Biochem. J. (1982) **208**, 1–7
Printed in Great Britain

REVIEW ARTICLE

Transfers and exchanges of esterified cholesterol between plasma lipoproteins

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Introduction

Knowledge concerning the formation and transport of esterified cholesterol in plasma is fundamental to an understanding of the mechanisms by which cholesterol is delivered to peripheral tissues from the liver and removed from these tissues for recycling or excretion through the liver.

Hepatic cholesterol enters the plasma in man predominantly in the unesterified, or free, form as a component of lipoproteins secreted by the liver. In the plasma most of this cholesterol is converted to cholesteryl esters which have their origin within the plasma high density lipoproteins (HDL) in a transesterification reaction catalysed by the enzyme lecithin:cholesterol acyltransferase (LCAT) (Glomset, 1968). The newly formed cholesteryl esters are subsequently incorporated into the hydrophobic core of lipoprotein particles in all plasma lipoprotein fractions. In the postabsorptive state, esterified cholesterol accounts for about 70% of the cholesterol in human plasma and is transported primarily in low density lipoproteins (LDL) (Goodman, 1965). In most other mammalian species, however, HDL are the major transport vehicle for plasma esterified cholesterol (Goodman, 1965).

This process of esterification and incorporation of cholesteryl esters into the hydrophobic core of lipoproteins enables the delivery of cholesterol to peripheral tissues to be controlled by the regulated uptake of plasma lipoproteins by tissues.

An esterification of plasma cholesterol is required also for the removal of cholesterol from peripheral tissues. Free cholesterol equilibrates rapidly between cell membranes and the surface monolayer of plasma lipoproteins (Hagerman & Gould, 1951). A continuing esterification of the free cholesterol on the surface of plasma lipoproteins maintains a concentration gradient which can drive a net movement of free cholesterol from cell membranes to plasma lipoproteins and thus provide a mechanism for the removal of cholesterol from tissues.

This review is concerned generally with the fate of the esterified cholesterol newly formed in the LCAT reaction and specifically with the process by which it is transferred to lipoproteins other than HDL. Questions relating to the mechanism of such transfers are of more than academic interest, since the LDL fraction, which transports the largest proportion of the esterified cholesterol in human plasma, is also the lipoprotein fraction most implicated in the genesis of human atherosclerosis. This review seeks to document the current state of knowledge and to integrate the process of esterified cholesterol transfers between plasma lipoproteins into an overall scheme of esterified cholesterol transport in plasma.

Abbreviations used: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; ECTEP, esterified cholesterol transfer/exchange protein.

Transfers of esterified cholesterol between plasma lipoproteins

When human plasma is incubated *in vitro* at 37°C there is a net mass transfer of esterified cholesterol from both HDL (Nichols & Smith, 1965) and LDL (Nichols & Smith, 1965; Barter *et al.*, 1980) to very low density lipoproteins (VLDL). However, when such transfers are studied using lipoproteins labelled isotopically in the esterified cholesterol moiety, it is apparent that esterified cholesterol is transferred in both directions between VLDL and HDL, between VLDL and LDL and between LDL and HDL (Barter & Lally, 1979). The exchange is mediated by an esterified cholesterol transfer/exchange protein (ECTEP) (Pattnaik *et al.*, 1978). Provided that esterified cholesterol is not being synthesized, the transfers between LDL and HDL are equal in each direction and a net mass transfer does not result (Sniderman *et al.*, 1978; Barter & Jones, 1979).

The rate of these bidirectional transfers, or exchanges, *in vitro* of esterified cholesterol between human LDL and HDL ranges from 100 to

0306-3283/82/100001-07$01.50/1

300nmol/h per ml of plasma, representing an hourly exchange of 8–12% of the LDL and 7–31% of the HDL pools of esterified cholesterol (Barter & Jones, 1979). By contrast, the rates *in vivo* of catabolism of human LDL and HDL (measured as the catabolism of lipoproteins isotopically labelled in the protein moiety) are 1–2% and less than 1%, respectively (Langer *et al.*, 1972; Blum *et al.*, 1977). In other words, the rate of transfer of esterified cholesterol between human HDL and LDL is great enough, relative to the rate of lipoprotein catabolism, to achieve a virtually complete equilibration of esterified cholesterol between the two fractions during their residence in the plasma *in vivo*; hence, a net mass transfer of esterified cholesterol in either direction is not evident during incubations *in vitro*. In the case of the more rapidly catabolized VLDL, however, the situation is different.

The hourly rate of transfer of esterified cholesterol into and out of VLDL is of the order of 20–30% of the VLDL pool (Hopkins & Barter, 1980). The rate of catabolism of human VLDL *in vivo*, however, is as high as 40% per h (Sigurdsson *et al.*, 1975). VLDL particles are therefore catabolized so rapidly that there is insufficient time for complete equilibration with other fractions to occur. A net mass transfer of esterified cholesterol into VLDL from both HDL and LDL during incubations *in vitro* may therefore result from the continuation of a process of equilibration which was commenced but not completed *in vivo*. To complete the equilibration *in vitro* requires 5–8h of incubation at 37°C (Hopkins & Barter, 1982*a*).

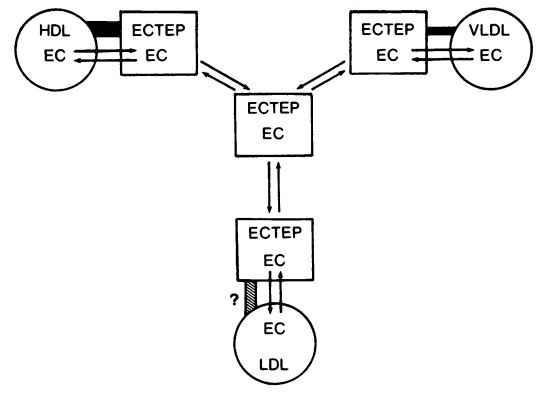

Fig. 1. *Schematic diagram of a mathematical model of the exchanges of esterified cholesterol (EC) between plasma lipoproteins promoted by an esterified cholesterol transfer/exchange protein (ECTEP)*

The ECTEP is shown as existing in two forms: a fraction bound to lipoprotein particles and an unbound fraction. The shaded band between the ECTEP and LDL is intended to indicate that, if there is such a complex, it has a very high dissociation constant.

Model of esterified cholesterol exchanges

The exchanges of esterified cholesterol between human lipoprotein fractions, incubated under conditions of mass equilibrium and with no production of esterified cholesterol, have been described recently in terms of a mathematical model (shown schematically in Fig. 1) which was able to predict experimental results with great accuracy (Barter & Jones, 1980, Barter *et al.*, 1982). The model assumes that the ECTEP (Pattnaik *et al.*, 1978) (see below) interacts with lipoprotein particles into which it deposits and from which it picks up molecules of esterified cholesterol. Assuming that the ECTEP has no 'memory' of the lipoprotein particle from which it has most recently collected esterified cholesterol, the model allows esterified cholesterol to be either redeposited back into the same lipoprotein particle from which it was picked up, deposited in a different particle in the same lipoprotein fraction or deposited in a particle in a different fraction. According to this model a number of predictions can be made.

(i) The ECTEP promotes an equilibration of esterified cholesterol not only between different lipoprotein fractions, but also between different particles within each fraction. Direct experimental support for this concept has been obtained from the observed exchange of esterified cholesterol, without net mass transfer, between human HDL subfractions (Barter *et al.*, 1981).

(ii) More than half of the total transfer activity in human plasma may be involved in exchanges of esterified cholesterol between particles within the HDL fraction.

(iii) Given equal concentrations of esterified cholesterol in each lipoprotein fraction, the probability of the ECTEP picking up (or depositing) a molecule of esterified cholesterol in HDL or VLDL or LDL is in the proportion of approx. 30:7:1. A greater probability of picking up from HDL may reflect no more than the greater number of HDL particles given the same esterified cholesterol concentrations in each fraction. However, the difference between VLDL and LDL cannot be so explained and implies a preferential interaction of the ECTEP with VLDL, a conclusion consistent with earlier direct experimental observations of a preferential incorporation of esterified cholesterol into VLDL relative to LDL (Akanuma & Glomset, 1968).

(iv) At physiological concentrations of lipoproteins, more than half of the ECTEP may exist 'bound' to HDL, a prediction consistent with direct experimental observation (Pattnaik & Zilversmit,

1979). Similarly, both the model and direct studies indicate minimal or no binding to LDL. However, a prediction of the model that the ECTEP binds to VLDL was not supported by direct experimental observation (Pattnaik & Zilversmit, 1979).

These studies of esterified cholesterol exchanges between plasma lipoproteins provide a picture of an ECTEP promoting an equilibration of esterified cholesterol between all particles in all fractions. However, they provide little insight into the more physiological state existing before equilibration is complete and when LCAT is actively synthesizing esterified cholesterol. The issue of how the LCAT reaction interrelates with the transfer/exchange process and by what mechanism newly formed esterified cholesterol becomes incorporated into the equilibrating system of pools in the different lipoprotein fractions is addressed in a later section of this review.

Transfer proteins

The first indication of an involvement of a transfer protein in transfers of esterified cholesterol between plasma lipoproteins was obtained in 1975 when it was reported that a factor in the lipoprotein-free fraction of rabbit plasma promoted an exchange of esterified cholesterol between rabbit VLDL and LDL (Zilversmit *et al.*, 1975). Subsequently, a comparable factor in human plasma (Pattnaik *et al.*, 1978) was shown to promote not only an exchange of esterified cholesterol between all plasma lipoprotein fractions (Barter & Lally, 1979) but also a net mass transfer from HDL to VLDL (Marcel *et al.*, 1980).

The transfer factor in rabbit plasma was partially purified and characterized as a high M_r globulin with an isoelectric point of 5.2 (Zilversmit *et al.*, 1975). A much more pure preparation of this ECTEP has since been isolated from human lipoprotein-free plasma and characterized as a glycoprotein with an apparent M_r of 80000 and an isoelectric point 5.0 (Pattnaik *et al.*, 1978). In more functional studies it has been reported that the human ECTEP binds to HDL (Pattnaik & Zilversmit, 1979), an observation consistent with one of the predictions of the mathematical model described above and which implies that some proportion of the protein may circulate *in vivo* as a complex with HDL.

It has been reported also that apolipoprotein D, a minor HDL apoprotein, promotes an irreversible transfer of esterifed cholesterol from human HDL to both VLDL and LDL (Chajek & Fielding, 1978). Other groups, however, have been unable to confirm that apolipoprotein D functions as a transfer protein (Morton & Zilversmit, 1981*a*; Albers *et al.*, 1981). It has been proposed subsequently that apolipoprotein D and LCAT exist physiologically as components of a small molecular complex which picks up and esterifies free cholesterol from various lipoproteins; the subsequent delivery of esterified cholesterol from the complex to lipoproteins has been attributed to a transfer protein role of apolipoprotein D (Fielding & Fielding, 1980*a*).

In terms of the identity of the protein promoting transfers and exchanges of esterified cholesterol between plasma lipoproteins, the overwhelming evidence favours that described by Zilversmit and his colleagues (Zilversmit *et al.*, 1975; Pattnaik *et al.*, 1978). However, despite the negative reports regarding the transfer protein function of apolipoprotein D, there is sufficient evidence implicating this apoprotein in plasma esterified cholesterol metabolism to warrant further investigation of its role.

As a final comment on the issue of esterified cholesterol transfer proteins, it should be noted that recently yet another protein, this time one which inhibits transfers and exchanges of esterified cholesterol between lipoproteins, has been identified in human plasma (Morton & Zilversmit, 1981*b*). How such an inhibitor interacts with the ECTEP or with apolipoprotein D is not known and it is premature even to speculate on its physiological significance.

Species differences in transfer protein activity

Before Zilversmit *et al.* (1975) reported the existence of an ECTEP in rabbit plasma it had been widely accepted that esterfied cholesterol did not exchange between plasma lipoprotein fractions. This belief was based largely on studies of rats (Roheim *et al.*, 1963; Gidez *et al.*, 1965). It has been shown recently, however, that, in contrast to man and rabbit, rat plasma is deficient in ECTEP activity (Barter & Lally, 1978), an observation which highlights the danger of drawing generalized conclusions from studies of just one species.

A wide range of ECTEP activity has been observed in a recent examination of the plasma of 16 vertebrate species (Ha & Barter, 1982). Three groups were identified: a high activity group including the rabbit, possum and trout, an intermediate activity group including man, wallaby, snake, toad, lizard, turkey, chicken and guinea-pig and a group including the rat, pig, dog, cow and sheep with either no or very low activity. There was no significant correlation between transfer activity and the rate of formation of esterified cholesterol, nor with the concentrations of esterified cholesterol in LDL or HDL. Transfer activity did, however, correlate with the concentration of esterified cholesterol in VLDL (Ha & Barter, 1982). Some of the physiological implications of this species variation in ECTEP activity are described below.

Inter-relationship between esterified cholesterol synthesis and transfers

Role of the ECTEP in the distribution of newly synthesized esterified cholesterol between different lipoprotein fractions

The LCAT reaction provides the major proportion of the esterified cholesterol in all lipoprotein fractions in postabsorptive human plasma (Glomset *et al.*, 1970). However, this does not necessarily imply an action of LCAT on all fractions, since the equilibration promoted by the ECTEP will result in a distribution of LCAT-derived esterified cholesterol to all lipoprotein fractions, even if it were incorporated initially into just one. In fact, available evidence suggests that LCAT activity is confined largely to the HDL fraction (Fielding & Fielding, 1971) and that the equilibration process is thus necessary for an incorporation of esterified cholesterol into VLDL and LDL.

When purified LCAT is incubated *in vitro* with isolated human lipoproteins, esterification of cholesterol occurs only with HDL (Fielding & Fielding, 1971). Similarly, when unfractionated human plasma is incubated *in vitro* with isotopically labelled cholesterol, most of the LCAT-derived labelled esterified cholesterol is recovered initially in HDL (Akanuma & Glomset, 1968; Rose, 1978). It is only with longer incubation, as the specific activity of esterified cholesterol in HDL increases, that the labelled esterified cholesterol becomes more evenly distributed among all lipoprotein fractions (Rose, 1978), precisely as would be predicted if an incorporation into VLDL and LDL were achieved as a consequence of an equilibration with HDL. In the absence of such equilibration, however, esterified cholesterol of LCAT origin remains predominantly in the HDL fraction, as evidenced by studies of pig plasma which is deficient in activity of the ECTEP (Barter *et al.*, 1981).

When pig plasma is incubated with labelled cholesterol most of the labelled esterified cholesterol formed is still recovered in HDL after 24h of incubation (Barter *et al.*, 1981). However, when pig plasma is supplemented with rabbit lipoprotein-free plasma, a rich source of the ECTEP, the LCAT-derived esterified cholesterol becomes distributed evenly between all lipoprotein fractions, comparable to that in incubations of human plasma (Barter *et al.*, 1981). Thus, in the absence of ECTEP activity, most of the esterified cholesterol formed in the LCAT reaction is incorporated into and remains with the HDL fraction. However, in the presence of the ECTEP the concentration gradient created by an incorporation into HDL drives a net mass transfer of esterified cholesterol into VLDL and LDL; ultimately, the newly synthesized esterified cholesterol will equilibrate between all fractions. Recently, however, this view has been challenged by a suggestion that previous conclusions about the site of action of LCAT may have been an artefact of the ultracentrifugal separation process (Fielding & Fielding, 1980*a*).

It has been proposed that, physiologically, LCAT exists as a complex with two HDL apolipoproteins: apolipoprotein A1, its obligatory cofactor, and apolipoprotein D, to which has been ascribed the role of transfer protein (Fielding & Fielding, 1980*a*). According to this view, a 'transferase–transfer' complex interacts with all lipoprotein fractions, picking up free cholesterol, esterifying it and delivering it back in ester form. It was concluded by these workers that VLDL and LDL, rather than HDL, were the major donors of free cholesterol and, in turn, were also the major recipients of the esterified cholesterol so formed. Such a scheme relegates the ECTEP to a minor or non-existent role in the distribution of LCAT-derived esterified cholesterol to VLDL and LDL. However, it is difficult to reconcile this view with the finding that the HDL fraction accounts for about 70% of the labelled esterified cholesterol formed during the first hour of an incubation of unfractionated human plasma with labelled cholesterol (Akanuma & Glomset, 1968), an observation that cannot be dismissed as an artefact of ultracentrifugation. The possibility that such labelled esterified cholesterol may be associated predominantly with a complex of LCAT–apolipoprotein A1–apolipoprotein D, rather than residing in the major HDL pool, is quite inconsistent with an implied transience of any esterified cholesterol pool associated with the complex. The results of pig plasma incubations (see above) further negate any major initial incorporation of LCAT-derived esterified cholesterol directly into VLDL and LDL via a pathway which bypasses the major HDL pool.

The proposition that VLDL and LDL, rather than HDL, may be the major initial recipients of the esterified cholesterol formed in the LCAT reaction (Fielding & Fielding, 1980*b*) was based on the observation that during incubations *in vitro* of human plasma the increase in esterified cholesterol concentration in VLDL and LDL was much greater than that in HDL. However, such a finding is equally compatible with an initial incorporation into HDL with subsequent displacement of esterified cholesterol into VLDL and LDL as a simple consequence of the equilibration process. Nevertheless, evidence favouring HDL as the major initial recipients of the esterified cholesterol formed in the LCAT reaction does not necessarily exclude the existence of an LCAT–apoprotein complex as the active transferase unit; it implies only that, if there is such a complex, it interacts predominantly with HDL particles. This would not be surprising when considering that, physiologically, HDL particles are

present in much greater number than either VLDL or LDL (Eisenberg & Levy, 1975). The concept of an LCAT–apoprotein complex is attractive and is certainly consistent with reports that the lipoprotein 'substrate' for LCAT represents only a very small subpopulation of particles within the HDL fraction.

Postulated scheme

The following hypothetical scheme proposes a role for both apolipoprotein D and the ECTEP in plasma esterified cholesterol formation and distribution. According to this scheme a physiological complex of LCAT with apolipoprotein A1 and apolipoprotein D interacts randomly with particles in all lipoprotein fractions. Because of the greater number of HDL particles, there is a predominance of such interactions within the HDL fraction. During an interaction, free cholesterol on the lipoprotein surface is esterified and the esterified cholesterol formed is internalized into the lipoprotein core. Since free cholesterol equilibrates rapidly between different lipoprotein fractions (Goodman, 1964), a greater rate of esterification in HDL will create a concentration gradient down which free cholesterol will move from other fractions, so that, in net mass terms, the larger pool in the VLDL and LDL will contribute most of the free cholesterol for a reaction taking place predominantly in the HDL. Although much less frequent than with HDL, there may also be interactions of the LCAT–apoprotein complex with VLDL and LDL, resulting in a direct esterification of their free cholesterol and a direct incorporation of esterified cholesterol into particles in these fractions. In the absence of the ECTEP, as in pig plasma, the process stops at this point with about 90% of the new esterified cholesterol in HDL and about 10% in VLDL and LDL (Barter *et al.*, 1981). However, in the presence of ECTEP activity, as in human plasma, the much greater initial incorporation into HDL creates a concentration gradient and a consequent shift in the equilibrium which drives a net mass transfer of esterified cholesterol from HDL to other fractions and, ultimately, an even distribution between all fractions.

A role of esterified cholesterol transfers in the regulation of plasma esterified cholesterol synthesis

It has been suggested that the rate of production of plasma esterified cholesterol is regulated by the capacity of VLDL and LDL to act as recipients of the esterified cholesterol formed in the postulated LCAT–apolipoprotein A1–apolipoprotein D complex (Fielding & Fielding, 1980*b*). However, the validity of this view is seriously undermined by the evidence that it is HDL, rather than VLDL or LDL, which are the major initial recipients of newly formed esterified cholesterol. Nevertheless, it is known that VLDL, if not LDL, stimulate plasma esterified cholesterol production (Rose & Juliano, 1976; Wallentin, 1977) and it is tempting to seek an explanation in terms of the esterified cholesterol transfer process.

As discussed in an earlier section, the equilibration of esterified cholesterol between different human lipoprotein fractions results in a net mass transfer of esterified cholesterol from both HDL and LDL to VLDL. The magnitude of this transfer is, in part, a function of VLDL concentration (Nichols & Smith, 1965); the greater the VLDL concentration, the greater the draining of esterified cholesterol from HDL and LDL. It may be this capacity of VLDL to drain esterified cholesterol from HDL that accounts for the stimulatory effect of VLDL on plasma esterified cholesterol synthesis.

Whether esterified cholesterol is formed in the postulated LCAT–apolipoprotein A1–apolipoprotein D complex or whether it is formed by the action of LCAT on specific substrate HDL particles, the rate of esterification may be influenced by the capacity of HDL particles to receive esterified cholesterol. If the recipient capacity of an HDL particle were an inverse function of the amount of esterified cholesterol already present, such a capacity would be enhanced in particles depleted of esterified cholesterol during exposure to elevated concentrations of VLDL whether *in vivo* or during incubations *in vitro*. It is significant that the enhanced rate of esterified cholesterol production in the plasma of subjects with elevated VLDL concentrations has been shown to persist even after the VLDL fraction has been removed (Patsch *et al.*, 1978). Furthermore, when human plasma containing added VLDL is preincubated at 37°C in the presence of a reversible inhibitor of LCAT before subsequently removing the VLDL and reversing the LCAT inhibition, the rate of esterification in the reisolated VLDL-deficient plasma is markedly enhanced when compared with controls preincubated at 37°C without added VLDL or with added VLDL at 4°C (Hopkins & Barter, 1982*b*). These findings are consistent with a proposition that VLDL influences the esterification reaction by depleting HDL particles of esterified cholesterol rather than by acting as immediate recipients of the esterified cholesterol as it is being formed.

Inter-relationship between transfers of esterified cholesterol and its removal from the plasma

The ECTEP provides a mechanism by which esterified cholesterol formed in the LCAT reaction may be removed from the plasma as a tissue uptake of lipoprotein particles in all lipoprotein fractions. Conversely, in the absence of the ECTEP, LCAT-derived esterified cholesterol must, for the duration of its residence in the plasma, remain within the

lipoprotein particles in which it was initially deposited. Since the major proportion of LCAT-derived esterified cholesterol is incorporated initially into HDL particles, the presence or absence of ECTEP activity may determine the quantitative importance of a tissue uptake of HDL as a pathway for removing esterified cholesterol from the plasma.

In the rat, which lacks ECTEP activity, the rate of formation of esterified cholesterol in the LCAT reaction is about 50nmol/h per ml (Stokke, 1974). Given an esterified cholesterol concentration in rat HDL of the order of 1000nmol/ml (Ugazio & Lombardi, 1965) and rate of removal *in vivo* of rat HDL of 5–7% per hour (Roheim *et al.*, 1971), it is apparent that a tissue uptake of HDL can account for virtually all of the esterified cholesterol formed in the LCAT reaction in this species. An identical conclusion may be drawn from studies of pigs (Ha *et al.*, 1981), another species deficient in ECTEP activity. The situation in man, however, is different.

The rate of formation of esterified cholesterol in the LCAT reaction in human plasma is 50–100nmol/h per ml (Glomset, 1968; Barter, 1974), comparable to that in rat plasma. The concentration of esterified cholesterol in human HDL, of the order of 1000nmol/ml (Barter & Jones, 1979) is also similar to that in the rat. However, human HDL is removed from the plasma *in vitro* at a rate of only 0.5–1.0% per hour (Blum *et al.*, 1977) and can thus account for a removal of only 10–20% of the esterified cholesterol formed in the LCAT reaction. The major proportion of the LCAT-derived esterified cholesterol in human plasma is transferred from HDL to other lipoprotein fractions, predominantly (in mass terms) to VLDL (Nichols & Smith, 1965). Under normal conditions human VLDL are not removed from the plasma, but as a consequence of catabolism within the plasma are converted quantitatively to LDL (Reardon *et al.*, 1978) which are taken up by tissues (Goldstein & Brown, 1974). In a normal human subject with an LDL esterified cholesterol concentration of 2000–3000nmol/ml and an LDL removal rate *in vivo* of about 2% per hour (Langer *et al.*, 1972) a tissue uptake of LDL can account for the removal of 40–60nmol of esterified cholesterol/h per ml, representing the major proportion of that formed in the LCAT reaction. Thus it appears that in man, in contrast with rat and pig, LCAT-derived esterified cholesterol is delivered to tissues by the atherogenic LDL rather than the non-atherogenic HDL.

Relationship between the transfers of esterified cholesterol and other lipids

Coincident with net mass transfer *in vitro* of esterified cholesterol from HDL to VLDL there is a net mass transfer of triacylglycerol in the reverse direction (Nichols & Smith, 1965). This has been interpreted as a molecular exchange of one moiety for the other, a conclusion which has been reinforced recently with a report that there is a mole-for-mole exchange of esterified cholesterol and triacylglycerol between HDL and VLDL (Chajek & Fielding, 1978). It has been shown also that net mass transfers of esterified cholesterol from LDL to VLDL are accompanied by a reciprocal transfer of triacylglycerol from VLDL to LDL (Barter *et al.*, 1980; Hopkins & Barter, 1982*a*). Despite these observations, however, there is mounting evidence that the transfers of esterified cholesterol and triacylglycerol may be at least partially independent processes.

Like esterified cholesterol, triacylglycerol transfers are bidirectional (Barter *et al.*, 1979*a*). They also require the presence of a transfer protein which, in a partially purified form, closely resembles the ECTEP (Rajaram *et al.*, 1980). On the basis of activity in different species (Barter *et al.*, 1979*b*) and patterns of interaction with different lipoprotein fractions (Rajaram & Barter, 1980), there is a strong possibility that a single protein is responsible for the transfers of both esterified cholesterol and triacylglycerol. However, the transfers of the two lipids can be dissociated. Mercurial thiol-group inhibitors such as *p*-chloromercuriphenylsulphonate markedly inhibit triacylglycerol transfers (Hopkins & Barter, 1980) while having virtually no effect on the transfer of esterified cholesterol (Zilversmit *et al.*, 1975). There is also a dissociation in the time course of the transfers *in vitro* of the two moieties, in that a net mass transfer of triacylglycerol may continue for several hours after net mass transfers of esterified cholesterol have ceased (Hopkins & Barter, 1982*a*). Since the transfers of triacylglycerol are bidirectional it is probable that, as with esterified cholesterol (see above), the net mass transfers *in vitro* of triacylglycerol may represent a process of equilibration that had not been completed *in vivo*. Although the equilibration of both triacylglycerol and esterified cholesterol may be promoted by a single protein, it is apparent that molecular movements of the two lipid moieties are not obligatorily linked.

Transfers and exchanges of phospholipids between plasma lipoproteins are also promoted (in part) by a transfer protein (Brewster *et al.*, 1978). In a recent study it was reported that a partially purified preparation of human ECTEP promoted transfers of phospholipid, although phospholipid transfer activity was also found in the absence of esterified cholesterol transfer activity in a comparable protein preparation isolated from rat plasma (Ihm & Harmony, 1980). The general issue of whether there is a single protein which functions to transfer a variety of lipid moieties or whether there exists a series of specific transfer proteins is not yet resolved and must await further work.

Concluding comments

The recent studies of esterified cholesterol transfers between plasma lipoproteins and of the proteins that promote them have provided major insights into plasma esterified cholesterol metabolism. However, there remain significant areas of uncertainty and ignorance which must receive further investigation in order to confirm or refute much of what has, of necessity, been speculative in this review. The issue of transfer protein identity will have to be resolved before attempting studies of the precise molecular mechanism of action. At a physiological level, to determine the importance of the ECTEP and apolipoprotein D in the distribution of LCAT-derived esterified cholesterol to different lipoprotein fractions will require an examination of plasma esterified cholesterol formation and incorporation into plasma lipoproteins in a system uncomplicated by concurrent transfers and exchanges between the different fractions. Finally, at a clinical-pathological level, it will be necessary to develop suitable assays to examine the clinical implications of variations in transfer protein activity.

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