrations. J. Lab. clin. Med. 1965, **66**, 582.
6. Glomset, J. A., F. Parker, M. Tjaden, and R. H. Williams. The esterification *in vitro* of free cholesterol in human and rat plasma. Biochim. biophys. Acta (Amst.) 1962, **58**, 398.
7. Glomset, J. A. Further studies of the mechanism of the plasma cholesterol esterification reaction. Biochim. biophys. Acta (Amst.) 1963, **70**, 389.
8. Goodman, D. S., D. Deykin, and T. Shiratori. The formation of cholesterol esters with rat liver enzymes. J. biol. Chem. 1964, **239**, 1335.
9. Swell, L., M. D. Law, and C. R. Treadwell. Role of the liver in the metabolism of exogenous cholesterol esters. Arch. Biochem. 1964, **105**, 541.
10. Goodman, D. S. The *in vivo* turnover of individual cholesterol esters in human lipoproteins. J. clin. Invest. 1964, **43**, 2026.
11. Folch, J., M. Lees, and G. H. Sloane-Stanley. A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem. 1957, **226**, 497.
12. Nestel, P. J., E. Z. Hirsch, and E. A. Couzens. The effect of chlorophenoxyisobutyric acid and ethinyl estradiol on cholesterol turnover. J. clin. Invest. 1965, **44**, 891.
13. Mangold, H. K. Thin layer chromatography of lipids. J. Amer. Oil Chem. Soc. 1961, **38**, 708.
14. Sperry, W. M., and M. Webb. A revision of the Schoenheimer-Sperry method for cholesterol determination. J. biol. Chem. 1950, **187**, 97.
15. Morris, L. J. Fractionation of cholesterol esters by thin-layer chromatography. J. Lipid Res. 1963, **4**, 357.
16. Goodman, D. S. Cholesterol ester metabolism. Physiol. Rev. 1965, **45**, 747.
17. Deykin, D., and D. S. Goodman. The hydrolysis of long-chain fatty acid esters of cholesterol with rat liver enzymes. J. biol. Chem. 1962, **237**, 3649.
18. Klein, P. D., and R. A. Martin. Heterogeneity in the turnover rates of liver cholesterol esters. J. biol. Chem. 1959, **234**, 1685.
19. Klein, P. D., and R. A. Martin. Some transitory changes in cholesterol metabolism induced by dietary cholesterol. J. biol. Chem. 1959, **234**, 3129.
20. Sugano, M., and O. W. Portman. Fatty acid specificities and rates of cholesterol esterification *in vivo* and *in vitro*. Arch. Biochem. 1964, **107**, 341.
21. Nestel, P. J., and E. A. Couzens. The influence of diet on the composition of plasma cholesterol esters in man. J. Lipid Res. In press.
22. Chobanian, A. V., and W. Hollander. Body cholesterol metabolism in man. I. The equilibration of serum and tissue cholesterol. J. clin. Invest. 1962, **41**, 1732.