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EVIDENCE FOR A WATER-SOLUBLE INTERMEDIATE IN EXCHANGE OF CHOLESTEROL BETWEEN MEMBRANES

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The mechanism of inter-membrane cholesterol exchange has been a matter of some debate. Evidence from kinetic studies indicates that cholesterol must transfer to and from membranes in a water-soluble form. In this study attempts have been made to demonstrate that this occurs using either dialysis membranes or a barrierless multiphase polymer system to physically separate the membranes. In both systems small amounts of cholesterol were seen to transfer from one membrane pool to another using both liposomes and erythrocyte membranes as donors or acceptors. The cholesterol transfer was shown to be independent of the movement of other membrane components. The amount of transfer observed was limited by the physical properties of the systems employed. The barrier to cholesterol transfer in the dialysis membrane system is primarily the pore size of the membrane, while in the multiphase polymer system the transfer was limited by the viscosity of the medium and the distance between the lower and upper phases containing the membranes. Nevertheless, the results provide evidence that cholesterol transfer is by a dissociation of molecules from membranes into the aqueous medium and does not require the formation of a collision complex between the membranes.

Introduction

Cholesterol and phospholipids are major constituents of biological membranes and lipoproteins where they are organized into bilayers and monolayers. These are dynamic structures in which the lipid molecules can move laterally, translocate across a bilayer or even exchange with like molecules in other membranes and lipoproteins. The mechanism of this exchange process has been a matter of controversy. It was originally proposed that the exchange of cholesterol, which is generally more rapid than that of the phospholipids, occurs via a water-soluble intermediate [1], even though the solubility of cholesterol in water is low. An alternative proposal was the formation of a collision complex between the cholesterol-bearing particles [2] and that a temporary fusion of the outer halves of the bilayer leaflets may afford a suitable hydrophobic channel for the transfer of cholesterol [3]. There were objections to the collision theory on two grounds. Firstly, the net negative charge of the membrane glycocalyx may prevent the formation of such a complex. Secondly, the kinetics of the exchange showed it to be a first-order process [4] although this was not the case in exchange between lipoproteins and membranes [5]. However, Bojesen [6] provided evidence that these differences could still be reconciled on the basis of

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cholesterol exchange by desorption of molecules into the aqueous environment.

Cholesterol has an apparent solubility in water of 4.6 μM [7] although this appears to be unrelated to the rate of exchange; the critical micellar concentration (approx. 25 nM) may be more relevant [8]. There have been difficulties in demonstrating directly the existence of soluble cholesterol in exchange experiments [9]. Significant exchange was observed through a dialysis membrane, but only after days of incubation when the integrity of the membrane may have been in doubt [4].

In this study we have tried different approaches: (1) to enhance the solubility of cholesterol monomers with agents known to increase exchange [8] and to observe transfer across the dialysis membrane, (2) to dialyse cholesterol through membranes with larger pore-sizes and (3) to use multi-phase polymer systems to remove all mechanical barriers to exchange.

In each case the amount of exchange was small, but sufficient to show that the cholesterol could transfer through the aqueous phase.

Materials and Methods

[4-^{14}C]Cholesterol, [7α-^{3}H]cholesterol, cholesteryl[1-^{14}C]oleate, $^{3}H_2O$ and $NaB^{3}H_4$ were purchased from Amersham International, Amersham, Bucks., U.K. Egg phosphatidylcholine, cholesterol, sodium periodate, cyanocobalamin, Methylene blue and sodium borohydride were obtained from Sigma Chemical Co. (U.K.) Ltd., Poole, Dorset. Poly(ethylene glycol) 6000 (PEG 6000) was supplied by BDH Ltd., Poole, Dorset; Ficoll 400 and Dextran T500 were from Pharmacia AB, Uppsala, Sweden.

(a) Preparation of liposomes

'Donor' liposomes were prepared from a mixture of dried lipids containing 5 mg egg phosphatidylcholine, 2.5 mg cholesterol, 10 μCi [7α-^{3}H]cholesterol and in some cases 1 μCi cholesteryl[1-^{14}C]oleate (spec. act. 58 mCi/mmol). These lipids were suspended in 5 ml 0.11 M sodium phosphate buffer (pH 6.8)/0.05 mM NaN_3 with a vortex mixer and sonicated for 12 min at 4°C with a 150 W Ultrasonic Disintegrator (M.S.E., Crawley, Sussex, U.K.) fitted with a titanium probe of 19 mm diameter. After sonication, the liposomes were centrifuged at 4°C for 30 min at 17500 $\times g$ in an M.S.E. 65 centrifuge and the supernatant was used for experiments. The specific activity of the liposome cholesterol was calculated from the radioactivity which was counted in a liquid scintillant (Scintran Cocktail T, BDH Ltd., Poole, Dorset, U.K.) using a Packard Tricarb Spectrometer Model 3385 with dual channel counting where appropriate, and the mass was determined by enzymatic analysis [10]; the liposomes were used on the day of preparation. Unlabelled 'receptor' liposomes were prepared with 5 mg egg phosphatidylcholine alone by a similar procedure.

(b) Preparation of erythrocyte ghosts

(i) Unlabelled ghosts. These were prepared from human blood collected in acid citrate/dextrose anti-coagulant [11]. The blood was centrifuged at 1000 $\times g$ for 10 min and washed in saline (9 g NaCl/l) by repeated centrifugation. Ghosts were prepared by the method of Dodge et al. [12].

(ii) [4-^{14}C]Cholesterol-labelled ghosts. 7 ml washed intact erythrocytes were incubated for 4 h at 37°C with 7 ml of [4-^{14}C]cholesterol-labelled plasma. The plasma was labelled, following a preincubation at 56°C for 1 h, by incubating at 37°C for 16 h with 10 μCi [4-^{14}C]cholesterol (spec. act. 54.8 mCi/mmol). After labelling, the erythrocytes were washed free of plasma by repeated centrifugation at 1000 $\times g$ for 10 min until the supernatant was free of label. Ghosts were then prepared as described above.

(iii) Surface-labelled ghosts. Unlabelled ghosts were resealed in a final volume of 5 ml 0.15 M NaCl/ 0.01 M sodium phosphate (pH 7.4) (buffered saline), and labelled by a modification of the method of Gahmberg and Andersson [13]. An equal volume of 2 mM $NaIO_4$ in buffered saline was added to the ghosts and incubated for 10 min at 22°C. 1 ml 0.1 M glycerol was then added to the incubation mixture. The ghosts were washed four times in buffered saline by centrifugation at 11000 $\times g$ in a Sorvall RC-5B Superspeed centrifuge (Du Pont U.K. Ltd., Stevenage, Herts.) The ghosts were then labelled by addition of 2.5 mCi $NaB^{3}H_4$ (spec. act. 261 mCi/mmol) for 30 min at 22°C in 5 ml buffered saline after which 1.25 mg of unlabelled $NaBH_4$ were added and the ghosts

were washed again in buffered saline. Unincorporated label was removed by washing the ghosts five times in 10 mM Tris/HCl buffer (pH 7.4) to prevent entrapment in sealed ghosts. They were finally resuspended in 2 ml 0.11 M sodium phosphate/0.05 mM NaN_3 (pH 6.8).

(c) Dialysis experiments

4 ml liposomes were prepared as described above and the 'donor' liposomes were introduced into dialysis sacs of Visking tubing (8/32 inch width, pore diamater 4.8 nm). The sacs were then rinsed thoroughly in saline to avoid external radioactive contamination and incubated at 37°C in 20 ml 'receptor' liposomes in capped plastic 'universal' tubes. The external medium was agitated with a magnetic bar which did not touch the dialysis membranes. Aliquots of the receptor liposomes were removed at intervals, the lipids were extracted [14] and separated by thin-layer chromatography (petroleum ether/diethyl ether/acetic acid, 70 : 20 : 4, v/v). The cholesterol spot was rechromatographed in another TLC system (ethylacetate/*n*-heptane, 1 : 1, v/v) to establish that the radioactivity was associated with cholesterol and not its oxidation products [15]. In all cases 90% or more of the radioactivity was associated with cholesterol.

In some experiments nitrocellulose dialysis thimbles, (UH 100/75, Schleicher and Schull GmbH, D-3354 Dassel, F.R.G.) were used, which permit the passage of molecules of up to M_r 75 000. The procedure was essentially the same as for the dialysis sacs.

(d) Three-phase polymer systems

7% Dextran T500, 7% Ficoll 400 and 6% poly(ethylene glycol) 6000 w/v in 0.11 M sodium phosphate (pH 6.8)/0.05 mM NaN_3 were mixed and left to separate for 72 h at 4°C. Individual phases were collected and stored at 4°C. The bottom and top phases were diluted (350 μl/g) before use by the addition of ^{14}C-labelled cholesterol, liposomes or erythrocyte ghosts to the bottom phase and of unlabelled 'receptor' liposomes, erythrocyte ghosts or phosphate buffer to the top phase. The phases remained viscous despite the dilution. The three-phase system was reconstituted by overlaying the diluted bottom phase (2 g) with middle phase (1.5 g) and diluted top phase (2 g) to give respective depths of 1.2, 0.8 and 1.4 cm with two interfaces of 1.54 cm^2 each. The three phases remained separate.

The bottom phase was agitated gently during incubation at 37°C by a magnetic stirrer in some cases and samples (at least 50% of total phase volume) were removed after a fixed time from either the top phase alone, or from the top and middle phases. Separate incubations were used for each time interval. The lipids were then extracted [6] and assayed for radioactivity and cholesterol as described above. Samples of ghosts were washed free of poly(ethylene glycol) before extraction by repeated centrifugation in saline (9 g NaCl/litre) at 11 000 × *g* for 10 min.

Results

The dialysis of liposomes containing labelled cholesterol

After long periods of dialysis in Visking tubing, up to 110 h, very small amounts of radioactivity (below 0.3% of the total counts) penetrated the membrane (Fig. 1A). This radioactive material did

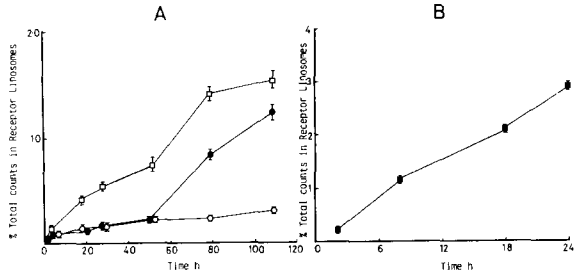

Fig. 1. The transfer of [^{3}H]cholesterol from donor liposomes through dialysis membranes to acceptor liposomes. Donor liposomes were prepared from [7α-^{3}H]cholesterol, egg phosphatidylcholine and cholesterol (see Materials and Methods) in 4 ml 0.11 M sodium phosphate (pH 6.8) (phosphate buffer) and introduced into Visking dialysis sacs (A) or nitrocellulose dialysis thimbles (B). The appearance of radioactivity in unlabelled egg phosphatidylcholine liposomes outside the sacs or thimbles was observed for several days and the identity of the extracted radioactivity was determined by thin-layer chromatography. The transfer of counts was measured from dialysis sacs in the presence of phosphate buffer (○), in the presence of 1.7 M acetone in phosphate buffer (●) and in the presence of 2.6 M acetone in phosphate buffer (□). From dialysis thimbles, only transfer in phosphate buffer was measured (■). Each point is the mean of six observations ± S.D.

co-chromatograph with cholesterol on thin-layer chromatograms. In similar dialyses in which the medium contained 1.7 M or 2.6 M acetone there was a significant increase in the transfer of cholesterol after 110 h. In the case of 2.6 M acetone this was true throughout the incubation period, but with 1.7 M acetone the higher rates of transfer were only apparent after 80 h (Fig. 1A).

In order to show that there was no restriction to the movement of molecules through the dialysis membrane after these long periods, the reverse dialysis of Methylene blue (M_r 374), and cyanocobalamin (M_r 1057) was demonstrated by spectroscopic measurements in all the dialyses. Conversely, leaks in the dialysis membrane were indicated by the appearance of cholesteryl[1-^{14}C]oleate, a non-exchangeable marker incorporated into some 'donor' liposomes, outside the dialysis sac. In the absence of acetone, leakage was negligible. However, if acetone was present, after 80 h there was an increase in the escape of ^{14}C counts, reaching 89% of the [^{3}H]cholesterol counts after 110 h with 2.6 M acetone (not shown). Therefore, in most cases, the exchange of cholesterol[^{4}C]oleate is much less than that of [^{3}H]cholesterol, indicating a separate exchange. Further experiments are required to study the effect of acetone on cholesterol ester exchange after long periods.

In nitrocellulose dialysis thimbles the pore sizes are larger (9 nm diameter) which permits the passage of compound or aggregates of up to M_r 75 000. Within 24 h, 3% of the total [^{3}H]cholesterol counts appeared in the acceptor liposomes outside the thimbles (Fig. 1B).

The diffusion of cholesterol through a multiphase polymer system

The multi-phase polymer systems are more generally considered to be a means of cell separation and much has still to be learned about their physical properties [17]. To evaluate their usefulness for exchange experiments it was necessary to show (a) that there is mobility of the solute, i.e., water in this relatively viscous medium and (b) that cholesterol would distribute throughout a multi-phase system. A three-phase system was established and ^3H_2O was injected into the bottom layer (Fig. 2). The free movement of solvent was indicated by the equilibration of ^3H_2O between the three layers within 8 h. A similar result was obtained without stirring the bottom layer.

The distribution of [^{14}C]cholesterol in this system was observed in two ways. Firstly, labelled cholesterol was added to the polymer mixture before it separated into the constituent phases. The three phases resolve in the volume ratio 1.1 : 1.0 : 2.0, bottom, middle, top, respectively, yet 91% of the cholesterol as found in the bottom phase with only 8.5 and 0.55% in the middle and top phases, respectively. The second approach was to determine whether cholesterol introduced into the bottom phase could diffuse into the two upper phases. Reconstituted three-phase systems were prepared (see Materials and Methods) and in each one the bottom phase contained 0.05 μCi [^{14}C]cholesterol (spec. act. 54.8 mCi/mmol) dispersed from an ethanol solution. The top phase was diluted with buffer and the bottom phase was stirred gently during incubation at 37°C. Samples were taken from the middle and top phases at 24, 48 and 72 h and assayed directly for [^{14}C]-cholesterol. In the middle phase counts appeared

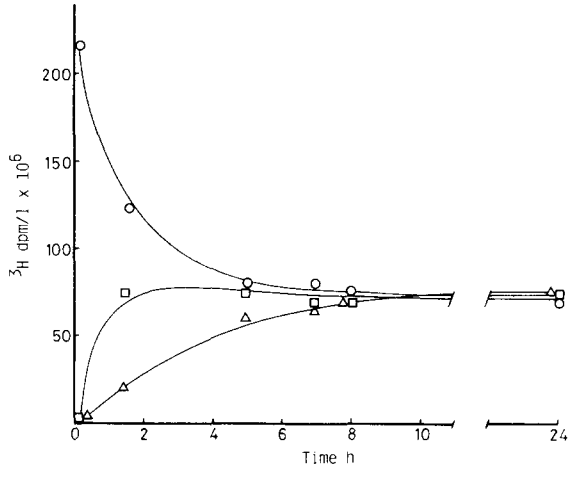

Fig. 2. The multiphase polymer system and the transfer of water between the phases. A multiphase polymer system containing Dextran T500, Ficoll 400 and poly(ethylene glycol) 6000 was established as described in the Materials and Methods section. 12 μCi ^3H_2O, (24 μl) was injected into the lower phase (2 ml) which was stirred slowly with a flea magnet. Small aliquots of the bottom (○), middle (□) and upper (△) phases were taken at intervals up to 24 h. Each point is a mean of two separate determinations.

after 24 h, but were less than 1% of the total in the system (Fig. 3A). This increased to over 14% after 72 h. The apparent non-linear diffusion is probably attributable to the heterogeneous size of cholesterol particles in the bottom phase which move at different rates into the middle phase. No significant counts were detected at any time in the upper phase.

In a similar experiment, an 'acceptor' pool of membranes was introduced into the upper phase in the form of erythrocyte ghosts containing a total of 200 μg cholesterol. Again, counts due to [^{14}C]cholesterol were detected in the middle phase, up to 9% of the total radioactivity after 72 h (Fig. 3B). At this time the upper phase was removed and the ghosts were washed free of upper-phase

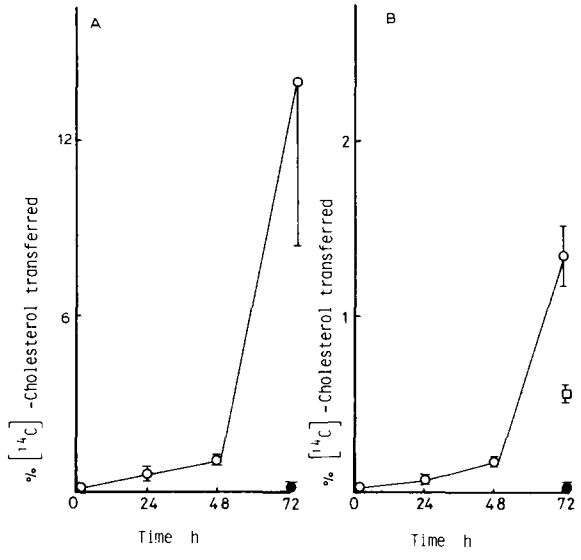

Fig. 3. The transfer of dispersed [^{14}C]cholesterol from the bottom to the top phase of a multi-phase system. The bottom, middle and top phases of a multi-phase system containing Dextran T500, Ficoll 400 and poly(ethylene glycol) 6000 were reconstituted in the weight ratio 2:1.5:2, respectively. The bottom phase contained 0.05 μCi [^{14}C]cholesterol dispersed from ethanol and to the top phase was added either buffer (A) or erythrocyte ghosts (200 μg cholesterol) in buffer (B). The appearance of counts in the middle phase was determined for a period up to 72 h. After 72 h the upper phase was removed and the ghosts (B) were washed free of the medium by repeated centrifugation in buffer at 11000 × g for 10 min. The ghosts lipids were extracted [16] and the radioactive counts in the middle phase (○), in the top-phase medium (●) and (B) in the top-phase ghosts (□) were identified as [^{14}C]cholesterol by TLC. Each point is a mean ± S.D. of three samples.

medium as described in Materials and Methods. [^{14}C]Cholesterol (3.6% of the original counts) was found associated with the ghosts, but none with the medium (Fig. 3B). Therefore, the presence of an exchangeable pool of cholesterol permitted the transfer of labelled cholesterol into the upper phase.

In the following experiments the transfer of labelled cholesterol from membranes in the lower phase to membranes in the upper phase was demonstrated using both liposomes and erythrocyte ghosts. The labelled cholesterol sources were placed in the bottom phase and the 'acceptor' membranes in the top phase to eliminate the possibility of contamination, due to gravity, of the 'acceptor' membranes by the labelled membranes. Also, since the distribution of the soluble cholesterol in this three-phase system strongly favours the bottom phase, the appearance of labelled cholesterol in the top phase would be particularly significant.

In the case of the liposomes the 'donors' in the bottom phase contained both [^{3}H]cholesterol and cholesteryl[^{14}C]oleate, the latter serving as a non-exchangeable marker to indicate the movement of whole liposomes. The amount of cholesterol transfer from the bottom phase into the phospholipid 'receptor' liposomes in the top phase was assayed after incubation at 37°C for 72 h. There was no transfer of cholesteryl[^{14}C]oleate into the top phase during the incubation, but 2% (equivalent of 10 μg) of the [^{3}H]cholesterol was transferred (results not shown). These ^{3}H counts could not be positively identified as pure cholesterol, because poly(ethylene glycol) contamination caused the cholesterol spot to streak on TLC. However, none of the counts migrated further than the cholesterol spot, as may be expected if the ^{3}H which had transferred to the top phase represented cholesterol oxidation products rather than cholesterol itself.

In experiments with erythrocyte ghosts the 'receptor' ghosts in the top phase were washed free of poly(ethylene glycol) by centrifugation before extraction of the lipids so that the radioactivity could be readily identified with cholesterol by TLC. The transfer of [^{14}C]cholesterol from 'donor' ghosts in the bottom phase to those in the top phase was observed within a period of 72 h (Table I). On average, less than 1% of the total cholesterol counts were transferred within 72 h (approx. 2 μg of

TABLE I

THE TRANSFER OF [^{14}C]CHOLESTEROL FROM ERYTHROCYTE GHOST MEMBRANES IN THE BOTTOM LAYER OF A MULTI-PHASE POLYMER SYSTEM TO MEMBRANES IN THE TOP LAYER

Ghost membranes for the bottom phase were prepared from erythrocytes which had been labelled by incubation with [^{14}C]cholesterol-enriched plasma, while the erythrocyte ghosts for the top phase were prepared from unlabelled cells (see Materials and Methods). Each membrane pool contained 200 μg cholesterol. The reconstituted systems were incubated at 37°C, without stirring of the bottom phase, and samples of the top and middle phases were removed at 24 and 72 h. The lipids were then extracted from the solution (middle phase) and ghost membranes (top phase) and the radioactivity was assayed and confirmed to co-chromatograph with cholesterol by TLC. The results are expressed as means ± S.D. ($n = 10$) in terms of (a) percent of total [^{14}C]cholesterol transferred and (b) the calculated total mass of transferred cholesterol. Similar results were obtained when the bottom phase was stirred during the incubation.

Time	% of total label transferred	Total cholesterol transferred (μg)
Top phase		
24 h	0.34 ± 0.09	0.65 ± 0.16
72 h	1.05 ± 0.39	2.04 ± 0.74
Middle phase		
24 h	0.06 ± 0.02	0.14 ± 0.04
72 h	0.09 ± 0.04	0.18 ± 0.08

cholesterol) and even smaller amounts were found in the middle phase. No counts were observed in samples from the middle and top phases at zero time (0 h), indicating that the transfer observed after 24 and 72 h, though small, is real.

It was also necessary to demonstrate that 'donor' ghosts labelled with [^{14}C]cholesterol had not migrated intact or as fragments into the top phase. In separate experiments erythrocyte ghosts were labelled by the galactose oxidase-NaB^3H_4 procedure (Materials and Methods) and placed in the bottom phase as described above. No trace of radioactive label was found in the middle or top phases after 72 h, indicating that cholesterol had transferred independently through an aqueous medium between two physically separate membrane pools.

Discussion

In most biological membranes and in model membrane systems, cholesterol exchanges rapidly between cholesterol pools which are normally associated with phospholipids. Cholesterol generally has a more rapid rate of exchange than the major phospholipid classes [3]. This sterol is sparingly soluble in water, or at least can be dispersed as aggregates of a molecular weight range 10 000 × 500 000 [7] and the critical micellar concentration or, perhaps more correctly, the critical aggregate concentration is 25 nM. Cholesterol molecules have a molecular dimension of 0.52 × 0.62 × 1.89 nm [18] and form crystals of different dimensions in the anhydrous and hydrated forms [19,20]. It was deduced that the length of a cholesterol micelle or aggregate M_r 200 000 may be 100 nm with a diameter of 2 nm. The probability of such a particle penetrating conventional dialysis tubing (mean pore diameter 4.8 nm) is not great, but it should be permeable to cholesterol monomers and possibly very small aggregates. Haberland and Reynolds [7] showed that an average pore diameter of 200 nm was required to achieve full equilibration of cholesterol aggregates.

In the experiments described above, the transfer of cholesterol across a dialysis membrane was achieved after a long period of incubation, but to a much smaller extent than that found by Backer and Davidowicz [4] even though cyanocobalamin, with a molecular weight 4-times greater than that of cholesterol passed through readily. In contrast, no exchange or transfer was found by Quarfordt and Hilderman [21] in comparable experiments. The rate of transfer of cholesterol was enhanced by the presence of acetone which increases the critical micellar concentration [8] and therefore the chances of a successful transfer. An increased rate of transfer was also achieved when the pore size was increased using nitrocellulose thimbles, showing that cholesterol can be transferred in significant amounts within a few hours. The validity of these experiments depends on the absence of mechanical ruptures or weaknesses in the membranes, which can be monitored by the presence of markers which are not exchangeable, e.g., cholesterol esters. The presence of acetone in the system caused some cholesterol ester transfer,

which may indicate that the dialysis membrane has become leaky or that acetone promotes cholesterol ester exchange.

In order to exclude the problems involved in dialysis and to confirm the results obtained, the multiphase polymer system was used at concentrations of polymers known to permit cell separation without damage to cell function [17]. The phases containing the two membrane cholesterol pools were separated by a third phase so that there could be no physical contact between them during incubation or sampling. The interfacial tension between the phases, 7 $\mu N \cdot m^{-1}$ between bottom and middle phases and 1.3 $\mu N \cdot m^{-1}$ between middle and top phases, measured by the rotating drop method [22], is high and prevents the passage of intact membranes between the phases (Crowe, J. and Gascoine, P., unpublished observations). However, the high viscosity of the medium reduces the movement of the small solutes and solvent so that water required 8 h to equilibrate throughout the system, and 90% of added soluble cholesterol partitioned into the bottom phase. Nevertheless, a small but significant transfer of cholesterol occurred from the bottom to the top phase. This transfer was not attributable to the transfer of whole liposomes, membrane fragments or to the formation of more polar oxidation products of cholesterol. Since no experiments were performed to investigate the exchange of phospholipids in erythrocyte ghosts, the possibility that cholesterol may transfer in association with specific types of phospholipids e.g., lysophospholipids cannot be excluded. The transfer of cholesterol to the top phase was more rapid (3.6% after 72 h) when the labelled cholesterol diffused from dispersed cholesterol in the bottom phase rather than from membranes (less than 1%). The amount of cholesterol in the middle phase was also greater. This may be due to limitations imposed by the dissociation of cholesterol from the membranes rather than from aggregates of cholesterol molecules.

In these experiments cholesterol moved from the bottom to the top phases apparently independently of other membrane constituents, and the assumption has been made that this movement was due to free diffusion of cholesterol molecules. Water molecules were mobile between all phases. Although the polymers resolve into separate phases after mixing, minor amounts of each polymer can be found in each of the phases, and an association between cholesterol monomers or aggregates with the polymers themselves cannot be excluded. However, if this were to occur, it would retard rather than accelerate the transfer of cholesterol, due to the slower rate of diffusion of a large particle. In any case, the occurrence of such an association requires the prior dissociation of cholesterol molecules from the donor membranes and therefore does not invalidate the proposition that cholesterol exchange involves a movement of molecules out of the membrane and into the aqueous medium. Any interaction of polymer with cholesterol would be confined to the free sterol and not cholesterol ester, which did not transfer to the top phase. When a small amount of cholesterol is dispersed in the polymer mixture to give a final concentration of less than one third of the maximum cholesterol solubility in water, and when the solution is allowed to resolve into its three phases, 91% of the cholesterol partitions into the bottom phase. The low affinity of cholesterol for the middle phase is indicated by its concentration there, which was slightly less than 9% of the total cholesterol present, but still above the critical micellar concentration. Cholesterol has an even lower affinity for the top phase where it can only be found associated with membranes, with negligible amounts free in the aqueous medium. The reason for this uneven distribution of cholesterol between the phases, which is unrelated to the distribution of the three polymers, is unknown, but may possibly be related to the relative hydrophobicity of the phase constituents.

The evidence given above, supporting that from kinetic analysis, indicates that cholesterol can be exchanged or transferred through an aqueous environment. In biological systems the cholesterol monomer or small aggregate may need to travel only a few nanometers to its acceptor and therefore the time to achieve equilibrium will be far shorter than in experimental systems with physical barriers or long distances through which diffusion must take place. The rate-limiting step for cholesterol exchange between cells is considered to be the dissociation of cholesterol from the membranes [4]. In the multi-phase polymer system, this

may not be the case and the rate of diffusion of the cholesterol through the viscous medium may be rate limiting. The main intention of this work has been to show that cholesterol transfers between membrane pools via a water-soluble intermediate.

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