review

The plasma lecithin:cholesterol acyltransferase reaction

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ABSTRACT Evidence for the existence of a plasma lecithin: cholesterol acyltransferase is reviewed with emphasis not only on the lipid reactants, but also on the lipoprotein "substrates" and "products." The cholesteryl esters of all major lipoprotein classes become labeled when plasma is incubated with cholesterol-^{14}C. However, the smaller, lecithin-rich high density lipoproteins appear to be preferred substrates. Most studies of factors that influence the acyltransferase reaction have not adequately distinguished between effects on the enzyme and effects on the lipoprotein substrates. However, the fact that cholesterol esterification is diminished in plasma from eviscerated animals or from patients with reduced liver function suggests that the liver may regulate both the level of the enzyme and that of the substrates. Several indications exist that the acyltransferase reaction is the major source of plasma esterified cholesterol in man. Furthermore, the reaction may have a broader, extracellular function. One possibility is that it plays a role in the transport of cholesterol from peripheral tissues to the liver.

KEY WORDS acyltransferase · plasma · lecithin · cholesterol · cholesteryl ester · lysolecithin · lipoproteins · cholesterol transport · peripheral tissues · liver

A NET ESTERIFICATION of cholesterol occurs when plasma, serum, or whole blood is incubated at 37°C. The enzyme that catalyzes the reaction is present in higher concentrations in plasma than in other tissues, and mainly transfers fatty acids from the 2-position of lecithin to cholesterol. For this reason it can be regarded as a plasma lecithin:cholesterol acyltransferase, although the character of the reaction may depend as much on the nature of the lipoprotein substrates as on the specificity of the enzyme.

The acyltransferase reaction is of interest for several reasons. For example, it appears to be a physiologically important source of plasma cholesteryl esters; an inborn error of metabolism in which the reaction does not occur has recently been discovered; and the reaction shows promise as a tool for studying lipoprotein structure. The purpose of the present review is to discuss the available evidence concerning the mechanism of the reaction, the properties of the enzyme, and the factors and conditions that influence the reaction. In addition, the possible physiological significance of the reaction will be considered.

MECHANISM OF THE REACTION

Lipid Reactants

The plasma cholesterol esterification reaction was first described by Sperry (1). He found that maximal esterification of the cholesterol of human plasma occurred at about pH 8, and that the change in cholesterol could be prevented by prior heating of the plasma at 50–60°C. At first he believed that the reaction was catalyzed by a plasma cholesterol esterase, and suggested that the necessary fatty acids might be derived from plasma phospholipids by the action of a lipase. Later, however, Sperry and Stoyanoff postulated the existence of a special cholesterol-esterifying enzyme to explain their observations (2–4) of the divergent effects of bile salts on the cholesteryl esters of dog, human, and monkey sera. At concentrations of 4–6 μmoles/ml, bile salts inhibited cholesterol esterification in the sera of all three species, but at higher concentrations they promoted the hydroly-

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; DEAE-cellulose, diethylaminoethyl cellulose.

sis of cholesteryl esters in dog serum alone. Because of these findings, it was concluded that dog serum contains separate cholesterol-esterifying and cholesteryl ester-hydrolyzing enzymes, whereas human and monkey sera contain only the former.

Sperry's observation of the increase in esterified cholesterol during the incubation of serum or plasma was soon confirmed (5, 6), as was his finding (4) that the change could not be accounted for by bacterial contamination (7). Furthermore, Le Breton and Pantaléon (7) obtained evidence that plasma phospholipids indeed might be the source of fatty acids in the esterification reaction. They showed that acetone-precipitable lipid decreased during the incubation of serum, that this decrease and the decrease in unesterified cholesterol could be prevented by adding phlorizin or monobromoacetate, and that adding ovolecithin increased the amount of cholesterol esterified. Because serum incubated for 72 hr did not hemolyze red cells, and because they could not detect acetone-precipitable lipid in some samples of incubated serum, they concluded that the decrease in "phosphoaminolipid" was caused by a lecithinase B. Finally, they proposed a mechanism for the plasma cholesterol esterification reaction that involved the coupled action of the lecithinase B and a plasma cholesterol esterase:

$$\text{lecithin} \xrightarrow{\text{lecithinase B}} 2 \text{ fatty acids} + \text{glycerophosphoryl choline};$$

$$\text{cholesterol} + \text{fatty acid} \xrightarrow{\text{cholesterol esterase}} \text{cholesteryl ester}.$$

Later, their observation concerning the effect of added lecithin was confirmed by Wagner (8–10) and Murphy (11); and Etienne and Polonovski (12–14) directly demonstrated a decrease in lecithin and an increase in glycerophosphoryl choline in extracts of incubated serum.

During their experiments, Etienne and Polonovski (12–14) also found indications that the changes in plasma phospholipid might be more complicated than originally envisaged by Le Breton and Pantaléon. They showed that lysolecithin transiently increased and that ultimately free choline increased when human serum was incubated for 72 hr. These findings are compatible with the possibility that more than one enzyme may be involved in the breakdown of phospholipid during prolonged incubations of plasma. Moreover, my colleagues and I (15, 16) subsequently obtained results which led us to propose that the initial breakdown of lecithin is not caused by a hydrolase, but by an acyltransferase. We observed (15) that labeled cholesteryl esters were formed in plasma incubated with linoleoyl-^{14}C lecithin, but not in plasma incubated with linoleate-^{14}C complexed to albumin. We showed (16) that the relative amount of arachidonic acid released from rat plasma lecithin by *Crotalus adamanteus* venom was similar to that which became esterified to cholesterol during the incubation of rat plasma. Finally, we noted (16) that, when human plasma was incubated, the molar change in lecithin was similar to that in unesterified cholesterol. On the basis of these findings we postulated the following:

$$\text{lecithin} + \text{unesterified cholesterol} \xrightarrow{\text{acyltransferase}} \text{lysolecithin} + \text{cholesteryl ester}.$$

Furthermore, we suggested that the enzyme mainly transfers fatty acids from the 2-position of lecithin.

Although this reaction formulation subsequently received support from Shah, Lossow, and Chaikoff (17), data exist that do not completely fit it. 10% or more of the fatty acids transferred to cholesterol are saturated (16), a much higher percentage than is released from lecithin by snake venom. Therefore, saturated fatty acids (mostly palmitic acid) may largely be derived from a source other than the 2-position. Plasma triglyceride might be one such source, since cholesteryl palmitate-^{14}C is formed when plasma is incubated with tripalmitin-^{14}C (15, 17). Moreover, the transfer from triglyceride to cholesterol may proceed via lecithin, since considerable labeled phosphatide is also formed when plasma is incubated with emulsified tripalmitin-^{14}C (17). The 1-position of mono- or diacyl glycerophosphoryl choline also might be a source of saturated fatty acid, although no evidence of this exists as yet.

A second observation that does not fit our reaction formulation is that only about one-half the predicted amount of lysolecithin can be demonstrated in human plasma after a 24 hr incubation (18). However, if lysolecithin can function as a donor of saturated fatty acids, as suggested above, or if one or more lysolecithin hydrolases are present in plasma (19), this discrepancy might be explained.

A third objection to our concept is provided by the results of Goodman (20) and of Portman and Sugano (21). Goodman noted that less cholesteryl arachidonate is formed during the incubation of *human* plasma than would be expected from the composition of the plasma lecithin. This has been confirmed, and contrasts with the good agreement existing with respect to linoleate.[1]

[1] Glomset, J., W. King, and Y. Akanuma. Unpublished experiments. We incubated LDL-free human plasma (22) with cholesterol-^{14}C and compared the pattern of labeled cholesteryl esters formed during a 6 hr incubation with the composition of the 2-fatty acids of lecithin extracted from the unincubated lipoproteins. The cholesteryl esters were separated on thin-layer plates of $AgNO_3$-treated silica gel (23). The 2-fatty acids were prepared by treatment of the chromatographically purified lecithin (22) with *Crotalus adamanteus* venom (24), and were subsequently analyzed by gas–liquid chromatography. The relative distribution of radioactivity in the cholesteryl esters was: 12.3% for the saturated esters, and 20.6, 56.6, and 10.5% for the mono-, di-, and tetraunsaturated esters, respectively. The corresponding figures for the 2-fatty acids were 3.5, 14.5, 56.5, and 25.4%.

Therefore, Goodman's observation suggests that the acyltransferase of human plasma may be able to distinguish between different fatty acids in the 2-position of lecithin. The results of Portman and Sugano (21) also support this possibility. They incubated mixtures of fresh human plasma with heat-inactivated rat plasma labeled with cholesterol-^{14}C, and fresh rat plasma with heat-inactivated human plasma labeled with cholesterol-^{14}C and studied the composition of the resulting cholesteryl-^{14}C esters. In both kinds of experiment they found that the newly formed esters more nearly resembled the preexisting cholesteryl esters of the fresh plasma than those of the unincubated mixture. They concluded that the pattern of cholesteryl esters formed during the incubation was related to the specificity of the enzyme (in the fresh plasma) rather than the composition of the fatty acid source (the lipoproteins of the mixed heated and fresh plasmas). This conclusion appears to be compatible with Goodman's results, because of the implication that the cholesterol-esterifying enzymes of human and rat plasma preferentially transfer linoleate and arachidonate, respectively.

Role of Lipoproteins

Another interpretation of the data of Goodman and of Portman and Sugano is that the specificity of the acyltransferase depends primarily on lipoprotein structure. It is not certain that linoleoyl lecithin and arachidonoyl lecithin occupy similar positions in human plasma lipoproteins. If they do not, they may differ in accessibility to the enzyme, and this, rather than fatty acid specificity per se, could explain Goodman's results. Similarly, the results of Portman and Sugano are compatible with the possibility that the enzymes of human and rat plasma selectively react not with specific fatty acids, but with homologous lipoproteins. Thus, their incubation mixtures contained substrate lipoproteins from both species, and the rat and human lipoproteins differed in relative content of linoleic and arachidonic acid. Therefore, preferential formation of homologous enzyme–substrate complexes could have led to the transfer of different amounts of linoleate and arachidonate. Fortunately, enzyme preparations can now be obtained that contain little lipoprotein (see later discussion). Use of these enzyme preparations instead of whole, fresh plasma should help to determine whether the enzymes of rat and human plasma preferentially react with specific fatty acids or with specific lipoproteins.

That human plasma acyltransferase *can* react preferentially with specific lipoproteins is suggested by the following. After a 1 hr incubation of human plasma with cholesterol-^{14}C, the specific activity of HDL cholesteryl esters greatly exceeded that of VLDL or LDL cholesteryl esters.[2] Furthermore, when human or baboon plasma was incubated with cholesterol-^{14}C, the specific activity of the esterified cholesterol also varied among HDL subfractions (22). These differences in cholesteryl ester specific activity probably reflect differences in the enzyme's rate of reaction with different lipoprotein substrates. In fact, calculations of the average rate of cholesterol esterification indicated that four times as much HDL cholesterol was esterified as LDL cholesterol (22). Moreover, the difference may have been even greater, since the calculations did not take into account the transfer of newly formed cholesteryl esters from HDL to LDL. Thus, these studies indicate that the acyltransferase readily differentiates between different lipoproteins.

Lipoprotein structure may influence not only the specificity and rate of the reaction, but also its direction. A significant proportion of the lysolecithin formed by the acyltransferase reaction apparently is not retained by the parent lipoproteins, but rather becomes bound to albumin (18, 25). This not only causes a net loss of phospholipid from the lipoproteins, but could be one reason why esterified cholesterol and lysolecithin are formed at the expense of lecithin. If lysolecithin is not retained by the part of the lipoprotein surface that reacts with the enzyme, reverse transacylation from esterified cholesterol to lysolecithin might be greatly diminished. If this is true, cholesterol esterification might occur less readily in the absence of albumin. This possibility should be explored when albumin-free preparations of plasma acyltransferase become available.

Implicit in the above discussion has been the concept that "substrate" and "product" lipoproteins exist. If the acyltransferase reaction is a physiologically important source of plasma cholesteryl esters (see later discussion), then recently secreted lipoproteins that have not reacted with the enzyme might contain relatively large amounts of lecithin and unesterified cholesterol, whereas lipoproteins that have circulated in the plasma long enough to react maximally with the enzyme might contain less lecithin and more esterified cholesterol. My colleagues and I (22) have reported evidence that lipoproteins of these types are present in plasma. We filtered HDL from human and baboon plasma through columns of Sephadex G-200 and measured the ratios of

[2] Akanuma, Y., and J. Glomset. Results to be submitted. After a 1 hr incubation of whole human plasma with cholesterol-^{14}C, VLDL, LDL, and HDL were prepared by ultracentrifugation and chromatography on hydroxylapatite. Lipids were extracted and cholesteryl esters were isolated by thin-layer chromatography (22). In three experiments mean cholesteryl ester specific activities relative to HDL esterified cholesterol were: HDL cholesteryl esters, 1.00; VLDL cholesteryl esters, 0.27; and LDL cholesteryl esters, 0.13.

lecithin to sphingomyelin and of esterified to unesterified cholesterol in the effluent. We also incubated whole blood with labeled cholesterol, filtered the HDL through Sephadex, and measured the specific activity of the esterified cholesterol in the effluent. We found that the HDL subfractions that emerged initially had lower ratios of lecithin to sphingomyelin than those that emerged later. Furthermore, the specific activity of the esterified cholesterol in the initial subfractions was lower than in the later subfractions. On the basis of these results we tentatively identified the initial and final subfractions of the HDL peak as "products" and "substrates" of the acyltransferase reaction, respectively.

The highly provisional nature of this identification can be seen from the following. First, the ratio of esterified to unesterified cholesterol in the initial and final subfractions did not appear to fit our concept, and we had to assume that secondary changes in lipoprotein cholesterol occur in the circulation. Secondly, we assumed that lecithin that has been degraded by the acyltransferase is not replenished, and that the sphingomyelin content does not change while lipoproteins remain in the circulation. Both of these assumptions remain to be verified. Furthermore, both should be considered in the light of the physical exchange known to occur among the plasma lipids. Newly formed cholesteryl esters transfer from HDL to LDL and VLDL (26, 27); unesterified cholesterol probably transfers to HDL from both cells and lipoproteins after HDL cholesteryl esters have been formed by the acyltransferase reaction (11, 22, 28); phospholipids can exchange among lipoproteins (29, 30); and, as already mentioned, much of the lysolecithin formed by the acyltransferase reaction appears to move away from lipoproteins onto albumin (18, 25).

Despite these problems of interpretation, we are continuing our efforts to identify and isolate "substrate" and "product" lipoproteins and are presently focusing our attention on the LDL. Although the acyltransferase appears to react preferentially with HDL, it also reacts with LDL. Therefore, "substrate" and "product" LDL should also exist.

Closely related to the problem of isolating and identifying "substrate" and "product" lipoproteins is the question of the effect of the acyltransferase reaction on the physical properties of lipoproteins. Although this question still has not received much attention, one of the first studies was performed several years ago by Tayeau and Nivet (31, 32). They found that some inhibitors of the acyltransferase reaction prevented or partially prevented the increase in electrophoretic mobility of α- and β-lipoproteins which is well known to occur during incubation or storage (33, 34). On the basis of these results, they concluded that the change in mobility might be caused by the cholesterol esterification reaction. A second study has already been mentioned, i.e., our study of HDL subfractions (22). We interpreted our results as evidence that the acyltransferase reaction causes aggregation of smaller "substrate" HDL into larger "product" HDL. Both studies emphasize the possibility that the acyltransferase reaction may influence the physical properties of lipoproteins. However, in both cases the conclusions should be regarded as tentative until the existence of experimental artifacts has been ruled out more thoroughly. For example, it is possible that the change in electrophoretic mobility noted by Tayeau and Nivet was caused by the release of fatty acids during the incubation and the subsequent binding of these fatty acids to lipoproteins.

Fortunately, it may soon be possible to test the validity not only of the above concepts, but also of the basic idea of the existence of "substrate" and "product" lipoproteins. Norum and Gjone (35–37) have recently described a new inborn error of metabolism characterized by the apparent absence of acyltransferase activity and by the near absence of cholesteryl esters from plasma. If, indeed, the primary defect in this disease is the absence of plasma lecithin:cholesterol acyltransferase activity and not some related, but independent disorder of lipoprotein structure, the lipoproteins in the blood of these patients should closely resemble the "substrates" postulated to be present in the plasma of normal individuals. One observation, consistent with this possibility, has already been made by Norum and Gjone, i.e., that the ratios of lecithin to sphingomyelin and unesterified to esterified cholesterol in the plasma of these patients are considerably higher than normal.

PROPERTIES OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE

Distribution

Kaplan and I (38) studied the distribution of lecithin: cholesterol acyltransferase-like activity in rat tissues. We used an assay system that consisted of heat-inactivated plasma and an albumin-stabilized emulsion of cholesterol-^{3}H, and measured the esterifying activity of tissue homogenates as compared to that of plasma. To reduce contamination of the tissues by blood, we perfused the animals with saline before removing the various tissues, and to compensate for possible extraneous tissue effects, we incubated known amounts of acyltransferase activity with tissue homogenate controls. We found that the concentration of acyltransferase-like activity in blood plasma was several-fold higher than that in any of the tissues. Moreover, the true plasma:tissue ratios may have been even higher because the tissues were contaminated with extracellular fluid. The low level of enzyme activity that we found in the liver is of interest (see later discus-

sion), since this organ possibly may be the source of the plasma enzyme. If the liver does secrete the enzyme into the plasma, it probably does not store the enzyme in active form. The low level of activity in the liver is also of interest in view of the results of Akiyama, Minari, and Sakagami (39), who found that the supernatant fraction of rat liver homogenates contained cholesterol-esterifying activity similar in several respects to the acyltransferase activity of rat plasma. Akiyama et al. (39) did not measure the relative activity of the liver acyltransferase compared to that of plasma. Therefore, the presence of enzyme in their liver homogenates may simply have been a reflection of contamination with extracellular fluid. On the other hand, the pH optimum of the liver supernatant enzyme was 6.5 as opposed to 7.3, the optimum they found for rat plasma. Consequently, a specific liver enzyme may exist. This possibility should be explored, and one of the first experiments performed should be to redetermine, with a *common* substrate, the pH optima of the rat liver and plasma enzymes.

Purification

Because the plasma acyltransferase has been only partially purified, comparatively little is known about its physical or chemical properties. However, it is more strongly adsorbed to DEAE-cellulose than most plasma proteins are (40). Furthermore, the cholesterol-esterifying activity of human plasma migrates as an α_1-globulin on zone electrophoresis (the enzyme activity has a somewhat slower mobility than that of α_1-lipoprotein cholesterol), and it emerges from Sephadex G-200 columns in the same position as α_1-lipoprotein (40, 22). The last-named observations suggest that the enzyme is either very similar to HDL or is complexed to HDL. That an HDL–enzyme complex can exist is further supported by the results of Lossow, Shah, and Chaikoff (41), who found that significant enzyme activity accompanied the HDL upon ultracentrifugal flotation in a solution of sucrose in D_2O. However, the enzyme can be separated from HDL by centrifugation in KBr solution that has a density of 1.21 g/ml (40).

A method for partial purification of the enzyme that has been suggested (40) is based on a combination of chromatography on DEAE-cellulose and hydroxylapatite, precipitation with ammonium sulfate, and separation of the enzyme from HDL by ultracentrifugation. This method yielded an enzyme preparation that contained only about 20% of the activity of the original plasma, and was only about 30-fold purified. At this stage the principal contaminant appeared to be albumin, as judged by electrophoresis and gel filtration. Clearly, a much more highly purified preparation of the enzyme would be desirable.

FACTORS THAT INFLUENCE THE ACYLTRANSFERASE REACTION

Methods of Assay

In most studies of the plasma acyltransferase reaction, the esterification of cholesterol has been measured by one of three methods. (*a*) Plasma or serum is incubated for 1–3 days at 37°C, and the change in unesterified cholesterol is determined. (*b*) Plasma is incubated for 1–3 hr at 37°C, and the initial rate of change in unesterified cholesterol is determined. (*c*) A relatively small amount of fresh plasma is incubated for 1–6 hr at 37°C with a substrate composed of heat-inactivated plasma and labeled, unesterified cholesterol added as an albumin-stabilized emulsion (37) or coated onto Celite particles (21). Unfortunately, none of these methods are ideal and therefore the results discussed below must be considered with caution. The first method measures essentially the extent of esterification, not the rate. The amount of substrate available (especially lipoprotein lecithin), particularly in the 3-day incubations, is more critical than the level of enzyme activity. Furthermore, when a relatively large amount of unesterified cholesterol is present, and the percentage of change rather than the absolute change in unesterified cholesterol is given, a mistaken impression even of the extent of the reaction can result. The second method depends on the level of enzyme activity and also on the nature of the endogenous, substrate lipoproteins. This is an advantage if the method is to be used to estimate the rate of esterification of plasma cholesterol in vivo, but not if the method is to be used to assay enzyme activity per se. In the third method the formation of labeled cholesteryl esters is measured. Two fundamental assumptions are made, i.e., that the labeled, unesterified cholesterol in the substrate mixture has equilibrated completely with the unlabeled, unesterified cholesterol of the lipoproteins, and that the enzyme reacts equally well with the preheated substrate lipoproteins and with the endogenous lipoproteins present in the fresh plasma itself. In support of these assumptions, Portman and Sugano (21) have reported that the calculated amount of cholesterol esterified is close to that actually obtained during the incubation of fresh plasma. Nevertheless, the third method can not be regarded as completely independent of endogenous substrate, except when essentially lipoprotein-free preparations of the enzyme are assayed. Moreover, adequate standardization of the third method is difficult because of the limited stability of the heated, substrate lipoproteins.

Factors That Affect the Reaction In Vitro

Tables 1 and 2 list the factors reported to affect the esterification of plasma cholesterol in vitro. Several deserve special comment. Exogenous lecithin is interesting (Table 1) because its effect of markedly increasing cho-

TABLE 1 STIMULATION OF CHOLESTEROL ESTERIFICATION IN VITRO

Factor	Method of Measurement	Remarks	Reference
1. Lecithin (exogenous)	Net esterification 1–3 days	1 mg/ml of ovolecithin added to sheep or calf serum	7
" "	Net esterification 1–8 days	9–14 mg of commercial soybean phosphatide preparation gave maximal effect	8
" "	Net esterification 1–3 days	2.7 mg/ml of synthetic dimyristoyl lecithin gave maximal effect. Use of higher concentrations not reported	9
" "	"	1 mg/ml of dimyristoyl lecithin stimulated, but dipalmitoyl lecithin and "dipalmitoyl cephalin" did not	11
2. Extracts of Group A streptococci	Net esterification 3 days	Both rate and extent of reaction increased; accompanied by increased opalescence of serum	42
3. Polyvalent anions	Radioactive cholesterol assay	Phosphate, sulfate, citrate, and EDTA all stimulated in concentrations up to 0.2 N	40

TABLE 2 INHIBITION OF CHOLESTEROL ESTERIFICATION IN VITRO

Factor	Method of Measurement	Remarks	Reference
1. Heating at 55–60°C	Net esterification 3 days	1–2 hr	1
" "	Radioactive cholesterol assay	30 min	40
2. Hemolyzed blood	Net esterification 3 days	Human blood incubated, then serum analyzed. Partial inhibition	1
3. Sodium salts of free and conjugated bile acid	Net esterification 1–2 days	In human and dog serum complete inhibition with 4 mM glyco-, taurodeoxycholate; 6 mM taurocholate, cholate, deoxycholate	2, 3
Sodium taurocholate	Radioactive cholesterol assay	Inhibition by 5 mM of the bile salt reversed by dialysis (human serum)	40
4. Phlorizin	Net esterification 3 days	1 g/liter partially inhibited (dog and human serum)	7
5. Monobromoacetate	"	0.2 g/liter completely inhibited (dog and human serum)	7
6. *p*-Hydroxymercuribenzoate	Net esterification 1 day	1–2 mM solution completely inhibited. Reversed by 0.01 M mercaptoethanol	16
7. Thymol	Net esterification 3 days	1 g/liter partially inhibited	7
8. Cholesterol	"	Exogenous cholesterol dissolved from glaze on side of incubation tube	8
9. Phospholipase A	Incorporation of labeled precursors into cholesterol esters by extracts of rat plasma acetone powders	Preincubation of 2 ml of extract with 0.5 mg of *Crotalus adamanteus* venom at 37°C for 20 hr completely inhibited	17
"	Net esterification 1 day	Fresh human serum incubated with *Crotalus adamanteus* venom completely inhibited	43
10. Phospholipase C	"	Commercial preparation of *Clostridium welchii* phospholipase C completely inhibited	43
11. Saponin	Net esterification 3 days	5 g/liter said to inhibit, but no details given.	44
12. Urea	Radioactive cholesterol assay	4 M urea partially inhibited	40
13. Diisopropyl fluorophosphate	Net esterification 1 day	5×10^{-4} M completely inhibited	42
14. Para-oxon	Estimation of change in lysolecithin on chromatograms after a 15 hr incubation	0.1 mM appeared to inhibit completely	45
15. Polyvalent cations	Radioactive cholesterol assay	Ca^{++} and Mg^{++} inhibited increasingly in concentrations up to 0.2 N	40

lesterol esterification suggests that the amount of endogenous lecithin in plasma is an important determinant of the reaction.[3] Since the rate of the reaction in fresh plasma decreases long before the lipoprotein lecithin is exhausted, the stimulatory effect of exogenous lecithin further suggests that only a limited amount of the lecithin present can serve as a donor of fatty acids. This obviously may have important implications with respect to lipoprotein structure.

The fact that the acyltransferase is inhibited by sulfhydryl-blocking agents, and reversibly inhibited by *p*-hydroxymercuribenzoate is of interest for practical as well

[3] This is also suggested by the inhibitory effect of lecithinase shown in Table 2.

as theoretical reasons. For example, inhibition of the enzyme is frequently useful during the preparative ultracentrifugation of plasma lipoproteins. Also, the reversible inhibition by *p*-hydroxymercuribenzoate suggests that one or more sulfhydryl groups either form a part of the active center of the acyltransferase or are important in maintaining its configuration.

Physiological and Experimental Conditions In Vivo

Relatively few studies have been done on how the preexisting state of individual subjects or animals influences the rate of cholesterol esterification in their isolated plasma. Moreover, even fewer studies have been reported in which changes in enzyme activity are effectively differentiated from changes in lipoprotein substrates. Wagner and Poindexter (46) studied the effect of age on the extent of esterification during a 3 day incubation. They reported that the ratio of esterified to unesterified cholesterol after the incubation was higher in 17–20-yr old human females than in "middle aged" females. Unfortunately, they did not include detailed information about the latter. Therefore, whether the actual amounts of cholesterol esterified differed significantly is difficult to determine. Later, Gherondache (47), who reported more detailed results from a similar study, found that both the absolute and relative amounts of cholesterol esterified during a 2 day incubation were greater in 20–39-yr olds than in 40–99-yr olds. Consequently, his results substantiate those of Wagner and Poindexter although they do not distinguish between an effect of age on enzyme activity and an effect of age on the lipoprotein substrates. Therefore, further investigation of the effect of ageing is needed.

Monger and Nestel (48) studied the relation between plasma cholesterol level and rate of esterification in a group of individuals with widely different plasma cholesterol concentrations. They employed an assay system comprised of preheated endogenous lipoproteins, labeled cholesterol, and fresh, "active" plasma; and they found evidence of a direct relationship between the level of cholesterol-esterifying activity and the concentration of plasma cholesterol. However, they did not attempt to determine whether the endogenous lipoproteins in the different plasma samples influenced the apparent level of esterifying activity. The problem of interpreting results such as these, that depend both on the level of enzyme activity and on the type and level of lipoprotein substrate present, is illustrated by the apparent discrepancy between the results of Monger and Nestel (48) and those of Gherondache (47). Gherondache found that less esterification occurred during a 2 day incubation in the sera of older than in that of younger females, even though the older females had higher concentrations of cholesterol. This discrepancy might be related to differences in HDL levels in the two series since HDL is important in the initial phases of the reaction (22). In any case, the value of separate measurements of enzyme activity and lipoprotein levels should be apparent.

A variable relation appears to exist between sex and plasma cholesterol esterification. Aftergood and Alfin-Slater (49) incubated rat sera for 5 hr, and found that significantly more cholesterol became esterified in the sera of mature females than in that of mature males. On the other hand, Gherondache (47), who measured the extent of esterification in the sera of human males and females, reported data that show no significant sex difference. Finally, Sugano, Chinen, and Wada (50) measured the level of esterifying activity in chicken plasma, using cholesterol-^{14}C and preheated plasma as a substrate, and found that the level was higher in males than in females (Table 4, below).

The most thorough study of the effect of diet reported is that of Sugano and Portman (51). They investigated the effect of essential fatty acid deficiency (Table 3) by comparing rats fed a fat-free diet, or a diet containing hydrogenated coconut oil, with rats fed a corn-oil diet. They found that more cholesterol became esterified during a 5 hr incubation of the plasma of the deficient rats, even though the deficient plasma contained less than half as much esterified and unesterified cholesterol at the start of the incubation. Aftergood and Alfin-Slater (49) subsequently confirmed this finding.

In order to follow up their initial observation, Sugano and Portman (51) compared the levels of esterifying activity in the essential fatty acid-deficient and control rats. They incubated aliquots of plasma from both kinds of rats with preheated, substrate plasma from both, and found that the level of esterifying activity was higher in the deficient rats irrespective of the substrate used. They also reported that the lipoprotein substrates differed in their capacity to react with the enzyme. Finally, they found that the increased esterification in vitro was correlated with an increased turnover of lipoprotein cholesterol in vivo.

So far, no comparable studies of the effect of diet in other species have been reported. Favarger (52) reported some studies of the effect of oral fat loads in man which led him to conclude that the rate of esterification of cholesterol in the plasma was increased during the absorption of fat. However, he used too few measurements during the first few hours of incubation to yield reliable results for the initial rate of esterification. Therefore, additional studies will be necessary before his conclusions can be accepted.

Several experimental conditions that affect the esterification of cholesterol in plasma are listed in Table 3. Of these, the effect of evisceration deserves particular mention. Brot, Lossow, and Chaikoff (54) removed the liver,

TABLE 3 EFFECT OF EXPERIMENTAL CONDITIONS IN VIVO ON THE ESTERIFICATION OF CHOLESTEROL IN VITRO

Factor or Condition	Method of Measurement	Remarks	Reference
1. Essential fatty acid deficiency	Net esterification 5 hr	Increased in rat plasma	51
" " " "	"	" " "	49
" " " "	Esterifying activity on labeled cholesterol + heated substrate	" " "	51
2. Phlorizin	Net esterification 3 days	Decreased in plasma of dogs injected with phlorizin during previous 2 days	7
3. Ethionine	Esterification of labeled cholesterol	Decreased in rat plasma 1 day after injection of 200 mg	53
4. Evisceration	" " "	Decreased in rat plasma 11 hr after operation	54
5. Ligation of bile duct	Net esterification	Increased in dog plasma 1 wk after ligation; decreased after 3–4 wk	55

gastrointestinal tract, pancreas, and spleen from rats; they obtained blood from the animals 11 hr after the final stage of the operation, and studied the esterification of cholesterol-^{14}C by the isolated plasma. They found that the plasma from the eviscerated rats esterified less labeled cholesterol, and that this decrease in esterifying activity could not be ascribed to the presence of inhibitors or to the absence of activators. They concluded that their results were most probably due to a diminution in enzyme activity, and suggested that this diminution had been caused by the hepatectomy in view of the known effect of this procedure (56) on the level of cholesteryl esters in plasma. In other words, they inferred that the liver is the source of plasma lecithin:cholesterol acyltransferase. This interesting possibility obviously needs to be substantiated by direct experimentation. For example, the plasma of partially eviscerated animals could be compared with that of animals that have also been hepatectomized, or perfused livers might be used to obtain direct evidence of hepatic synthesis and secretion of the enzyme. A second implication of the results of Brot et al. (54) which should also be explored further is that the acyltransferase of rat plasma is either unstable per se or is rapidly degraded by mechanisms that do not involve the liver, gut, pancreas, or spleen.

Effect of Pathological Conditions

The possibility that the liver plays a role in the synthesis and secretion of plasma lecithin:cholesterol acyltransferase is suggested not only by the experiments of Brot et al. (54) and those of Friedman and Byers (56), but also by the changes in plasma cholesterol in patients with liver disease. In disorders that obstruct the flow of bile, the concentrations of unesterified cholesterol and lecithin in the plasma are increased (57), and the relative, but not necessarily the total, concentration of esterified cholesterol is decreased. In advanced liver failure, the concentrations of all the plasma lipids decrease, especially that of esterified cholesterol. Therefore, plasma acyltransferase activity conceivably might be reduced in either or both of these conditions, and evidence with respect to both possibilities has been reported.

The possible course of events in biliary obstruction has been indicated by the experiments of Castro Mendoza and Jimenez Diaz (55). They ligated the bile ducts of dogs and studied the acute and chronic effects of this procedure on the plasma cholesterol esterification reaction. 1 wk after the operation the ratio of unesterified to esterified cholesterol had increased, and the absolute concentrations of both had increased. Furthermore, when the plasma of these dogs was incubated for 24 hr, more cholesterol became esterified. However, when they repeated their measurements in two of the dogs after 3 and 4 wk, respectively, the concentration of esterified cholesterol had decreased, and less cholesterol became esterified in vitro. Castro Mendoza and Jimenez Diaz (55) also thought they could discern a similar trend in humans with obstructive jaundice. In most of their patients who had normal or high absolute concentrations of plasma esterified cholesterol, a normal or greater than normal amount of cholesterol became esterified in vitro. On the other hand, when the plasma of two patients with low concentrations of esterified cholesterol was incubated for 24 hr, less esterification occurred. Furthermore, in patients with kala-azar, who had low serum cholesteryl ester concentrations, little cholesterol became esterified in vitro. Because kala-azar often affects the liver, both findings suggest a correlation between the acyltransferase reaction, serum cholesteryl ester concentrations, and liver function.

Turner and his colleagues (58, 59) also studied patients with a number of liver diseases. In general their results in patients with obstructive jaundice appear to agree with those of Castro Mendoza and Jiminez Diaz. However, they expressed their results in terms of the percentage decrease in serum unesterified cholesterol during a 24 hr incubation, and did not include the actual values for cholesterol. Therefore, their results are not strictly comparable. In patients with acute hepatitis they found that the percentage decrease in unesterified cholesterol was

always low during the 1st wk of the illness, whereas esterification increased as the patients improved. Furthermore, they found that esterification was decreased in the sera of many patients with impaired liver function caused by chronic hepatitis, cirrhosis, metastasis, or Hodgkin's disease. Again, however, they expressed their results on the basis of the percentage decrease in unesterified cholesterol during a 24 hr incubation. Since they defined one "enzyme unit" as that "amount of activity capable of producing a 1% decrease in the original level of free cholesterol," the absolute amounts of cholesterol esterified may have been normal or even high in some of their patients. The general problem of interpreting results of this type was briefly discussed earlier. In any study of the effect of physiological or pathological conditions on the acyltransferase reaction an attempt should be made to differentiate between alterations in the plasma lipoprotein substrates and alterations in the level of acyltransferase activity. Moreover, in pathological conditions the presence of inhibitors should also be ruled out. The possibility that soluble cholesteryl ester hydrolase activity, released from tissues as a result of cell damage, might be present should be kept in mind. Both pancreas and liver contain enzymes of this type (60, 61) so that in disorders such as acute hepatitis, hydrolase activity that could offset the activity of the esterifying enzyme might be present in the plasma.

Familial Plasma Cholesteryl Ester Deficiency

This newly discovered inborn error of metabolism (35–37) has already been mentioned briefly. The three sisters who have this disease have normal or increased amounts of cholesterol in their plasma, but less than 10% of it is esterified. Furthermore, no plasma acyltransferase activity is demonstrable either with the patients' own plasma or with heated plasma substrate from normal individuals. Radioactive cholesteryl esters appeared in the plasma of two of these patients after they had ingested cholesterol-^{3}H, but only traces appeared after one of the patients was given an intravenous injection of mevalonate-^{3}H. These findings suggest that the primary defect in this disease may be a genetically determined lack of plasma acyltransferase activity. However, the levels of plasma HDL are abnormally low in this disease, so that the primary defect could involve HDL instead, or some other related, but independent defect in the liver. Despite this uncertainty, the clinical findings are particularly provocative. They are: proteinuria, anemia, hyperlipidemia, and corneal arcus. Moreover, histopathological specimens from bone marrow and kidney contain foam cells.[4] These findings raise interesting questions about the physiological role of the acyltransferase reaction.

PHYSIOLOGICAL ROLE OF THE ACYLTRANSFERASE REACTION

Formation of Plasma Cholesteryl Esters In Vivo

A number of reasons exist for believing that the acyltransferase reaction is a physiologically important source of plasma cholesteryl esters. These are:

1. In human plasma the rate of cholesterol esterification in vitro agrees well with the calculated rate of esterification in vivo (Table 4). In other species in which comparisons have been made the rates of esterification in vitro are at least half the calculated rates in vivo (Table 4).

2. The pattern of cholesteryl esters formed by the acyltransferase reaction during the incubation of human plasma is very similar to the pattern of cholesteryl esters in fresh plasma (21). No other enzyme has been shown to yield this pattern. Similarities also exist in the rat (15, 28), but in contrast to man (64), the composition of the VLDL cholesteryl esters differs appreciably from that of the LDL and HDL cholesteryl esters, and does not appear to be compatible with the specificity of the acyltransferase (15, 16, 28, 65). This is particularly true for the cholesterol-fed rat, in which the composition of the VLDL cholesteryl esters, like that of liver cholesteryl esters, is characterized by a predominance of palmitic and oleic acids (65). Both this and the results of tracer experiments (66) suggest that all or nearly all of the VLDL cholesteryl esters may be derived from the liver.[5]

3. The relative incorporation of labeled cholesterol into the cholesteryl esters of human plasma HDL, VLDL, and LDL in vivo (20) is similar to that found in vitro.[2] Also, the incorporation of labeled cholesterol into the cholesteryl esters of HDL subfractions in the baboon is similar in vivo and in vitro (22).

4. Acyltransferase activity and esterified cholesterol are both absent or nearly absent from plasma in familial plasma cholesteryl ester deficiency. This is clearly significant, even though it has not yet been established whether the mechanisms of cholesterol esterification in the liver are normal in this disease.

5. The concentration of lecithin:cholesterol acyltransferase is considerably higher in plasma than in tissues

[4] It is worth noting that patients with Tangier disease (familial HDL deficiency) have foam cells that contain cholesterol (62).

[5] Nevertheless, the results of Heimberg, Van Harken, and Brown (67) may also be mentioned. They perfused livers from rats fed low-cholesterol diets, and found that VLDL released into the perfusate contained few if any cholesteryl esters. Since VLDL prepared from the fresh sera of the same rats did contain cholesteryl esters, these in vitro experiments raise the possibility that many of the cholesteryl esters of rat plasma VLDL may be derived from the plasma in vivo.

TABLE 4 RATES OF ESTERIFICATION OF PLASMA CHOLESTEROL IN VIVO AND IN VITRO

Species	Rate In Vivo	Rate In Vitro	Reference
	μmole/ml/hr		
Man	0.12*		63
		0.11†	16
		0.12‡	21
		0.06‡	48
Cebus monkey	0.17*	0.24‡	28
Rat	0.16*	0.08‡	28
Chicken			
Male White Leghorn		0.13‡	50
Female White Leghorn		0.08‡	50
Female White Leghorn (laying)		0.04‡	50

* Calculated on basis of data obtained on injection of mevalonic acid-^{3}H.

† Difference in unesterified cholesterol in fresh plasma.

‡ Assayed with heated plasma–cholesterol-^{14}C substrate.

(38), which suggests that the enzyme may have a specific function in plasma.

Reasons for believing that the acyltransferase reaction is *not* an important source of plasma cholesteryl esters have also been advanced. In particular, the liver has seemed the most likely source of the cholesteryl esters of plasma lipoproteins. Plasma cholesteryl ester formation is greatly reduced in hepatectomized animals (56); good evidence exists that most of the plasma lipoproteins are synthesized in the liver (68); the liver contains an active cholesterol-esterifying enzyme system (69); and some investigators have reported that liver cholesteryl esters have a higher specific activity than plasma cholesteryl esters after the injection of labeled cholesterol (70).

However, the effect of hepatectomy is no longer relevant in view of the evisceration experiments of Brot and his colleagues (54) discussed earlier. Furthermore, the existence of plasma lipoproteins that are essentially cholesteryl ester-free in the patients with familial plasma cholesteryl ester deficiency indicates that lipoproteins of this type *can* be synthesized and secreted. The most studied liver cholesteryl-esterifying enzyme, which apparently is the most active (69), preferentially forms cholesteryl oleate and palmitate; these esters do not occur in large amounts in plasma. The importance of the second cholesterol-esterifying enzyme in rat liver (39, 71) as a source of plasma cholesteryl esters remains to be established. Finally, with respect to the isotopic evidence of Swell and Law (70) that the specific activities of cholesteryl linoleate and arachidonate were higher in rat liver than in rat plasma after the injection of unesterified cholesterol-^{14}C, Sugano and Portman (28) performed similar experiments and reached almost exactly opposite conclusions.

The recent experiments of Gidez, Roheim, and Eder (66) demonstrate the difficulty of interpreting results based on studies of unfractionated plasma and liver. They injected rats with labeled mevalonate and subfractionated the cholesteryl esters of individual plasma lipoproteins and rat liver organelles after approximately 2 and 8 hr. They showed that the cholesteryl esters of the liver microsomes had the highest specific activities at the earlier time period, and that the specific activities of the saturated and monounsaturated esters were several times higher than those of the most active plasma lipoproteins, the VLDL. On the other hand the specific activity of the microsomal tetraenoic cholesteryl esters was only slightly higher than that of the corresponding HDL esters. The authors concluded that liver microsomes might be the main source of the saturated and monounsaturated cholesteryl esters of the VLDL. However, they also concluded that their data were "not inconsistent with an extrahepatic synthesis of cholesteryl arachidonate on the $d > 1.063$ lipoproteins" because so much more cholesteryl arachidonate was present in plasma than in the liver.

Role of Plasma Acyltransferase in Cholesterol Transport

If the acyltransferase is a physiologically important source of plasma cholesteryl esters, an important question remains to be answered, i.e., why should a specific cholesterol-esterifying enzyme exist in the plasma? One highly tentative possibility is that the acyltransferase reaction plays a role in the transport of cholesterol from peripheral tissues to the liver, and therefore, in a sense, in membrane homeostasis. Some mechanism probably exists for the transport of cholesterol from peripheral tissues, because most peripheral cells can synthesize cholesterol (72), whereas none are known that can catabolize or excrete it.[6] Furthermore, the experiments of Murphy (11) suggest that the acyltransferase may be part of such a mechanism. He incubated erythrocytes in serum that first had been separately incubated at 37°C for 24 hr, and showed that a net transfer of unesterified cholesterol from the erythrocytes to the serum occurred. He also showed that the loss of erythrocyte cholesterol was related to the amount of esterified cholesterol formed in the serum during the preincubation, and concluded that this loss was due to a shift in the equilibrium between erythrocyte and lipoprotein unesterified cholesterol caused by the esterification reaction. If a similar relation exists between lipoproteins and the cells of tissues such as muscle and kidney, the conditions for a cholesterol transport mechanism might be satisfied. The following evidence is compatible with this possibility:

1. Courtice and Morris (73) have shown that both α_1- and β-lipoproteins are present in the peripheral lymph of the rabbit; and Voigt, Apostolakis, and Beyer (74)

[6] Cholesterol can, however, be lost from the body through sloughing of the skin and intestinal mucosa.

have shown that human peripheral lymph also contains soluble lipid that is presumably protein-bound.

2. Peripheral lymph of both humans and monkeys contains cholesterol-esterifying activity, as shown by incubation experiments with labeled cholesterol.[7]

3. The unesterified cholesterol of most peripheral tissues appears to be in equilibrium with that of the plasma lipoproteins (75–77). Therefore, the following sequence of events can be postulated. Plasma lipoproteins (particularly HDL) and plasma lecithin:cholesterol acyltransferase enter the interstitial fluid of peripheral tissues along with other plasma proteins such as albumin (78) and 6.6S γ-globulin (79); lipoprotein cholesterol is esterified by the acyltransferase reaction; the "product" lipoproteins subsequently pick up unesterified cholesterol from cell membranes; finally, the lipoproteins reenter the plasma via the lymph and release their excess cholesterol in the liver. This hypothetical concept is illustrated in Fig. 1.

One objection to this mechanism is that erythrocytes are the only cells for which a net transfer has so far been demonstrated in vitro, and a net loss of erythrocyte cholesterol has not yet been demonstrated in vivo. A loss may be found when better methods for separating young and old erythrocytes become available. However, it is also possible that very little loss normally occurs because of compartmentation within the body. Thus, the cells of the blood and liver can be viewed as occupying one compartment which is partially separated by capillary endothelium from a second compartment which contains cells of other tissues. Blood cells and liver cells can be included in the same compartment for present purposes because of the relatively unrestricted diffusion of plasma proteins between the hepatic intravascular space and the space of Disse (80). On the other hand diffusion between the first and the second compartment is limited, particularly in the case of the larger plasma proteins (81). Both compartments contain plasma lipoproteins and plasma acyltransferase, and in both of them unesterified cholesterol in cell membranes is in equilibrium with the unesterified cholesterol of circulating lipoproteins. The concept, then, is that the deficit in lipoprotein unesterified cholesterol in the first compartment is largely made up by the liver cells, whereas the deficit in lipoprotein cholesterol in the second compartment is made up by peripheral cells because the circulating interstitial fluid proteins remain out of contact with the liver for relatively long periods (82).

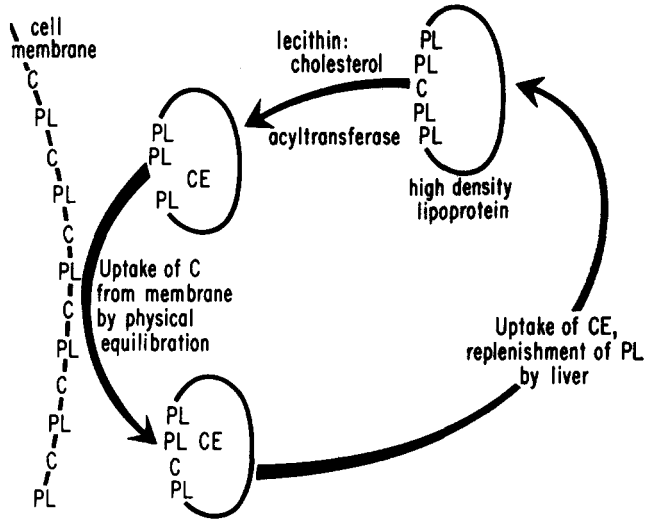

FIG. 1. Postulated mechanism for the transport of cholesterol from membranes of peripheral cells to the liver. Lecithin:cholesterol acyltransferase reacts with circulating lipoproteins to form cholesteryl esters from unesterified cholesterol and lecithin. The lipoproteins subsequently pick up unesterified cholesterol from cell membranes, circulate through the liver, and release esterified cholesterol. C, unesterified cholesterol; CE, cholesteryl ester; PL, phospholipid.

Other Roles of the Acyltransferase Reaction

Since the acyltransferase reaction is often referred to as the "plasma cholesterol esterification reaction," a role of the reaction in cholesterol transport might seem logical. However, the possibility that esterified cholesterol is only a by-product, and that the principal physiological role of the reaction is connected with the changes in lipoprotein phospholipid, should also be considered. It would be surprising if the conversion of lecithin to lysolecithin and the subsequent loss of lysolecithin from lipoproteins did not have important effects on lipoprotein structure and metabolism. Furthermore, the lysolecithin formed by the reaction is probably metabolized rapidly by cells of the liver and other tissues (83); and studies have only just begun of the physiological role of this lysolecithin. Finally, the fact that patients with familial plasma cholesteryl ester deficiency have anemia and proteinuria introduces the possibility that the acyltransferase reaction may affect the still poorly understood mechanisms that control erythrocyte destruction and the handling of plasma proteins by the kidney.

CONCLUSIONS

The data discussed in this review indicate that the plasma cholesterol esterification reaction is catalyzed by a plasma lecithin:cholesterol acyltransferase, and support the con-

[7] Glomset, J. Unpublished results. Peripheral lymph was obtained from the leg of a patient with a lymph fistula. An aliquot was incubated with 9 volumes of substrate composed of cholesterol-^{14}C and heated human plasma (40), and the amount of labeled cholesteryl ester formed was compared with that formed by a similarly incubated aliquot of the patient's plasma. The lymph:plasma ratio of cholesterol esterifying activity was 1:22. A similar experiment was also performed with subcutaneous lymph obtained from the leg of a pigtail monkey. In this experiment, the ratio of lymph enzyme to plasma enzyme activity was 1:10.

clusion that the reaction is a physiologically important source of plasma cholesteryl esters. However, they leave many questions unanswered. One question concerns the role of lipoproteins in the reaction, and the effect of the reaction on lipoproteins. If the interaction between the enzyme and its lipoprotein substrates is relatively specific, the enzyme may be useful in the study of natural and reconstituted lipoproteins. A second question concerns the nature of the factors that coordinate the formation and breakdown of plasma cholesteryl esters with the formation and breakdown of plasma lipoproteins. Considerably more information will be required to explain how a relatively constant ratio of esterified to unesterified cholesterol is maintained in the plasma. Finally, the physiological role of the reaction remains to be clarified. If plasma cholesteryl esters are formed extracellularly, it would be logical to infer that the reaction has a broader, extracellular function. At present little is known about the factors that control the metabolism of plasma lipoproteins once they have entered the circulation, and knowledge of the metabolic interaction between circulating lipoproteins and the lipoproteins of cell membranes is almost as limited. The plasma lecithin:cholesterol acyltransferase reaction could play a role in either or both of these areas.

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References

1. Sperry, W. M. 1935. *J. Biol. Chem.* **111:** 467.
2. Sperry, W. M., and V. A. Stoyanoff. 1937. *J. Biol. Chem.* **117:** 525.
3. Sperry, W. M., and V. A. Stoyanoff. 1937. *J. Biol. Chem.* **121:** 101.
4. Sperry, W. M., and V. A. Stoyanoff. 1938. *J. Biol. Chem.* **126:** 77.
5. Klein, W. 1938. *Z. Physiol. Chem.* **254:** 1.
6. Pantaléon, J. 1943. *Compt. Rend. Soc. Biol. (Paris).* **137:** 609.
7. Le Breton, E., and J. Pantaléon. 1947. *Arch. Sci. Physiol.* **1:** 199.
8. Wagner, A., and L. Rogalski. 1952. *J. Lab. Clin. Med.* **40:** 324.
9. Wagner, A. 1955. *Circ. Res.* **3:** 165.
10. Wagner, A. 1960. *Quart. Bull. Northwestern Univ. Medical School.* **33:** 282.
11. Murphy, J. R. 1962. *J. Lab. Clin. Med.* **60:** 86.
12. Etienne, J., and J. Polonovski. 1959. *Bull. Soc. Chim. Biol.* **41:** 805.
13. Etienne, J., and J. Polonovski. 1959. *Bull. Soc. Chim. Biol.* **41:** 813.
14. Etienne, J., and J. Polonovski. 1960. *Bull. Soc. Chim. Biol.* **42:** 857.
15. Glomset, J. A., F. Parker, M. Tjaden, and R. H. Williams. 1962. *Biochim. Biophys. Acta.* **58:** 398.
16. Glomset, J. A. 1962. *Biochim. Biophys. Acta.* **65:** 128.
17. Shah, S. N., W. J. Lossow, and I. L. Chaikoff. 1964. *Biochim. Biophys. Acta.* **84:** 176.
18. Glomset, J. A. 1963. *Biochim. Biophys. Acta.* **70:** 389.
19. Dawson, R. M. C. 1956. *Biochem. J.* **64:** 192.
20. Goodman, D. S. 1964. *J. Clin. Invest.* **43:** 2026.
21. Portman, O. W., and M. Sugano. 1964. *Arch. Biochem. Biophys.* **105:** 532.
22. Glomset, J., E. Janssen, R. Kennedy, and J. Dobbins. 1966. *J. Lipid Res.* **7:** 638.
23. Goodman, D. S., and T. Shiratori. 1964. *J. Lipid Res.* **5:** 578.
24. Saito, K., and D. J. Hanahan. 1962. *Biochemistry.* **1:** 521.
25. Switzer, S., and H. A. Eder. 1965. *J. Lipid Res.* **6:** 506.
26. Rehnborg, C. S., and A. V. Nichols. 1964. *Biochim. Biophys. Acta.* **84:** 596.
27. Nichols, A. V., and L. Smith. 1965. *J. Lipid Res.* **6:** 206.
28. Sugano, M., and O. W. Portman. 1964. *Arch. Biochem. Biophys.* **107:** 341.
29. Kunkel, H. G., and A. G. Bearn. 1954. *Proc. Soc. Exptl. Biol. Med.* **86:** 887.
30. Eder, H. A., J. H. Bragdon, and E. Boyle. 1954. *Circulation.* **10:** 603. (Abstr.)
31. Tayeau, F. 1955. *Exposés Ann. Biochem. Med.* **72:** 213.
32. Tayeau, F., and R. Nivet. 1956. *In* Biochemical Problems of Lipids. G. Popják and E. Le Breton, editors. Butterworth Scientific Publications, London. 365.
33. Armstrong, S. H., Jr., M. J. E. Budka, and K. C. Morrison. 1947. *J. Am. Chem. Soc.* **69:** 416.
34. Hoch, H., and A. Chanutin. 1954. *J. Biol. Chem.* **209:** 661.
35. Norum, K. R., and E. Gjone. 1967. *Biochim. Biophys. Acta.* In press.
36. Gjone, E., and K. R. Norum. 1967. *Acta Med. Scand.* In press.
37. Norum, K. R., and E. Gjone. 1967. *Scand. J. Clin. Lab. Invest.* In press.
38. Glomset, J. A., and D. M. Kaplan. 1965. *Biochim. Biophys. Acta.* **98:** 41.
39. Akiyama, M., O. Minari, and T. Sakagami. 1967. *Biochim. Biophys. Acta.* **137:** 525.
40. Glomset, J. A., and J. L. Wright. 1964. *Biochim. Biophys. Acta.* **89:** 266.
41. Lossow, W. J., S. N. Shah, and I. L. Chaikoff. 1966. *Biochim. Biophys. Acta.* **116:** 172.
42. Rowen, R., and J. Martin. 1963. *Biochim. Biophys. Acta.* **70:** 396.
43. Rowen, R. 1964. *Biochim. Biophys. Acta.* **84:** 761.
44. Tayeau, F., and R. Nivet. 1955. *Compt. Rend. Acad. Sci.* **240:** 567.
45. Vogel, W. C., and E. L. Bierman. 1967. *J. Lipid Res.* **8:** 46.
46. Wagner, A., and C. A. Poindexter. 1952. *J. Lab. Clin. Med.* **40:** 321.
47. Gherondache, C. N. 1963. *J. Clin. Endocrinol. Metab.* **23:** 1024.
48. Monger, E. A., and P. J. Nestel. 1967. *Clin. Chim. Acta.* **15:** 269.
49. Aftergood, L., and R. B. Alfin-Slater. 1967. *J. Lipid Res.* **8:** 126.
50. Sugano, M., I. Chinen, and M. Wada. 1965. *J. Biochem. (Tokyo).* **58:** 285.
51. Sugano, M., and O. W. Portman. 1965. *Arch. Biochem. Biophys.* **109:** 302.
52. Favarger, P. 1946. *Arch. Int. Pharmacodyn.* **72:** 1.

53. Lossow, W. J., S. N. Shah, M. Brot, and I. L. Chaikoff. 1963. *Biochim. Biophys. Acta.* **70:** 593.
54. Brot, N., W. J. Lossow, and I. L. Chaikoff. 1962. *J. Lipid Res.* **3:** 413.
55. Castro Mendoza, H., and C. Jimenez Diaz. 1949. *Bull. Inst. Med. Res.* (*Madrid*). **2:** 81.
56. Friedman, M., and S. O. Byers. 1955. *J. Clin. Invest.* **34:** 1369.
57. Phillips, G. B. 1960. *J. Clin. Invest.* **39:** 1639.
58. Turner, K. B., and V. Pratt. 1949. *Proc. Soc. Exptl. Biol. Med.* **71:** 633.
59. Turner, K. B., G. H. McCormack, Jr., and A. Richards. 1953. *J. Clin. Invest.* **32:** 801.
60. Hernandez, H. H., and I. L. Chaikoff. 1957. *J. Biol. Chem.* **228:** 447.
61. Deykin, D., and D. S. Goodman. 1962. *J. Biol. Chem.* **237:** 3649.
62. Fredrickson, D. S. 1966. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Co., New York. 2nd edition. 486–508.
63. Nestel, P. J., and E. A. Monger. 1967. *J. Clin. Invest.* **46:** 967.
64. Goodman, D. S., and T. Shiratori. 1964. *J. Lipid Res.* **5:** 307.
65. Gidez, L. I., P. S. Roheim, and H. A. Eder. 1965. *J. Lipid Res.* **6:** 377.
66. Gidez, L. I., P. S. Roheim, and H. A. Eder. 1967. *J. Lipid Res.* **8:** 7.
67. Heimberg, M., D. R. Van Harken, and T. O. Brown. 1967. *Biochim. Biophys. Acta.* **137:** 435.
68. Scanu, A. M. 1965. *Advan. Lipid Res.* **3:** 63.
69. Goodman, D. S., D. Deykin, and T. Shiratori. 1964. *J. Biol. Chem.* **239:** 1335.
70. Swell, L., and M. D. Law. 1966. *Arch. Biochem. Biophys.* **113:** 143.
71. Swell, L., and M. D. Law. 1967. *Biochem. Biophys. Res. Commun.* **26:** 206.
72. Dietschy, J. M., and M. D. Siperstein. 1967. *J. Lipid Res.* **8:** 97.
73. Courtice, F. C., and B. Morris. 1955. *Quart. J. Exptl. Physiol.* **40:** 138.
74. Voigt, K. D., M. Apostolakis, and H. K. Beyer. 1967. *Experientia.* **23:** 355.
75. Avigan, J., D. Steinberg, and M. Berman. 1962. *J. Lipid Res.* **3:** 216.
76. Chobanian, A. V., B. A. Burrows, and W. Hollander. 1962. *J. Clin. Invest.* **41:** 1738.
77. Field, H., Jr., L. Swell, P. E. Schools, Jr., and C. R. Treadwell. 1960. *Circulation.* **22:** 547.
78. Berson, S. A., R. S. Yalow, S. S. Schreiber, and J. Post. 1953. *J. Clin. Invest.* **32:** 746.
79. Solomon, A., T. A. Waldman, and J. L. Fahey. 1963. *J. Lab. Clin. Med.* **62:** 1.
80. Brauer, R. W. 1963. *Physiol. Rev.* **43:** 115.
81. Barth, W. F., R. D. Wochner, T. A. Waldmann, and J. L. Fahey. 1964. *J. Clin. Invest.* **43:** 1036.
82. Areskog, N.-H., G. Arturson, and G. Grotte. 1964. *Acta Physiol. Scand.* **62:** 209.
83. Stein, Y., and O. Stein. 1966. *Biochim. Biophys. Acta.* **116:** 95.