MOLECULAR PHARMACOLOGY, 21:694–700

Movement of Free Cholesterol from Lipoproteins or Lipid Vesicles into Erythrocytes

Acceleration by Ethanol *in Vitro*

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Received September 28, 1981; Accepted December 10, 1981

SUMMARY

Human erythrocytes were incubated with heat-inactivated plasma, and the transfer of cholesterol to the red cells was followed to equilibrium over 24 hr. When cholesterol-enriched plasma was used, there was a net flow of sterol into red cells. Ethanol, in a concentration-related manner, accelerated the cholesterol transfer without appreciably affecting the final sterol content of the erythrocytes at equilibrium. Ethanol also accelerated the exchange of tritiated cholesterol between normal or cholesterol-enriched plasma and red cells, whether or not there was a net cholesterol flow. Ethanol speeded up sterol transfer from several cholesterol donors, including prelabeled erythrocytes, low-density lipoproteins, high-density lipoproteins, and egg lecithin vesicles. Ethanol (0.35 M) increased the rate constant of the transfer by about 30–40% with different sterol donors. These observations may be related to the previously reported increase in cholesterol in the brain and red cell membranes of mice after chronic treatment with ethanol.

INTRODUCTION

Ethanol is known to disrupt the structure of membrane lipids, as shown by measurement of electron paramagnetic resonance (1, 2) or fluorescence polarization (3, 4). When mice are treated with ethanol for several days, they become behaviorally tolerant and their cell membranes become resistant to the disordering effects of ethanol *in vitro* (3, 5). An increased membrane content of cholesterol accompanies this *in vitro* tolerance, both in erythrocyte ghosts and in synaptosomal plasma membranes (3, 6). Chin and Goldstein (7) have shown that cholesterol can block the disordering effects of ethanol on model membranes, so the possibility exists that tolerance is partially mediated by increased cholesterol in neuronal membranes. The studies reported here were designed to examine the mechanism by which ethanol causes membrane cholesterol to increase. We began by testing the simplest possibility, namely that the mere presence of ethanol could affect the transfer of cholesterol into cell membranes from sterol carriers in the plasma. We report that ethanol accelerates transfer of unesterified cholesterol from any of several donors into red cell membranes, with little or no effect on the equilibrium value.

This research was supported in part by United States Public Health Service Grant AA-01066 and Training Grant GM 07149. The work was submitted in partial fulfillment of the requirements for a Ph.D. degree of C. K. D. Preliminary reports have been published [*Fed. Proc.* 39:744 (1980) and *Alcoholism Clin. Exp. Res.* 5:146 (1981)].

0026-895X/82/030694-07$02.00/0

METHODS

Preparation of media. As cholesterol donors, we used human plasma, cholesterol-enriched plasma, plasma lipoproteins, erythrocytes, or sonicated model membranes. A method modified from that of Shinitzky (8) was used to prepare cholesterol-enriched plasma. Plasma was separated from outdated blood obtained from a local blood bank. It was heated at 56° for 30 min to inactivate enzymes, including the plasma lecithin-cholesterol acyltransferase. After centrifugation (30 min at 5000 × *g*) to remove denatured protein, the supernatant was diluted with 9 volumes of isotonic buffer [137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4, (pH 7.4)]. Glucose (2 mg/ml), penicillin (100 units/ml), and streptomycin (100 μg/ml) (final concentrations) were included in this buffer when it was used for incubation media, but not when it was used for washing. Cholesterol enrichment was carried out by adding one-tenth volume of a solution of cholesterol (5.17 mM) in tetrahydrofuran-0.6% KCl (5:1). The cholesterol was added dropwise with stirring in a round-bottomed flask. Unenriched plasma was prepared similarly except that cholesterol was omitted from the tetrahydrofuran-KCl solution. In some experiments, tritiated cholesterol was included in the tetrahydrofuran-KCl solution to a specific activity of 10 μCi/ml. The preparation was lyophilized overnight and stored at −20° under nitrogen. When needed, it was reconstituted with distilled water to 10 times the original volume of the plasma. Turbidity was removed by centrifugation at

20,000 × *g* for 30 min. Unenriched plasma medium contained about 0.5 mM endogenous cholesterol; enriched medium contained about 1 mM, of which 70%–98% was recovered in the supernatant after centrifugation. If it is assumed that about 20% of endogenous plasma cholesterol is unesterified (9), a typical preparation of unenriched medium contained about 0.1 mM free cholesterol at a specific activity of about 10 μCi/mmole. Since all of the extra cholesterol (about 0.4 mM) in enriched medium was free, this type of preparation had about 0.5 mM free cholesterol at a concentration of 2 μCi/mmole.

VLDL,[1] LDL, and HDL were isolated from unenriched or cholesterol-enriched dilute heat-inactivated plasma by a method modified from Havel *et al.* (10), using differential centrifugation in NaCl-KBr solutions. For preparative purposes, the fractions were separated sequentially in repeated ultracentrifugations at successively higher densities. For analysis of the cholesterol-labeled media, three samples were centrifuged in parallel and the amounts in each density fraction were determined by difference. VLDL were defined as the material of density less than 1.006, LDL as density 1.006–1.063, and HDL as density 1.063–1.210. After dialysis to remove the salts, the fractions were made up in buffer to 10 times the original volume of plasma from which they had been isolated.

Sonicated vesicles were prepared from egg phosphatidylcholine, cholesterol, and [^{3}H]-cholesterol, by a method modified from Cooper *et al.* (11). Cholesterol and egg lecithin in chloroform-methanol (3:2) were mixed at a molar ratio of 1.0 for "low-cholesterol vesicles" or 1.6 for "high-cholesterol" vesicles. The lipids were dried on the walls of a flask under vacuum and subsequently hydrated in 10 ml of buffer. They were sonicated for 60–75 min at 85 W under nitrogen, with cooling (Heat Systems sonicator, Model W-225R, standard tip). Human serum albumin was added to a final concentration of 10 mg/ml, and the preparation was centrifuged at 106,500 × *g* for 30 min to remove Ti particles and aggregated lipids. Samples were taken for extraction and lipid assay.

Incubation. One volume of fresh, washed, human erythrocytes was incubated at 37° with shaking in 9 volumes of enriched or unenriched plasma medium or other cholesterol donors as noted. The red cells contained about 350 nmoles of cholesterol per 0.1 ml of packed cells. Blank incubation mixtures, lacking cells, were included to be sure that the cholesterol did not aggregate and spin out at the end of the incubation. Samples of cells taken at the start of the incubation contained only a few counts per minute (0.5% of the total), as did cells that were added at the end of the 24-hr incubation period and immediately harvested. This showed that little or no cholesterol was instantly bound to cells and also that aggregated cholesterol did not contaminate the red cell fraction. Hemolysis was negligible, less than 2% in the longest incubations. The addition of ethanol did not affect the amount of hemolysis.

In some experiments, red cells were used as cholesterol donors. They were preloaded with [^{3}H]cholesterol by incubating for 24 hr with cholesterol-labeled unenriched plasma medium, then washed before being used to study the efflux of cholesterol from cells into unlabeled plasma media.

Lipid extraction and assay. At intervals during the incubation, triplicate samples (1 ml) were centrifuged. The red cells were washed three times with buffer, and 50-μl samples of packed cells were lysed with distilled water. Lipids were extracted from red cells by the method of Rose and Oklander (12) and the extracts were assayed for cholesterol (13) and phospholipid (14). Lipids were extracted from plasma, lipoproteins, or sonicated vesicles by the method of Folch *et al.* (15).

In experiments with tritiated cholesterol, 10 ml of scintillation fluid (Instagel; Packard Instrument Company, Downers Grove, Ill.) was added to 100-μl samples of medium or 25 μl of packed, washed cells, and the samples were counted on a Packard Tri-Carb liquid scintillation spectrometer, using quench corrections by the external standard ratio method. To improve counting efficiency in samples that contained erythrocytes, the lysed cells were bleached with 1 ml of 30% hydrogen peroxide. The excess hydrogen peroxide was destroyed by heating at 55° for 15 min.

Ethanol was determined by the gas chromatographic method of Gallaher and Loomis (16). These assays established that ethanol did not evaporate from the stoppered incubation vessels during the 24-hr incubation.

Free cholesterol and its esters were separated by thin-layer chromatography, using Silica Gel G plates (Eastman Chromagen sheet with fluorescent indicator 6060), with hexane-diethyl ether-glacial acetic acid (80:20:1) as solvent. Cholesterol and cholesterol linoleate in chloroform-methanol (2:1) were used as standards. After the plates were developed, lipid spots were revealed by exposure to iodine vapor and extracted for assay of cholesterol or [^{3}H]cholesterol.

Materials. Cholesterol was purchased from Sigma Chemical Company (St. Louis, Mo.). Cholesterol linoleate and egg phosphatidylcholine (A grade) were obtained from Calbiochem (San Diego, Calif.). Tritiated cholesterol [7(*n*), 8–20 Ci/mmole] was obtained from Amersham Corporation (Arlington Heights, Ill.). Tetrahydrofuran was Gold Label grade (Aldrich Chemical Company, Milwaukee, Wisc.). Penicillin and streptomycin were added as PenStrep (Grand Island Biological Company, Grand Island, N. Y.).

RESULTS

Characterization of the incubation medium. Table 1 shows the distribution of cholesterol among lipoprotein fractions in normal and cholesterol-enriched, tritium-labeled media. About 10% of the total cholesterol was found in VLDL, 65% in LDL, and 20% in HDL fractions. The distribution was the same in enriched and unenriched media; thus the added free cholesterol distributed among the carriers in approximately the same proportions as in the original plasma. The over-all recovery in the three lipoprotein fractions was about 95%, indicating that less than 5% of the cholesterol was bound to plasma albumin. As expected, the added cholesterol did not become esterified. Thin-layer chromatographic separa-

[1] The abbreviations used are: VLDL, very low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins.

TABLE 1

Distribution of cholesterol enrichment and [^{3}H]cholesterol among lipoproteins

Duplicate 6-ml samples of prepared medium, enriched with cholesterol or not, and labeled with [^{3}H]cholesterol, were fractionated by centrifugation at densities of 1.006, 1.063, or 1.210 as described under Methods. The top portion of each tube (containing visible particulate material) was assayed for cholesterol (free plus esterified) and for ^{3}H (added free cholesterol). VLDL was defined as the material floating at density 1.006. LDL was defined as the difference between amount of material floating at density 1.063 and that at 1.006; similarly, HDL was the difference between the material floating at density 1.210 and that at 1.063. Data are percentage of total counts per minute or cholesterol, the means of two experiments that agreed within a few percentage points. The mean total cholesterol in the unenriched media was 0.57 mM; enriched media contained 1.05 mM total cholesterol.

Density	Fraction	cpm, % of total		Cholesterol, % of total	
		Unenriched	Enriched	Unenriched	Enriched
<1.006	VLDL	10	9	9	12
1.006–1.063	LDL	63	66	67	68
1.063–1.210	HDL	20	20	20	17

tion of the plasma medium showed essentially all of the tritium counts in the free cholesterol position.

Net transfer of cholesterol from cholesterol-enriched media to erythrocytes. When erythrocytes were incubated with cholesterol-enriched plasma medium, there was a net transfer of cholesterol to the cells, as Fig. 1 shows. The red cell phospholipid content did not change, and the cholesterol to phospholipid molar ratio increased from 1.04 to 1.68 over the 30-hr period. After 4 hr of incubation, the cholesterol content had already increased significantly. Cells incubated with unenriched plasma medium did not change their cholesterol to phospholipid molar ratio after 30 hr; the initial and final ratios (± standard deviation) for six samples were 1.05 ± 0.059 and 0.99 ± 0.029. The red cells used for most of these experiments were from a single donor whose erythrocyte cholesterol to phospholipid ratio (approximately 1.0) was slightly higher than the average reported values (0.8–0.9).

The addition of ethanol to the cholesterol-enriched incubation mixture caused a concentration-related increase in the amount of sterol transferred during the initial 4-hr period of incubation, as Fig. 2 shows. Here the cells had a cholesterol to phospholipid ratio of 1.04 initially. After 4 hr of incubation in cholesterol-enriched medium, the ratio had increased to 1.30 in the absence of ethanol. When ethanol was included in the incubation mixture, more sterol was transferred during the 4-hr period, bringing the ratio up to 1.49 (115% of control) at the highest ethanol concentration. The slope of the concentration-response curve for enriched medium was highly significant ($p < 0.001$). With unenriched media, the cholesterol to phospholipid ratio was not significantly affected by any concentration of ethanol (Fig. 2, *lower curve*). Furthermore, ethanol did not appreciably affect the amount of cholesterol in the cells at equilibrium (after 24 hr of incubation) regardless of which medium was used. For example, after 24 hr of incubation in cholesterol-enriched medium, the erythrocyte cholesterol to phospholipid ratio increased by 49% ± 4.7% (± SEM, $N = 6$), whereas in the presence of 0.35 M ethanol the increase was 54% ± 4.7%.

Effect of ethanol on the time course of cholesterol transfer. In order to follow the process more closely and

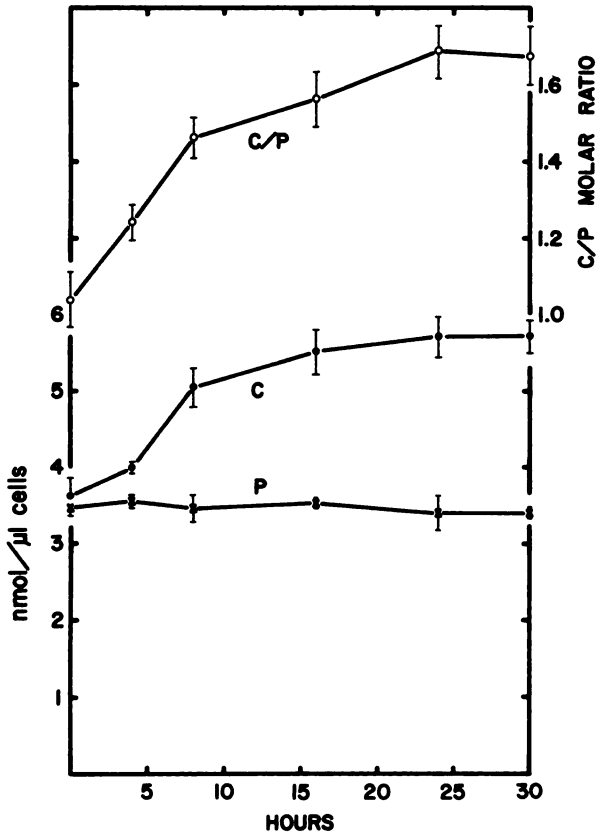

FIG. 1. *Net transfer of cholesterol to red cells during incubation with cholesterol-enriched medium*

Cells were incubated for 30 hr at 37°; samples taken at intervals were assayed in duplicate for cholesterol (*C*, ●) and lipid phosphorus (*P*, ×), as described under Methods. The *upper curve* (○——○) and *right ordinate* show the molar cholesterol to phospholipid (*C/P*) ratio. Points are means for six samples; bars represent standard deviation.

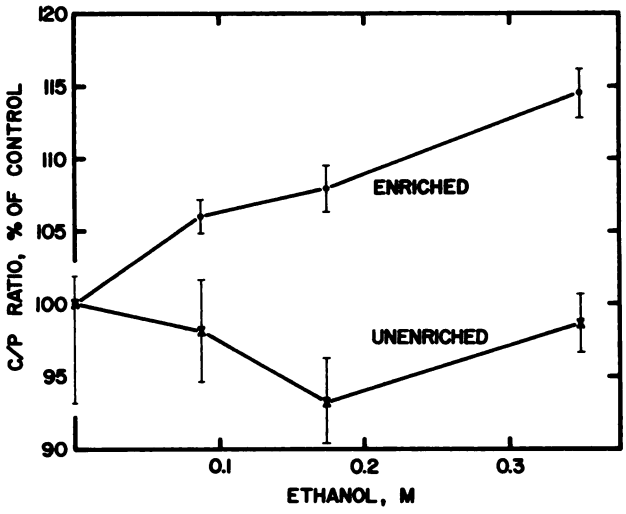

FIG. 2. *Effect of ethanol on the molar cholesterol to phospholipid ratio of erythrocytes after 4 hr of incubation with cholesterol-enriched or unenriched media*

Data are combined from two experiments and expressed as percentage of the cholesterol to phospholipid (*C/P*) ratio at 4 hr in the absence of ethanol. Points are means of six samples; bars represent standard error of the mean.

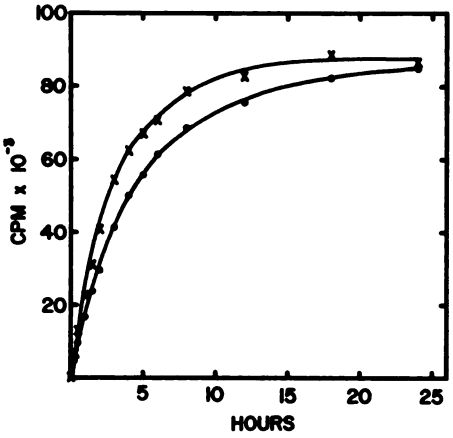

FIG. 3. *Effect of ethanol on transfer of [^{3}H]cholesterol from cholesterol-enriched plasma to erythrocytes*

Points are means of duplicate samples of washed red cells. ●, No ethanol; ×, 0.35 M ethanol.

to observe the exchange in the absence of net sterol flow, the remaining experiments were carried out with [^{3}H]-cholesterol. Figure 3 shows the time course of transfer of cholesterol from enriched medium to red cells and the effect of ethanol (0.35 M). Ethanol accelerated the sterol transfer without affecting the equilibrium value. Data from such experiments could be expressed as a fractional approach to equilibrium, using a closed two-compartment model where C_t is the concentration of unesterified cholesterol in red cells at time t per milliliter of incubation mixture, C_{eq} is the cellular cholesterol concentration at equilibrium and C is the total unesterified cholesterol concentration in the incubation mixture. The factors k_1 and k_2 are the rate constants for the forward and reverse transfers, respectively.

$$dC_t/dt = k_1(C-C_t)-k_2C_t$$

Integrating from $t = 0$ (where $C_t = 0$) to t,

$$\ln\frac{k_1C-(k_1+k_2)C_t}{k_1C} = -(k_1+k_2)t$$

But at equilibrium, $k_1C = (k_1+k_2)C_{eq}$. Substituting:

$$\ln\frac{C_{eq}-C_t}{C_{eq}} = -(k_1+k_2)t$$

The quantity $(C_{eq}-C_t)/C_{eq}$ can also be expressed as $(1-f)$, where f is the cellular cholesterol concentration as a fraction of its equilibrium value. Then

$$\ln(1-f) = -(k_1+k_2)t \qquad (1)$$

By using cholesterol disintegrations per minute per microliter of packed cells at times t and 24 hr to represent C_t and C_{eq}, respectively, the over-all rate constant of the transfer could be determined from a plot of Eq. 1. Such plots were linear on a semilog scale and were used to determine half-times. Data from Fig. 3 thus showed half-times of 3.1 hr in the absence of ethanol and 2.2 hr in the presence of 0.35 M ethanol, an increase of 41% in the rate constant. A similar equation described the loss of counts from cholesterol donors. The total recovery was over 90% and the rates of loss of counts from the medium and appearance in cells generally agreed well.

Despite some variability among different lots of plasma, it was clear that cholesterol transfer into cells was considerably faster from unenriched than from cholesterol-enriched plasma media (Table 2). In several experiments, the half-times were between 1 and 2 hr for unenriched and 3–4 hr for cholesterol-enriched media.

Transfer from specific cholesterol donors. The efflux of cholesterol from preloaded erythrocytes was also accelerated by ethanol. Cells were preincubated with tritium-labeled unenriched medium for 24 hr and were then washed and incubated in unlabeled enriched or unenriched medium. Figure 4 shows that the movement of cholesterol to either medium was similarly affected by ethanol. Table 2 shows the half-times.

LDL and HDL, isolated from cholesterol-enriched plasma media, transferred cholesterol to red cells with the same logarithmic approach to equilibrium as for the whole medium (Fig. 5). Ethanol (0.35 M) apparently affected the rate of transfer from HDL more than from

TABLE 2

Effect of ethanol on rate of cholesterol transfer

Erythrocytes (RBC) were incubated for 24 hr at 37° with heat-inactivated dilute plasma, isolated lipoproteins, or egg lecithin-cholesterol model membranes, labeled with tritiated cholesterol. Ethanol, when added, was present at a final concentration of 0.35 M. High-cholesterol vesicles had a cholesterol to phospholipid ratio (C/P) of 1.69; low-cholesterol vesicles, 0.95. The ^{3}H disintegrations per minute appearing in the acceptor were plotted according to Eq. 1. Least-squares lines were computed for determination of the half-times and their 95% confidence limits, shown in parentheses. The last column shows the acceleration due to ethanol, expressed as the percentage increase in rate constant (ln 2/half-time).

Donor	Acceptor	Half-time, hr		% Change
		Control	Ethanol	
Unenriched plasma	RBC	1.44 (1.41–1.47)	1.08 (1.01–1.15)	33
Enriched plasma	RBC	3.14 (3.05–3.23)	2.19 (2.08–2.30)	43
RBC	Unenriched plasma	1.90 (1.87–1.94)	1.37 (1.30–1.44)	39
RBC	Enriched plasma	2.72 (2.65–2.78)	1.90 (1.84–1.97)	43
HDL	RBC	1.61 (1.57–1.64)	1.15 (1.09–1.22)	40
LDL	RBC	3.40 (3.35–3.44)	2.75 (2.68–2.83)	24
Vesicles, low C/P	RBC	1.52 (1.40–1.66)	1.11 (1.09–1.14)	37
Vesicles, high C/P	RBC	4.03 (3.77–4.32)	3.10 (2.96–3.26)	30

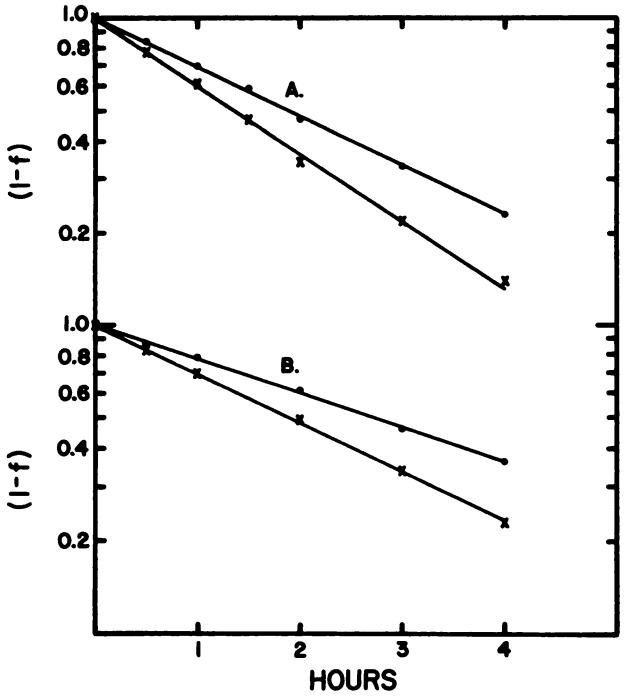

FIG. 4. *Effect of ethanol on transfer of [^{3}H]cholesterol from prelabeled red cells to plasma*

Data represent the appearance of ^{3}H in the plasma, plotted according to Eq. 1. A, Unenriched plasma; B, cholesterol-enriched plasma. ●, No ethanol; ×, 0.35 M ethanol in the incubation mixture.

LDL (Table 2). Ethanol decreased the LDL half-time by 24% in this experiment, versus 40% for HDL.

Finally, model membranes composed of egg lecithin and cholesterol were used as donors (Fig. 6). These

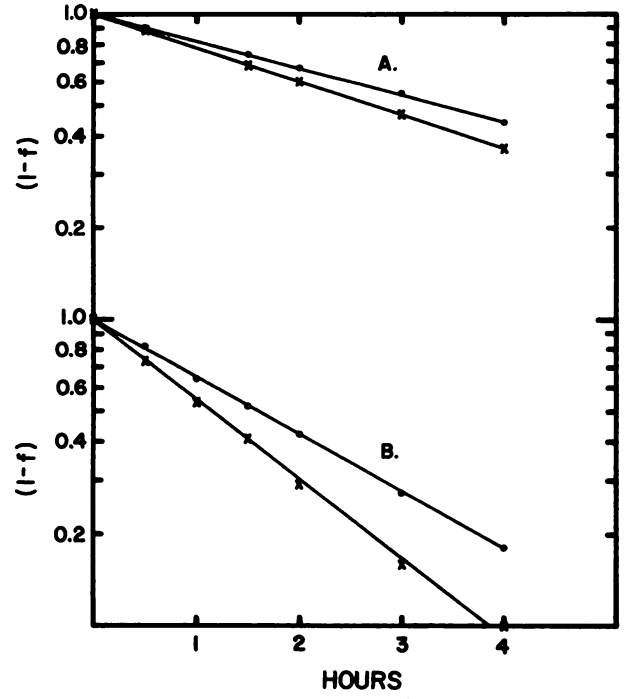

FIG. 5. *Effect of ethanol on transfer of [^{3}H]cholesterol to erythrocytes from isolated LDL (A) or HDL (B)*

Data represent the appearance of ^{3}H in the erythrocytes, plotted according to Eq. 1. ●, No ethanol; ×, 0.35 M ethanol in the incubation mixture.

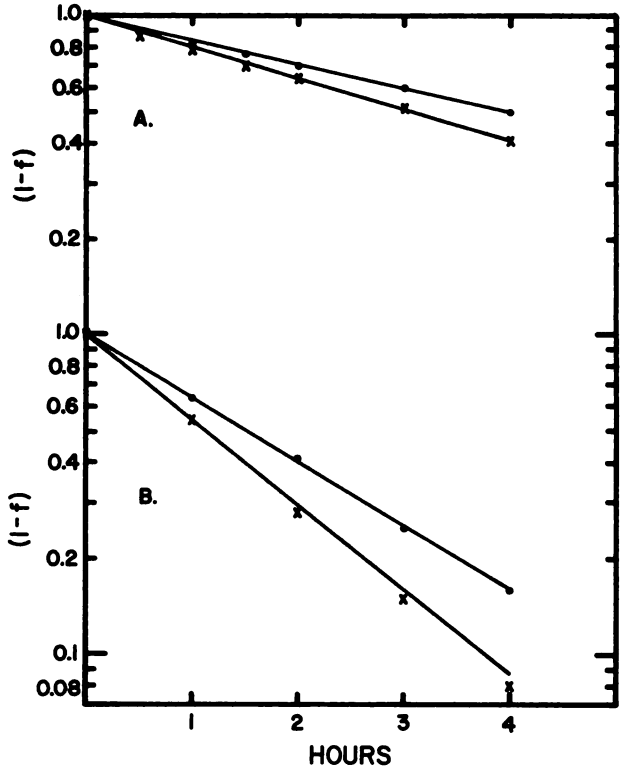

FIG. 6. *Effect of ethanol on transfer of [^{3}H]cholesterol to erythrocytes from high-cholesterol vesicles (A) or low-cholesterol vesicles (B)*

Data are plotted according to Eq. 1. ●, No ethanol; ×, 0.35 M ethanol in the incubation mixture.

sonicated vesicles were prepared either with a high cholesterol to phospholipid ratio of 1.69, similar to that of enriched medium or with a ratio of 0.95, similar to that of unenriched media. High-cholesterol vesicles transferred cholesterol to red cells with a half-time of about 4 hr. Low-cholesterol vesicles showed a faster transfer, with a 1.5-hr half-time. In either case, transfer was facilitated by ethanol (Table 2).

DISCUSSION

As Table 1 shows, the cholesterol in the incubation medium was distributed among various lipoproteins. Free cholesterol that was added in large amounts as enrichment and in trace amounts as label distributed among the lipoprotein classes like endogenous cholesterol. As expected, we found that the added cholesterol did not become esterified during preparation of the medium.

The rate constants of cholesterol transfer *in vitro* differed considerably among the donors we studied. Unenriched plasma, isolated HDL, and low-cholesterol vesicles had half-times around 1.5 hr, whereas cholesterol-enriched plasma, LDL, and high-cholesterol vesicles transferred cholesterol more slowly, with half-times above 3 hr (Table 2). This indicates a rough inverse correlation of rates with cholesterol content or with the ratios of free cholesterol to phospholipid in different donors. We did not measure unesterified cholesterol but could estimate (see Methods) that our cholesterol-enriched media had approximately 5 times as much free cholesterol as the unenriched media. LDL and HDL prepared from normal plasma have free cholesterol to

phospholipid molar ratios of about 0.7 and 0.3, respectively (17). Our LDL and HDL, prepared from cholesterol-enriched media, had greater amounts of cholesterol. The LDL still contained more than twice as much cholesterol as the HDL (Table 1). These lipoproteins transferred cholesterol with half-times of 3.4 hr (LDL) and 1.6 hr (HDL). Quarfordt and Hilderman (18) also reported slower transport from LDL than from HDL, with half-times of about 2.2 and 1.5 hr, respectively, but Gottlieb (17) found no difference between LDL and HDL transfer rates. We determined a free cholesterol to phospholipid ratio of 1.7 in our high-cholesterol sonicated vesicles and 1.0 in the low-cholesterol vesicles; their half-times were 4.0 and 1.5 hr, respectively. The latter agrees with the 1.25 hr reported by Poznanzky and Lange (19) and roughly with the 2.3 hr observed by Cooper *et al.* (20), but not with the slower rate (about 7 hr) seen by Giraud and Claret (21). Our exchange rate for high-cholesterol vesicles and erythrocytes (half-time 4 hr) agrees with that of Lange *et al.* (22). In red cells, used as donors in some experiments, all of the cholesterol is unesterified, and the cholesterol to phospholipid ratio was about 1.0. Our results with erythrocytes as donors are equivocal. The half-times should be the same in both directions, since back transfer is taken into account in our two-compartment model. But the variability between experiments prevented our demonstrating this clearly, and the half-times were intermediate between those of high-cholesterol and low-cholesterol donors.

Ethanol facilitated the transfer of cholesterol between plasma carriers and cell membranes. There was little or no effect on the equilibrium value, but the rate constants for transfer were increased by 24%–40% in 0.35 M ethanol. The effect seems to be general, since it does not vary much with different donors and is similar when red cells are used either as donors or recipients of the transferred cholesterol. Lecithin/cholesterol vesicles, lacking protein, showed the same ethanol effect.

Current theories of the mechanism of cholesterol transfer between membranes favor a model involving passage of the sterol through the water phase, rather than collision of cholesterol-bearing particles (23, 24). This model is based on the observation that rates of transfer are not proportional to the concentration of acceptor (17, 18, 23, 24), which argues against collision models, and on observed transfer through dialysis membranes (23, 24), a process that is slow but does fit theoretical calculations based on diffusion through the pores of the dialysis membrane. Presumably, the ethanol effect is a result of the disordering of membrane lipid bilayers that is known to be caused by anesthetic drugs, including alcohols. The disorder may weaken hydrophobic bonds so as to increase the probability of escape of cholesterol from the bilayer. Quarfordt and Hilderman (18) found that the addition of 0.15 M acetone to the incubation mixture accelerated passage of cholesterol from LDL (but not HDL) to red cells without affecting net transfer. Ethanol (5.8 M) was reported to speed up cholesterol transfer through a dialysis membrane (23).

When membrane fluidity is increased by other means, such as by raising the temperature or incorporating fatty acids with lower melting points in the lipids, transfer from sonicated lipid vesicles to other vesicles (25) or to erythrocytes (19, 26) is facilitated. Thus, it is clear that the physical properties of the bilayer can alter transfer rates. A possible additional factor that we cannot rule out is an ethanol-mediated increase in the aqueous solubility of cholesterol.

Our data suggest the possibility that cholesterol, by virtue of its ability to order biomembranes at physiological temperatures, may modify membrane fluidity sufficiently to affect its own transfer. If this were true, Eq. 1 might not hold exactly under conditions where there is a net transfer of cholesterol. The rate constants k_1 and k_2 might reflect the changing cholesterol content of the donor and acceptor membranes. But the two rates should change in opposite directions, and their sum, which determines the over-all transfer rate, might not change appreciably.

These results may be related to our studies *in vivo* in which chronic administration of ethanol caused a net increase in the cholesterol content of red cell and brain membranes (6). *In vivo*, when the lecithin/cholesterol acyltransferase is working and when various organs contribute or take up cholesterol from plasma, steady-state conditions prevail, rather than equilibrium. One may imagine that the addition of a compound that facilitates transfer of cholesterol among membranes would affect the steady-state level of the sterol in several compartments, but we lack enough knowledge of the controlling factors to be able to predict the direction or magnitude of the changes *in vivo*. Since chronic administration of ethanol increases plasma cholesterol levels in humans (27), a shift in the steady-state cholesterol content of cell membranes may be set in motion, assisted by the ability of the alcohol to accelerate sterol transfer.

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