Phosphatidylcholine–Cholesterol Acyltransferase in the Ultracentrifugal Residual Protein Fraction of Rat Plasma

By MICHIIHIRO SUGANO

Laboratory of Nutrition Chemistry, Department of Food Science and Technology, Kyushu University School of Agriculture, Fukuoka, Japan

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1. The phosphatidylcholine–cholesterol acyltransferase of rat plasma was dissociated from the plasma lipoproteins by ultracentrifugation and shown to be present in the plasma residual-protein fraction of density >1.210 . 2. The general properties of the acyltransferase were substantially unchanged in the residual fraction as judged from the effects of differences in the substrates and of overnight starvation on the formation of different cholesterol esters. 3. The enzyme from rats starved overnight, by comparison with the enzyme from fed rats, preferentially formed cholesteryl arachidonate at the expense of cholesteryl linoleate. 4. The results suggest that ultracentrifugal separation of the plasma residual fraction may be used as an initial step for the purification of the acyltransferase.

Plasma phosphatidylcholine–cholesterol acyltransferase (an enzyme belonging to the EC 2.3.1 group) appears to be a physiologically important source of plasma cholesterol esters, and may be concerned in the metabolic fate of the very-low-density lipoproteins (Goodman, 1965; Glomset, 1968; Schumaker & Adams, 1970). The reaction also shows promise as a tool for studying lipoprotein structure. However, it is difficult at present to distinguish between effects on the enzyme and effects on its lipoprotein substrates, because the enzyme in plasma is associated mainly with the lipoproteins (Goodman, 1965; Glomset, 1968).

Because the plasma protein fraction of $d > 1.210$ was shown to stimulate cholesterol esterification by plasma lipoprotein fractions, Glomset (1963) suggested that acyltransferase activity might be present in this protein fraction. Raz, Kummerow & Nishida (1969) have presented results showing that the acyltransferase in human plasma is associated exclusively with the protein fraction of $d > 1.210$. They suggest that incomplete separation of the plasma lipoproteins of $d < 1.210$ from the residual proteins is responsible for the presence of enzyme activity in the lipoprotein fraction. However, the study by Lossow, Shah & Chaikoff (1966) also suggests the possibility that exposure of plasma to the high concentration of sodium bromide necessary for the ultracentrifugal preparation of lipoproteins may dissociate the enzyme from the lipoproteins. The question therefore arises as to whether the enzyme in the residual fraction has the properties of that in the plasma.

The present paper considers this question and describes the effects of starvation and of different types of phosphatidylcholine on the reaction.

MATERIALS AND METHODS

Wistar rats fed on a commercial pellet ration (Oriental Rat Chow, type NMF; Tokyo, Japan) were used. Specification of the sex and body weight is given in each table and figure. Starvation was overnight (15–16 h). Rat plasma was separated from blood collected in heparin. Human plasma was obtained from healthy males aged between 21 and 28.

[1- 14 C]Palmitic acid, [1- 14 C]linoleic acid and [4- 14 C]-cholesterol were the products of Daiichi Chemical Co., Tokyo, Japan. [1- 14 C]Oleic acid and [1- 14 C]arachidonic acid were purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and Applied Science Laboratories Inc., State College, Pa., U.S.A., respectively. Phosphatidylcholines with labelled palmitic acid, oleic acid, linoleic acid and arachidonic acid in the 2-position were prepared by the method of Robertson & Lands (1962) as modified by Van den Bosch, Slotboom & Van Deenen (1969) with lysophosphatidylcholine prepared from microsomal phosphatidylcholine of rat liver. (L- α -Dipalmitoyl) phosphatidylcholine was purchased from Sigma Chemical Co., St Louis, Mo., U.S.A., and purified by t.l.c. (Arvidson, 1968). The position of the labelled fatty acids on the synthesized phosphatidylcholines was confirmed by treating them with snake venom (*Crotalus adamanteus*; Sigma Chemical Co.) under the conditions described by Robertson & Lands (1962). Of the radioactivity 95–100% was recovered as free fatty acids at the end of incubation. Molecular species of phosphatidylcholines from rat liver microsomes were fractionated

according to the degree of unsaturation by the method of Arvidson (1968).

The labelled or unlabelled substrates (cholesterol and phosphatidylcholines) were used in Tween solution, or in a complex with β -globulin or lipoproteins. The Tween solution was prepared by dissolving each substrate in an appropriate volume of a 0.5% solution of Tween 20 in ethanol, evaporating the alcohol in a water bath under nitrogen, and dissolving the residue in a volume of 0.9% NaCl equal to that of the Tween solution. The volume of the Tween solution was such that its concentration in the incubation mixture was 0.005%. When β -globulin (Cohn fraction III-human; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.) was employed as a carrier, Avigan's (1959) method, as outlined by Shah, Lossow & Chaikoff (1964), was followed. To prepare the lipoprotein substrate, labelled cholesterol was dispersed on Celite 545 and incubated with heat-inactivated plasma (60°C, 30 min) at 37°C for 5 h (Portman & Sugano, 1964). After removal of the Celite by centrifugation, the supernatant solution was used as a lipoprotein substrate containing labelled free cholesterol.

Ultracentrifugation was carried out in a Spinco model L2-65 preparative ultracentrifuge, with the 65 rotor (Bragdon, Havel & Boyle, 1956; Raz *et al.* 1969). The total plasma lipoprotein fraction was isolated by ultracentrifugation at 115000g at 10–12°C for 24 h after the density of the plasma had been adjusted to 1.210 with solid NaBr. The lipoprotein and residual fractions were used as a source of phosphatidylcholine-cholesterol acyltransferase after dialysis against 0.01 M-potassium phosphate buffer, pH 7.4, containing 0.001 M-EDTA, for 20 h below 4°C.

Lipoprotein and residual fractions were incubated in ground-glass stoppered tubes in a 37°C water bath with mechanical shaking for 6 h. Where the concentration of protein present in the enzyme fractions varied, it was adjusted to a constant value with phosphate buffer. The incubation was terminated with chloroform-methanol (2:1, v/v). Lipids were extracted with the same solvent and purified as described by Folch, Lees & Sloane-Stanley (1957).

Lipid extracts were finally dissolved in chloroform. Separation of individual cholesterol esters was carried out by t.l.c. The plate (0.3 mm thick layer of silica gel G; Merck) was developed in cyclohexane-benzene (4:1, v/v) twice consecutively at 15–17°C. Recovery tests with labelled cholesterol esters (palmitate, oleate, linoleate and arachidonate) showed that 96–98% of the radioactivity

was located at the corresponding zones. The cholesterol ester bands were located with iodine vapour and were scraped off into scintillation vials. The radioactivity of each sample was counted with a Beckman LS-250 liquid-scintillation system, in 10 ml of scintillator solution composed of 2 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene/litre of toluene. When the incubation system was constructed with the residual fraction and trace amounts of the substrates, the cholesterol ester mixture separated from the lipoprotein fraction of the same plasma was added before t.l.c. as a carrier. Cholesterol and lipid phosphorus were determined as described by Sperry & Webb (1950) and Gomori (1942) respectively. G.l.c. analyses of fatty acids were performed as previously described (Sugano, Imaizumi, Cho, Hori & Wada, 1969). Protein was determined either by measurement of E_{280} or by the Folin-Ciocalteu method, as modified by Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Lipid composition of the lipoprotein and residual fractions. Table 1 shows that the residual fraction of $d > 1.210$ separated from rat plasma contains detectable amounts of cholesterol and phospholipid. T.l.c. showed that the main phospholipid in the residual fraction was lysophosphatidylcholine, but phosphatidylcholine was also present in detectable amounts. The fatty acid compositions of these two phospholipids were similar to those of the same phospholipids in whole plasma and in lipoproteins.

Time-course of esterification. Fig. 1 shows the rate of cholesterol esterification by the rat plasma residual fraction at two concentrations of the lipoprotein substrate. Esterification proceeded linearly for 6 h and the distribution of the radioactivity in the different cholesterol esters was not influenced by the length of the incubation. If it is assumed that labelled cholesterol and non-labelled endogenous cholesterol participate equally in the reaction (cf. Portman & Sugano, 1964; Sugano & Portman, 1964), the amounts of cholesterol esterified correspond to 25–28 $\mu\text{g}/0.8 \text{ ml}$ of enzyme per 6 h.

Enzyme activity in lipoprotein and residual frac-

Table 1. *Chemical analyses of rat lipoprotein and residual fractions*

The values for the protein concentration of the residual fraction are the range determined throughout the experiments and those for the lipid components are the range of two determinations. The values for the lipoprotein fraction are the results of a single determination. Starvation was overnight (16 h).

Fractions	Protein (mg/ml)	Cholesterol ($\mu\text{mol/ml}$)	Phospholipid ($\mu\text{mol/ml}$)
Residual fraction			
Starved	43–49	0.019–0.023	0.17–0.20
Fed	45–50	0.021–0.026	0.20–0.23
Lipoprotein fraction			
Starved	7.9	2.98	2.65
Fed	8.0	3.08	2.90

Fig. 1. Effect of incubation time and substrate concentration on cholesterol esterification by the residual fraction of rat plasma. Pooled plasma was obtained from fed rats (male and female) weighing 230–435 g. The incubation system consisted of 0.8 ml of the residual fraction and either 0.2 ml (●) or 0.5 ml (○) of the substrate containing [4-¹⁴C]cholesterol in lipoproteins. Each point in (a) represents the average of two incubations. The mean distribution of radioactivity among the different cholesterol esters in these incubations is shown in (b): Δ, cholesteryl palmitate; ▲, cholesteryl oleate; ▼, cholesteryl linoleate; ▽, cholesteryl arachidonate; □, cholesteryl penta- and hexa-enoate.

tions. Table 2 compares [4-¹⁴C]cholesterol esterification by the lipoprotein and the residual fractions of rat plasma. The presence of activity in the lipoprotein fraction is in contrast to the report by Lossow *et al.* (1966), where ultracentrifugal separa-

tion of rat plasma lipoproteins by means of sodium bromide was reported to inhibit esterification seriously. The present results are, however, in accord with observations by Raz *et al.* (1969), who detected activity in unpurified lipoproteins from human plasma (see also Table 3). On the basis of unit amount of protein present in the enzyme fractions, the lipoprotein fraction had approximately 10 times the activity of the residual fraction, and more than half of the total activity remained in the lipoprotein fraction.

Table 2 also shows that the esterifying activity is not influenced by overnight starvation. However, starvation does affect the percentage distribution of radioactivity in the different cholesterol esters. With both the lipoprotein and residual fractions as enzyme sources, there is an increase in the percentage of radioactivity in the arachidonate esters and a decrease in that in the linoleate esters. These results are similar to those found in experiments with whole plasma (Swell & Law, 1967). Starvation causes similar changes in the fatty acid composition of plasma cholesterol esters and phosphatidylcholine fractions. Thus there is a decline in the percentage of linoleate and a rise in that of arachidonate (cf. Swell & Law, 1968b).

The results in Table 2 also suggest that the pattern of newly formed esters is determined by the enzyme rather than by the substrate. For example, the pattern of labelled esters formed by the residual fraction from the fed rat is independent of the source of the substrate.

Experiments similar to those carried out with rat plasma were performed with human plasma. The results in Table 3 indicate that both the lipoprotein and residual fractions are able to esterify cholesterol *in vitro*. However, the findings differ from those with rat plasma in two respects. First, the relative activity of the lipoprotein fraction is only twice that of the residual fraction, when comparison is made on the basis of unit concentration of protein present in the enzyme fractions. This observation, together with that made by Raz *et al.* (1969), suggests that dissociation of the acyltransferase from lipoproteins occurs more readily in human plasma than in rat plasma. Secondly, overnight starvation did not influence the esterification activity or the specificity of esterification by the two fractions. G.l.c. analyses of the fatty acid composition of plasma cholesterol esters and phosphatidylcholines also showed no differences between fed and starved subjects.

Effect of fatty acid composition of phosphatidylcholine substrate on esterification. In order to obtain more information about the enzyme in the residual fraction, different molecular species of phosphatidylcholines were separated from rat liver microsomes according to the degree of unsaturation of their

Table 2. *Cholesterol esterification in the lipoprotein and residual fraction of rat plasma in vitro*

Enzyme fraction (0.8 ml) was incubated with 0.2 ml of the lipoprotein substrate containing [4-¹⁴C]cholesterol at 37°C for 6 h. Male rats starved overnight and weighing 220–300 g were used as plasma donors for the enzyme preparation. The substrate was prepared from the plasma of male rats (380–490 g). Values are the means of four incubations and are corrected for heated control values obtained with preparations that had been kept at 60°C for 30 min. The percentage esterification in such controls was usually 0.02–0.03%. The cholesteryl palmitate also contained cholesteryl stearate, cholesteryl oleate also contained cholesteryl palmitoleate and cholesteryl arachidonate also contained cholesteryl penta- and hexa-enoate esters.

Enzyme		Source of lipoprotein substrate	[4- ¹⁴ C]Cholesterol esterified (%)	Radioactivity distribution in cholesterol esters (%)			
				Palmitate	Oleate	Linoleate	Arachidonate
Expt. 1							
Starved, plasma	Starved	32.8	7.2	4.3	25.0	63.5	
	Fed	37.9	7.1	4.1	22.5	66.3	
Fed, plasma	Starved	34.7	7.3	4.4	32.1	56.2	
	Fed	30.4	7.1	4.8	34.5	53.6	
Expt. 2							
Starved, residual fraction	Starved	7.9	9.5	6.7	19.6	64.2	
	Fed	8.3	6.8	7.9	20.7	64.6	
Fed, residual fraction	Starved	10.9	9.0	7.3	37.1	46.6	
	Fed	10.4	8.1	7.4	37.1	47.4	
Starved, lipoprotein	Starved	17.8	7.6	7.0	17.7	67.7	
	Fed	18.5	7.9	6.3	19.6	66.2	
Fed, lipoprotein	Starved	15.3	8.1	7.9	31.9	52.1	
	Fed	15.9	8.4	7.5	35.5	48.6	

Table 3. *Cholesterol esterification in the lipoprotein and residual fractions of human plasma in vitro*

The incubation conditions were as described in Table 2. Plasma for the enzyme and the substrate was obtained from healthy men (five persons per group). Starvation was overnight (16 h). Protein concentration of the lipoprotein and residual fractions was 4.8 mg and 42 mg per incubation respectively. Values are the means of three incubations and are corrected for heated controls.

Enzyme	Source of lipoprotein substrate	[4- ¹⁴ C]Cholesterol esterified (%)	Radioactivity distribution in cholesterol esters (%)			
			Palmitate	Oleate	Linoleate	Arachidonate
Starved, residual fraction	Starved	15.7	12.8	23.5	55.9	7.8
	Fed	16.2	13.0	23.8	55.4	7.8
Fed, residual fraction	Starved	15.6	13.4	23.7	55.0	7.9
	Fed	16.7	14.4	22.4	55.3	7.9
Starved, lipoprotein	Starved	3.6	11.7	21.9	58.2	8.2
	Fed	3.9	11.0	22.0	60.2	6.8
Fed, lipoprotein	Starved	4.1	11.7	22.4	58.7	7.2
	Fed	4.6	10.5	23.2	58.0	8.3

fatty acids (Table 4) and were added as a Tween 20 solution with [4-¹⁴C]cholesterol to the incubation mixture. Regardless of the substrates added, the rate of esterification of [4-¹⁴C]cholesterol was always higher with the residual fraction from the fed rats than with that from starved rats (Table 5). Even in the absence of any added phosphatidylcholine, some esterification of cholesterol occurred. This suggests that fatty acids in the residual fraction can be used for esterification of cholesterol. Addition of phosphatidylcholines with saturated or mono-unsaturated fatty acid at the 2-position lowered the

rate of esterification below this control rate. Phosphatidylcholine with a dioenic acid at the 2-position produced a slight increase in the rate above the control value, and those with tetra- and hexa-enoic acids gave marked increases in rate. Phosphatidylcholine appeared to be utilized as a substrate in preference to phosphatidylethanolamine.

The radioactivity distribution in the different cholesterol esters formed during the incubation showed that there was a distinct tendency for activity to be present in the ester fraction corresponding to the type of phosphatidylcholine added. Regard-

Table 4. *Fatty acid compositions of molecular species of phosphatidylcholines*

Rat liver microsomal phosphatidylcholine was fractionated according to the degree of unsaturation by argentation t.l.c. (Arvidson, 1968). Each species is classified by the fatty acids combined at the 2-position of the phosphatidylcholine.

Species	Fatty acids (%)								
	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:4}	C _{20:5}	C _{22:5}	C _{22:6}
Saturated*	98.9	—	1.1	—	—	—	—	—	—
Monounsaturated	36.4	3.2	19.8	38.6	2.0	—	—	—	—
Diunsaturated	16.8	0.6	30.5	2.1	50.0	—	—	—	—
Tetraunsaturated	9.5	0.4	38.5	1.0	0.9	49.6	—	—	—
Hexaunsaturated	17.6	Trace	38.2	1.0	0.3	2.3	4.7	1.2	34.7
Microsomal phosphatidylcholine	15.2	0.4	33.4	3.3	12.3	26.6	0.4	0.5	7.9
Microsomal phosphatidyl-ethanolamine	13.5	0.3	34.5	2.4	8.6	22.2	0.8	1.0	16.5

* (L- α -Dipalmitoyl) phosphatidylcholine commercially available.

Table 5. *Effects of phosphatidylcholines on cholesterol esterification by rat plasma residual fraction in vitro*

Residual fraction (1.0 ml) was incubated with 0.1 ml of the substrate in Tween 20 at 37°C for 6 h. The substrate contained 0.2 μ mol of [4-¹⁴C]cholesterol (0.2 μ Ci) and, where present, 0.4 μ mol of either various phosphatidylcholines or phosphatidylethanolamine. Male rats weighing 217–286 g were used as the source of the residual fraction. Starvation was overnight (16 h). Values are the means of two incubations and are corrected for heated controls. See also Tables 2 and 4. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Substrates	[4- ¹⁴ C]Cholesterol esterified (%)	Radioactivity distribution in cholesterol esters (%)				
		Palmitate	Oleate	Linoleate	Arachidonate	Penta- and hexa-enoate
Starved, residual fraction						
No PC added	0.47 ^a	13.0	8.2	23.1	47.4	8.3
Satd. PC	0.17	16.3	9.3	20.8	43.4	10.2
Monounsated. PC	0.24	18.3	27.6	19.4	29.0	5.7
Diunsatd. PC	0.57	11.4	9.7	43.0	30.5	5.4
Tetraunsatd. PC	5.34	4.1	3.0	5.3	84.5	3.1
Hexaunsatd. PC	5.79	7.0	3.5	9.6	32.5	47.4
Mixed PC*	1.04	10.6	10.2	13.8	50.9	14.8
Microsomal PC	3.00	6.0	5.8	15.1	62.3	10.8
Microsomal PE	1.15	8.6	7.3	15.9	56.4	11.8
Fed, residual fraction						
No PC added	0.71	12.1	9.8	32.3	32.1	13.7
Satd. PC	0.29	15.9	11.3	28.9	31.5	12.4
Monounsated. PC	0.47	15.2	25.1	26.6	22.8	10.8
Diunsatd. PC	1.11	10.4	9.3	45.6	25.3	9.4
Tetraunsatd. PC	7.61	4.6	3.4	12.0	74.3	5.7
Hexaunsatd. PC	7.88	6.7	5.3	18.2	27.2	42.6
Mixed PC*	2.07	9.5	9.7	22.9	42.8	15.0
Microsomal PC	3.75	6.4	6.3	22.8	51.9	12.6
Microsomal PE	2.21	8.2	7.6	24.7	44.9	14.6

* Equimolar mixture of satd., monounsated., diunsatd., tetraunsatd. and hexaunsatd. phosphatidylcholines.

less of the type of phosphatidylcholine added starvation resulted in preferential incorporation of cholesterol into the tetraenoic ester.

Similar types of experiments were performed with phosphatidylcholines containing different labelled fatty acids at the 2-position and with non-labelled cholesterol present as a complex with β -globulin. The results in Table 6 show that the relative rates

of esterification were higher with species of phosphatidylcholines containing polyunsaturated fatty acids than with those containing saturated or monounsaturated fatty acids. The patterns of radioactivity distribution among the different esters were identical in experiments with the residual fraction and with whole plasma from which the residual fraction was separated. Also, as shown in

Table 6. *Relative rate of cholesterol esterification with different labelled phosphatidylcholines in vitro*

Enzyme fraction (1.0 ml) was incubated with 0.1 ml of globulin-bound phosphatidylcholines labelled at the 2-position with different fatty acids at 37°C for 6 h. Male rats weighing 220–256 g were used as the enzyme source. Starvation was overnight (15 h). Values are the means of two incubations. See also Table 5. 'Dialysed plasma' was sham-treated with NaBr and dialysed as for the preparation of the residual fraction.

Relative rate of incorporation of fatty acids combined at the 2-position of phosphatidylcholines into cholesterol esters

Enzyme fractions	Palmitate	Oleate	Linoleate	Arachidonate	Mixed*
Fresh plasma					
Starved	1.00	1.54	1.64	3.03	1.82
Fed	1.00	1.57	1.82	2.44	2.22
Dialysed plasma					
Starved	1.00	Not run	1.67	2.72	Not run
Fed	1.00	Not run	1.87	2.23	Not run
Residual fraction					
Starved	1.00	1.03	1.59	2.40	1.53
Fed	1.00	1.16	1.77	2.01	1.73

* Equimolar mixture of palmitate, oleate, linoleate and arachidonate esters of phosphatidylcholines.

Table 7. *Relative rate of cholesterol esterification with [4-¹⁴C]cholesterol in vitro*

Enzyme fraction (1.0 ml) was incubated with 0.1 ml of the substrate that had been complexed with globulin. The substrate contained rat liver microsomal phosphatidylcholine (0.4 μ mol) and [4-¹⁴C]cholesterol (0.2 μ mol, 0.2 μ Ci). Male rats weighing 222–257 g were used as the enzyme source. Values are the means of two incubations. For experimental details, see Table 6.

		Radioactivity distribution in cholesterol esters (%)				
Enzyme sources	[4- ¹⁴ C]Cholesterol esterified (%)	Palmitate	Oleate	Linoleate	Arachidonate	Penta- and hexa-enoate
Phosphatidylcholine added						
Fresh plasma						
Starved	17.1	7.4	3.8	24.7	57.1	7.0
Fed	15.0	7.6	4.8	36.7	41.4	9.5
Dialysed plasma						
Starved	15.6	8.5	4.2	25.9	55.0	6.4
Fed	15.4	8.3	5.3	37.9	39.8	8.7
Residual fraction						
Starved	6.5	8.1	4.2	26.2	53.9	7.7
Fed	7.3	8.1	4.7	37.4	39.8	10.1
No phosphatidylcholine added						
Fresh plasma						
Starved	18.9	7.6	4.2	24.5	57.5	6.2
Fed	17.9	8.0	5.0	37.3	41.0	8.8
Dialysed plasma						
Starved	15.8	8.2	4.3	25.9	55.1	6.5
Fed	14.7	8.1	5.0	38.7	39.5	8.5

Table 7, exposure of plasma to sodium bromide and subsequent dialysis had no effect either on the relative rate of esterification of labelled cholesterol or on the distribution of radioactivity in the individual esters formed during the incubation. Free fatty acids (palmitic acid, oleic acid, linoleic acid and arachidonic acid) were not incorporated into cholesterol esters when either plasma or the residual fraction was used as enzyme source.

DISCUSSION

The present study is concerned with the presence of acyltransferase activity in a rat plasma fraction containing proteins of $d > 1.210$. The properties of the enzyme in this fraction are similar to those of the enzyme in whole plasma and in lipoproteins, in so far as the rate of transfer of different fatty acyl molecules combined at the 2-position of phosphatidylcholines and the effect of starvation on the

behaviour of the enzymes is concerned. These results suggest, therefore, that ultracentrifugal floatation may be applicable as an initial step for the purification of the acyltransferase. The lower recovery of the enzyme in the fraction of $d > 1.210$ may be overcome by repeating the centrifugation as indicated by Raz *et al.* (1969).

Starvation of rats overnight or for longer periods has been shown to lower the percentage of linoleate and to raise that of arachidonate in the plasma cholesterol esters and to alter the relative rates of synthesis of individual cholesterol esters by the plasma acyltransferase in a corresponding fashion (Albers & Gordon, 1962; Swell & Law, 1967, 1968a,b; Sugano, Hori & Wada, 1969). The fatty acid composition of the plasma phosphatidylcholines is changed in a similar manner (Swell & Law, 1968b). In view of the mechanism of cholesterol esterification, it would appear that the change in the fatty acid composition of the phosphatidylcholines is responsible for alterations in the composition of the cholesterol esters both *in vitro* (Swell & Law, 1967, 1968b; Sugano *et al.* 1969) and *in vivo* (Swell & Law, 1968a). Table 5 suggests that this also applies when the enzyme is present in the plasma residual fraction. However, Table 2 shows that the pattern of newly formed labelled cholesterol esters is also influenced by the source of the enzyme. These different observations may not be fully explained until more detailed kinetic data for the purified enzyme and with substrates in a physiological form are available.

The low rate of cholesterol esterification observed with (2-palmitoyl)- or (2-oleoyl)-phosphatidylcholine is consistent with the observation that (distearoyl)- and (dioleoyl)-phosphatidylcholine, but not (dimyristoyl) phosphatidylcholine, lower the rate of esterification by human serum (Wagner, 1959). However, the effects of the different types of phosphatidylcholines on the esterification reaction may also be due to differences in their form when they are added to the incubation systems.

In the present study, the relative rates of transfer of the different types of acyl molecules from the 2-position of the phosphatidylcholine molecules were not directly related to the concentration of the corresponding cholesterol esters present in the plasma. This finding is in accord with observations made by Shah *et al.* (1964) and reviewed by Stefanovich (1969) that, with acetone-dried-powder extracts of rat plasma as an enzyme source, the percentage compositions of labelled cholesterol esters formed *in vitro* from phosphatidylcholines with labelled palmitic acid, oleic acid and linoleic acid in the 2-position differed from the corresponding compositions of the cholesterol esters present in the plasma, except for cholesteryl palmitate. In contrast, the cholesterol ester pattern obtained by the

action of the acyltransferase of rabbit plasma *in vitro* is reported to be virtually identical with the fatty acid pattern of the cholesterol esters present in the plasma (Stefanovich, 1969). One possible explanation is that the concentration of cholesteryl arachidonate is much higher in rat plasma than in the plasma of other experimental animals. Also, there are species-specific differences in the relative rates of metabolism of cholesterol esters (cf. Goodman, 1965).

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