Cholesterol Esterification in Rabbit Plasma

BY V. STEFANOVICH

Department of Pathology, University Hospital and Boston University Medical School,
Boston, Mass. 02118, U.S.A.

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1. When [4-¹⁴C]cholesterol, attached to β -globulin or dispersed with Tween 20, was incubated with fresh rabbit (New Zealand albino females) plasma, 30–47% esterification was observed. The optimum pH was 6.8. This esterification was accomplished by the transfer of fatty acids from the C-2 position of lecithin (phosphatidylcholine) to cholesterol. 2. There was no evidence that triglycerides or free fatty acids participated directly in this reaction. Lecithins with labelled palmitic acid, oleic acid and linoleic acid in the 2-position yielded 3.2, 4.8 and 6.8% of cholesteryl esters respectively. This pattern reflects that which is normally observed in the cholesteryl esters of rabbit plasma and supports the concept that plasma cholesteryl esters originate from the plasma. 3. Snake venom (containing phospholipase A), sulphoevernan [an α -(1 $\rightarrow$ 3,1 $\rightarrow$ 4)-sulphopolyglucan with 12% sulphur], thiol-blocking agents (*p*-chloromercuribenzoate and *N*-ethylmaleimide), or an atherogenic diet (stock diet supplemented with 1% cholesterol for 8 weeks) were all effective inhibitors of this cholesterol esterification.

The esterification of the free cholesterol in human plasma *in vitro* was first reported by Sperry (1935). The studies by Glomset, Parker, Tjaden & Williams (1962) and Glomset (1962) showed that the linoleic acid from the C-2 position of lecithin (phosphatidylcholine) and the palmitic acid from the tripalmitin could esterify cholesterol in human plasma *in vitro*. Free fatty acids did not participate in this reaction. In later work (Glomset & Wright, 1964) some of the properties of this acyltransferase were established and a preliminary purification of the enzyme was achieved.

The mechanism of cholesterol esterification in rat plasma involving the transfer of fatty acids (palmitic acid, oleic acid and linoleic acid) from the 2-position of lecithin to cholesterol was established by Shah, Lossow & Chaikoff (1964). The role and the properties of a plasma lecithin-cholesterol acyltransferase were reviewed by Glomset (1968).

On the basis of these and other considerations, the acyltransferase reaction appears to be important for several reasons: (a) it is a reaction in which a prominent sterol, cholesterol, is esterified with fatty acids in humans and other animals; (b) it appears to be a significant and perhaps major source of plasma cholesteryl esters, which are believed to accumulate in early atherosclerotic lesions (Smith, 1965); (c) it has some promise as a tool for a study of lipoprotein structure (Glomset, Janssen, Kennedy & Dobbins, 1966); (d) changes in cholesterol-

esterifying activity have also been observed in other diseases (e.g. obstructive jaundice and kala-azar; Mendoza & Diaz, 1949) and a new inborn error of metabolism characterized by the lack of an acyltransferase has been reported (Norum & Gjone, 1967; Gjone, Torsvik & Norum, 1968).

The aim of my investigation was to study the properties of the enzyme responsible for cholesterol esterification in rabbit plasma; to determine if this enzyme is an acyltransferase, and then to prepare lecithins with various 1-¹⁴C-labelled fatty acids in the 2-position in order to compare the ratios of fatty acids in labelled cholesteryl esters formed with the normal pattern of cholesteryl esters present in rabbit plasma. Further, these experiments were carried out to investigate the possible role of triglycerides and free fatty acids in the esterification of cholesterol, and finally to search for specific inhibitors of cholesterol esterification as a possible means to study the control of cholesterol biosynthesis.

A preliminary report of this work has been presented (Stefanovich & Gore, 1968).

MATERIALS

[4-¹⁴C]Cholesterol, [1-¹⁴C]palmitic acid, [1-¹⁴C]oleic acid and [1-¹⁴C]linoleic acid were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. Lecithins with [1-¹⁴C]-palmitic acid, [1-¹⁴C]oleic acid and [1-¹⁴C]linoleic acid in the

2-position were prepared by the method of Robertson & Lands (1962) with lysolecithin obtained from Applied Science Laboratories, State College, Pa., U.S.A. [$1\text{-}^{14}\text{C}$]-Palmitoyl and [$1\text{-}^{14}\text{C}$]oleoyl triglycerides were obtained from Nuclear-Chicago Corp., Chicago, Ill., U.S.A. Rabbit β -globulin (fraction III), *p*-chloromercuribenzoate and *N*-ethylmaleimide were obtained from Mann Research Laboratories, New York, N.Y., U.S.A. Tween 20, EDTA and heparin (157 units/mg.) were obtained from Fisher Scientific Co., Fairlawn, N.J., U.S.A. *Crotalus adamanteus* (Eastern diamondback rattlesnake) venom was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Silica gel G was obtained from E. Merck A.-G., Darmstadt, Germany. Sulphoevernan was prepared from *Evernia prunastri* (oak moss) (Stefanovich, 1967).

METHODS

In a typical experiment 2 ml. of fresh rabbit plasma (obtained after centrifugation of blood for 15 min. at 1000g, with pH adjusted to 6.8 with 0.5M-HCl, drawn with a heparinized syringe from New Zealand albino female rabbits) was added to a test tube containing 1×10^5 d.p.m. of a labelled substrate previously dissolved in 0.2 ml. of ethanol containing 0.5% of Tween 20. The ethanol was evaporated under N_2 before the plasma was added. When β -globulin was employed as a carrier, Avigan's (1959) method, as outlined by Shah, Lossow & Chaikoff (1964), was followed.

The incubation was performed in a Dubnoff shaker at 37° for 20 hr. or as indicated. When the influence of a particular agent on the reaction was investigated, the incubation was carried out for 1 hr. with the agent but without the labelled substrate, the substrate was then added and incubation continued for 19 hr. The incubation was stopped by adding methanol (10 ml.) and centrifuging off the precipitate. After two additional extractions with chloroform (10 ml.) and methanol (10 ml.) the combined supernatants were dried over Na_2SO_4 , filtered and evaporated to dryness in a flash evaporator. The residue was dissolved by trituration with 2 ml. of methanol and with three 1 ml. portions of chloroform, transferred to a centrifuge tube and evaporated to dryness under N_2 . For t.l.c., the residue was dissolved in 0.5 ml. of methanol-chloroform (1:1, v/v). A portion was applied to a silica gel G t.l.c. plate and lipids were separated into classes by development with hexane-diethyl ether-acetic acid (225:70:6, by vol.). Lecithin had R_F 0.02, cholesterol R_F 0.21 and cholesteryl ester group R_F 0.93.

When all three labelled lecithins were employed simultaneously, the resulting cholesteryl esters were separated into the individual components by a subsequent t.l.c. of the cholesteryl ester fraction (Goodman & Shiratori, 1964). Corresponding lipid standards and a brief exposure to iodine vapour were employed to identify the position of substances of interest. After evaporation of the iodine, the silica gel layer with the desired lipid fraction was scraped into a glass gooch and the lipid eluted with methanol directly into the scintillation vial. Methanol was evaporated to dryness under N_2 and 20 ml. of toluene containing 0.3% of 2,5-diphenyloxazole and 0.01% of 1,4-bis-(5-phenyloxazol-2-yl)benzene was added. The radioactivity was measured in the Packard Tri-Carb liquid-scintillation spectrometer to an error of 3%. The quenching in each sample was calculated by the addition of a known quantity of internal standard. For the determination of free chol-

esterol and cholesteryl esters in rabbit plasma, lipids from plasma (2 ml.) were extracted by established procedures (Folch, Lees & Sloane-Stanley, 1957). A portion of the total lipid extract was subjected to t.l.c. on silica gel G with hexane-diethyl ether-acetic acid (225:70:6, by vol.) for development. Two fractions containing cholesteryl esters (R_F 0.95) and free cholesterol (R_F 0.25) were scraped and eluted twice with 3 ml. of methanol. After evaporation of the solvent to dryness under N_2 , cholesteryl esters were hydrolysed and their fatty acids methylated as follows: 33% (w/v) KOH (1 ml.) and ethanol (9 ml.) were added and samples placed in water bath at 55° for 30 min. After cooling to room temperature samples were acidified with 6M-HCl, extracted with two 5 ml. portions of light petroleum (b.p. 30–60°) and evaporated to dryness under N_2 . Fatty acids were methylated with 10% boron trifluoride in methanol (2 ml.) by heating in a boiling-water bath for 2 min. The fatty acid methyl esters were extracted with two 5 ml. portions of light petroleum (b.p. 30–60°), which was then concentrated under N_2 , transferred to a micro test tube and evaporated to dryness under N_2 . The residue was dissolved usually in 20 or 50 μl . of hexane and a sample subjected to g.l.c. An F & M model 402 gas chromatograph with a hydrogen flame ionization detector was employed in the analysis. For fatty acid methyl esters a glass column containing 6% of diethylene glycol succinate coated on Diatoport S (60–80 mesh) was employed; column oven temperature was programmed from 120° to 200°. For determinations of free cholesterol, a glass column with 6% SE-30 on Diatoport S (60–80 mesh) was employed. Column oven temperature was 230° and in both cases the carrier gas was N_2 . Reference compounds were run simultaneously with experimental samples to provide identification of compounds and calibration peaks for quantitative determinations.

In experiments with rabbits, 20 New Zealand albino female rabbits were divided into two groups: one group was fed on a stock diet (Purina rabbit chow) and the second group was fed on a cholesterol diet (Purina rabbit chow fortified with 1% cholesterol). Sulphoevernan (25 mg.) was administered (three times a week, subcutaneously) to five rabbits in each group, the remaining rabbits serving as controls. After 8 weeks plasma from both groups of rabbits was examined for cholesterol esterification. [$4\text{-}^{14}\text{C}$]-Cholesterol and the above method were employed.

RESULTS

Fig. 1 shows the extent of esterification of cholesterol at various times of incubations or at different pH values or concentrations of enzymes. The esterification activity is linear for more than 1 hr.; the pH optimum was about 6.75. The enzyme activity in rabbit plasma appears to increase linearly with enzyme concentration contributed by 0.4–2.0 ml. of fresh plasma added to 1.6–0.0 ml. of plasma inactivated by heating at 60° for 30 min.

Table 1 shows the influence of the various agents on cholesterol esterification in rabbit plasma. In the absence of an inhibitor, about 50% of the cholesterol is esterified. The significant inhibition of esterification with thiol blocking agents (*p*-

Fig. 1. Effect of incubation time (a), enzyme concentration (b) and pH (c) on cholesterol esterification in rabbit plasma. Experiments were conducted as described in the text. All points are averages of three determinations.

Table 1. Influence of various agents on cholesterol esterification in rabbit plasma *in vitro*

Experiments were conducted as described in the text. The results given are the means of four determinations.

Labelled substrate (10^5 d.p.m.)	Agent	Esterification (%)
[4- 14 C]Cholesterol	None	48
[4- 14 C]Cholesterol	Sodium taurocholate (10 mM)	0.3
[4- 14 C]Cholesterol	<i>p</i> -Chloromercuribenzoate (mM)	0.2
[4- 14 C]Cholesterol	<i>N</i> -Ethylmaleimide (mM)	0.2
[4- 14 C]Cholesterol	EDTA (10 mM)	62
[4- 14 C]Cholesterol	Heparin (500 units)	45
[4- 14 C]Cholesterol	Sulphoevernan (0.4 μ M)	3
[4- 14 C]Cholesterol	<i>Crot. adamanteus</i> venom (3 mg.)	0.5
(2-[1- 14 C]Oleoyl) lecithin	<i>Crot. adamanteus</i> venom (3 mg.)	0.4
(2-[1- 14 C]Oleoyl) lecithin	None	8

chloromercuribenzoate and *N*-ethylmaleimide) suggests that one or more of the thiol groups forms a part of the active centre of the enzyme. Snake venom (containing phospholipase A) completely prevented the formation of labelled cholesteryl esters regardless of whether [4- 14 C]cholesterol or lecithin with [1- 14 C]oleic acid in the 2-position was employed. This indicates that a fatty acid from the 2-position of lecithin is involved in cholesterol esterification. EDTA increased the degree of cholesterol esterification. Heparin was without effect. The sulphoevernan, a semi-synthetic α -(1 $\rightarrow$ 3,1 $\rightarrow$ 4)-sulphopolyglucan with molecular weight 20×10^3 and sulphur content 12%, inhibits cholesterol esterification. The esterification was also inhibited by 10 mM-sodium taurocholate.

Table 2 shows the percentage of 14 C in the cholesteryl esters. When [4- 14 C]cholesterol was combined with β -globulin, only 30% of cholesterol was esterified. When Tween 20 was used as a dispersing agent, esterification in the 50% range was usually accomplished. The lecithins with labelled fatty acids in the 2-position were found to

serve as donors of fatty acids for cholesteryl esters. On the other hand, neither triglycerides nor free fatty acids could serve directly as donors of fatty acids. A very small quantity of cholesteryl esters was sometimes observed when plasma was incubated with labelled triglycerides or with free fatty acids. This could be tentatively explained by a correspondingly small amount of labelled phospholipids that may serve as donors of fatty acids.

Studies of the influence of the inhibitors of the acyltransferase reaction in rabbit plasma showed that sulphoevernan inhibits cholesterol esterification in rabbit plasma *in vitro* (Table 1). Table 3 shows that administration of sulphoevernan significantly inhibits cholesterol esterification in the plasma of healthy rabbits and in the plasma of rabbits fed on an atherosclerotic diet for 8 weeks. Feeding with an atherogenic diet, however, results in an inhibition of cholesterol esterification in rabbit plasma *in vitro* to 10% of the extent of esterification observed in plasma from stock-fed animals.

The results in Table 4 show the fatty acid pattern in the cholesteryl ester fractions of a New Zealand

Table 2. *Incorporation of labelled substrates into cholesteryl esters by rabbit plasma in vitro*

Experiments were conducted as described in the text. The results given are means of four determinations.

Labelled substrate (5×10^4 d.p.m.)	Form in which substrate was added	Esterification (%)
[4- 14 C]Cholesterol	β -Globulin	30
[4- 14 C]Cholesterol	Tween 20	47
(2-[1- 14 C]Palmitoyl) lecithin	Tween 20	3.2
(2-[1- 14 C]Oleoyl) lecithin	Tween 20	4.8
(2-[1- 14 C]Linoleoyl) lecithin	Tween 20	6.8
[1- 14 C]Palmitoyl triglycerides	Tween 20	0.4
[1- 14 C]Oleoyl triglycerides	Tween 20	0.7
[1- 14 C]Palmitic acid	Tween 20	0.3
[1- 14 C]Oleic acid	Tween 20	0.2
[1- 14 C]Linoleic acid	Tween 20	0.2

Table 3. *Effect of sulphoevernan administration on esterification of cholesterol in rabbit plasma in vitro*

Experiments were conducted as described in the text. The results given are the means $\pm$ s.e.m. of five determinations.

	Esterification (%)	
	Stock diet	Cholesterol diet (1%)
Control group	48.54 $\pm$ 1.96	5.22 $\pm$ 0.43
Sulphoevernan group	38.90 $\pm$ 2.29*	2.20 $\pm$ 0.30†

* Differs significantly ($P < 0.05$) from control group on stock diet.

† Differs significantly ($P < 0.05$) from control group on cholesterol diet.

albino rabbit (female). Also presented is the pattern of the fatty acids of the cholesteryl esters obtained by the incubation of plasma of the same five rabbits with a mixture of three lecithins with labelled palmitic acid, oleic acid and linoleic acid in the 2-position. It is obvious from Table 4 that the cholesteryl ester pattern obtained by the action of the lecithin-cholesterol acyltransferase is virtually identical with the fatty acid pattern of the cholesteryl esters present in rabbit plasma *in vivo*. This indicates that the acyltransferase reaction might be the source of the cholesteryl esters in rabbit plasma.

DISCUSSION

The presence of a lecithin-cholesterol acyltransferase for mediating a transfer of a fatty acid from the 2-position of lecithin to cholesterol has been reported in human and rat plasma (Glomset *et al.* 1962; Shah *et al.* 1964). The present study is concerned with the presence of an acyltransferase in rabbit plasma. Although the enzyme in all

three species apparently shows the same reaction mechanism and is susceptible to inhibition by the same agents, the optimum pH of this enzyme varies [7.4 for rat plasma (Shah *et al.* 1964); 6.8 for rabbit plasma; 7.5–8.5 for human plasma (Glomset & Wright, 1964)]. Also, the reaction rate of cholesterol esterification appears to vary with the species (Table 5). Although it would be attractive to compare the rate of plasma cholesterol esterification with species susceptibility to atherosclerosis (e.g. the rate of cholesterol esterification *in vitro* is high in susceptible rabbits and low in rats, which are relatively resistant) the small number of species examined so far does not allow for any valid conclusions on this relationship.

The complete inhibition of cholesterol esterification in the plasma of rabbits maintained for 2 months on a laboratory chow diet fortified with 2% cholesterol and 6% corn oil has been reported (Shapiro, Jastremsky & Kritchevsky, 1968). The inhibition of cholesterol esterification in human plasma by extrinsic cholesterol was reported by Wagner & Rogalski (1952). In my experiments with rabbit plasma the esterification of cholesterol was inhibited 90% by an atherogenic diet given for 8 weeks. A definite explanation for the inhibition of cholesterol esterification by an excess of cholesterol or by a cholesterol diet is not possible at the present time. The inhibition of cholesterol esterification by administration of sulphoevernan observed in my experiments suggests that a study of sulphoevernan as a potential antiatherogenic agent could be worth while. Such studies should include the effects on cholesterol accumulation in other tissues.

When studying the mechanism and the physiological significance of the lecithin-cholesterol acyltransferase reaction in human plasma, Glomset *et al.* (1962) employed only one lecithin, labelled with [1- 14 C]linoleic acid in the 2-position, to study the transfer of the fatty acid from the 2-position of lecithin to cholesterol. Supporting evidence for

Table 4. *Fatty acids of cholesteryl esters of rabbit plasma in vivo compared with those formed by the acyltransferase reaction in vitro*

The results for rabbit plasma are given as percentages of fatty acids in a cholesteryl ester fraction (average of five animals), with the relative proportions of palmitic acid, oleic acid and linoleic acid in parentheses. These cholesteryl ester fatty acids were determined as described in the Methods section. The results for the acyltransferase reaction are given as the percentages of fatty acids transferred from the 2-position of lecithin to cholesterol during incubation *in vitro* with plasma of the same rabbits, with the relative proportions of palmitic acid, oleic acid and linoleic acid in parentheses. Experiments were conducted as described in the Methods section.

Fatty acid	...	...	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}
Fresh rabbit plasma			1.5%	16.9%	6.9%	4.6%	27.3%	42.7%
(proportions)				(1.0)			(1.6)	(2.5)
Acyltransferase reaction				3.2%			4.8%	6.8%
(proportions)				(1.0)			(1.5)	(2.1)

Table 5. *Rates of esterification of plasma cholesterol in vitro in different animal species*

Species	Rate (μmole/hr./ml.)
Man	0.097*
Cebus monkey	0.24†
Rat	0.08†
Rabbit	0.19‡
Chicken, White Leghorn, males	0.13§
Chicken, White Leghorn, females	0.08§
Chicken, White Leghorn, laying females	0.04§

* Mean values reported in different studies: Glomset (1962), Portman & Sugano (1964) and Monger & Nestel (1967).

† Sugano & Portman (1964).

‡ Determined after 4 hr. of incubation with [4-¹⁴C]-cholesterol; experimental conditions as described in the text.

§ Sugano, Chinen & Wada (1965).

this mechanism is also provided by the inhibition of cholesterol esterification by snake venom (containing phospholipase A, which hydrolyses lecithin to lysolecithin) and by depletion of plasma lecithin and an increase in lysolecithin after esterification of cholesterol (Rowen, 1964). The pattern of cholesterol esterification was also studied by incubating active human plasma with inactivated rat plasma and [¹⁴C]cholesterol and vice versa (Portman & Sugano, 1964). It was found that the pattern of newly formed labelled cholesteryl esters resembled that existing in active plasma more closely than that existing in the inactivated plasma acting as a substrate.

The only study using lecithins with labelled palmitic acid, oleic acid and linoleic acid, in the 2-position, employed acetone-dried-powder extracts of rat plasma (Shah *et al.* 1964). The percentage of labelled cholesteryl esters obtained after incubation (with Tween 20 as dispersing agent) was: cholesteryl palmitate, oleate and linoleate, 13.15, 12.90 and

24.35% respectively. However, these authors did not compare the pattern of labelled esterified cholesteryl esters formed during cholesterol esterification *in vitro* with the composition in fatty acids of cholesteryl esters present in normal rat plasma. From the two sets of data reported in the literature (Goodman, 1962; Apostolakis, Grimmer, Glaser & Voigt, 1962), the percentage of cholesteryl palmitate, oleate and linoleate in rat plasma was about 14, 17 and 33%. These values, with the exception of cholesteryl palmitate, differ from the above values obtained *in vitro* (Shah *et al.* 1964). It is difficult to evaluate such comparisons, but the possibility that some other enzyme besides plasma lecithin-cholesterol acyltransferase is contributing to the cholesteryl ester fraction of rat plasma cannot be discounted. The decrease of the acyltransferase activity seen in the eviscerated rat by Brot, Lossow & Chaikoff (1962) was taken by these authors to indicate that acyltransferase is synthesized in the liver. But one can also conclude that the cholesteryl esters in general are synthesized in the liver. Further, the liver has an active cholesterol-esterifying enzyme system (Akiyama, Minari & Sakagami, 1967; Goodman, Deykin & Shiratori, 1964). It has also been reported (Swell & Law, 1966) that, after the injection of labelled cholesterol, liver cholesteryl esters have a higher specific radioactivity than plasma cholesteryl esters.

However, my results obtained with rabbit plasma (Table 4) indicate that the plasma lecithin-cholesterol acyltransferase reaction might be a major source of rabbit plasma cholesteryl esters, but the hepatic origin of these esters cannot be discounted.

The role of lecithin as a donor of fatty acid in cholesterol esterification could be related to a complex-formation between lecithin and cholesterol. Dervichian (1958) has reported that cholesterol will bring about the condensation of a lecithin monolayer. The general inference from this observation seems to be that the packing of

Scheme 1. Hypothetical scheme of cholesterol esterification by lecithin-cholesterol acyltransferase in plasma.

lecithin and cholesterol molecules together is more efficient than the packing of lecithin alone. In experiments with a 1:1 mixture of cholesterol and a synthetic (1-stearoyl-2-oleoyl) lecithin, Van Deenen (1966) found that cholesterol decreases the cross-sectional (molecular) area of lecithin from $75\text{\AA}^2$ to $64\text{\AA}^2$. Chapman & Penkett (1966) have published n.m.r. data showing that the freedom of motion of the lecithin side chains is greatly diminished in the presence of cholesterol, suggesting intensive interaction of the hydrocarbon moieties. Also, Zull, Greanoff & Adam (1968) have reported i.r.-spectral data that indicate that in films cast from organic solvents cholesterol can form a 2:1 complex with lecithin involving the interaction of cholesterol hydroxyl groups with lecithin polar groups. The authors proposed a model that invokes hydrogen-bonding of cholesterol with the ester phosphate oxygen atoms of lecithin.

The role of the different plasma lipoproteins in the cholesterol esterification reaction was studied in human and baboon plasma by Glomset *et al.* (1966) and Glomset (1968). By incubating human plasma for 24 hr. (Glomset, 1963) and then fractionating the lipoproteins by ultracentrifugal flotation, it was found that the molar changes in free cholesterol, lecithin and lysolecithin were approximately proportional to the original concentrations of these compounds in each lipoprotein fraction. The total amount of these compounds did change, however. The major decrease in lecithin was found in the high-density lipoproteins, and the largest decrease in free cholesterol and increase in esterified

cholesterol was observed in the low-density lipoprotein fraction. Most of the lysolecithin, however, was found in the protein fraction with a density greater than 1.21. It could be assumed that a similar situation exists in human and rabbit plasma for the role of lipoproteins in lipid transport. The recent study of the composition of plasma lipoproteins in various animal species, including rabbits, has indicated general similarities in lipoprotein classes in animals and humans (Fried, *et al.* 1969).

Taking the above information into account, the following hypothetical scheme of cholesterol esterification can be suggested (Scheme 1). Lecithin and cholesterol are carried in relatively high amounts in the blood stream by α -lipoprotein and β -lipoprotein respectively and are brought into contact with the enzyme acyltransferase, which has at least one thiol group in the active site. The configuration of the active site will accept only the cholesterol-lycithin complex. The transfer of the acyl group from the 2-position of lecithin is accomplished via an S-acyl-enzyme intermediate. After the reaction the lysolecithin and the cholesteryl esters, which do not form a complex and therefore do not fit the configuration of the active site, are ejected from the enzyme. The lysolecithin is carried away by albumin (Switzer & Eder, 1965) and the cholesteryl esters, at least transiently, by α -lipoprotein (Glomset *et al.* 1966).

However, this scheme is clearly hypothetical and it should serve only as a model for future work on the esterification of cholesterol in plasma.

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