Quantitation of the in vitro free cholesterol exchange of human red cells and lipoproteins

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ABSTRACT The exchange of free cholesterol in vitro between human red blood cells and low density lipoproteins (LDL) was quantified. The flux of sterol between LDL and red cells was relatively constant over a wide range of concentrations of free cholesterol in lipoproteins. In a system containing a suspension of red blood cells in a mixed solution of high density lipoproteins (HDL) and LDL, the fractional rate of exchange of HDL cholesterol was most rapid followed by LDL and lastly, by red cells.

Increasing the ionic strength of the incubation media had no effect on the exchange of cholesterol between LDL and red cells. However, when both HDL and LDL were incubated with red cells in a buffer of increased ionic strength, total red cell cholesterol exchange was unaltered, but proportionately more exchange occurred with HDL and less with LDL. Addition of acetone to the buffer increased the exchange of cholesterol between LDL and red cells but produced no increment in red cell–HDL exchange.

SUPPLEMENTARY KEY WORDS flux · red blood cell · HDL · LDL

The free cholesterol of plasma lipoproteins readily exchanges with cholesterol in the red cell membrane both in vivo (1–3) and in vitro (4). Isotopic equilibration of labeled cholesterol in a closed system of red cells and lipoprotein occurs rapidly during in vitro incubation and precedes significant esterification or net transfer of free cholesterol (4–6). Complete equilibration has been seen for the free sterol of red cells and human LDL (5). Only partial equilibration was observed, however, for HDL and red cell cholesterol (6).

Abbreviations: LDL, low density lipoprotein(s); HDL, high density lipoprotein(s).

The present study is concerned with the quantification of the in vitro exchange of free sterol between red cells and HDL and red cells and LDL, and the exchange occurring when all three components are incubated together. Fractional exchange rates of red cell and lipoprotein cholesterol and free sterol flux (i.e., mass of cholesterol exchanged per minute) were derived for each study. Changes in the exchange process induced by altering the buffer conditions or the proportion of red cell to lipoprotein in the incubation media were evaluated.

MATERIALS AND METHODS

Preparation of Cholesterol-Labeled Lipoproteins and Red Cells

Whole blood was obtained from normal subjects 10 hr after an intravenous injection of 25–35 μCi of cholesterol-1-^{3}H, (Amersham/Searle Corp., Des Plaines, Ill.), which had been incorporated in vitro into plasma proteins by the method of Goodman and Noble (7). The blood was collected in disodium EDTA (1 mg/ml of whole blood), and the red cells were removed from the plasma after centrifugation at 795 *g* for 25 min in a Lourdes model LRA refrigerated centrifuge. Prior to the incubation the red cells were washed four times with 5 volumes of 0.1 M Tris buffer pH 7.4, per volume of red cells.

Very low density lipoproteins were removed from the plasma by ultracentrifugation for 16 hr at 105,000 *g* (d 1.006). Low density lipoproteins were isolated from the infranate after adjusting the density to 1.063 and after ultracentrifugation at 105,000 *g* for 20 hr. High density lipoproteins were recovered from the infranate of the low density lipoprotein preparations after a 24 hr ultracentrifugation at a density of 1.21. All ultracentrif-

ugations were performed in a Spinco model L with a 40 rotor.

HDL and LDL were dialyzed against 100 volumes of 0.1 M Tris buffer, pH 7.4. The purity of each lipoprotein preparation was determined by immunoelectrophoresis using a commercial antiserum to whole human serum (Hyland Laboratories, Los Angeles, Calif.) and antisera to human HDL and LDL prepared in the rabbit. The HDL and LDL which were used for the incubations were immunochemically pure.

Incubations

All incubations were performed in a Dubnoff metabolic shaker at a temperature of 37°C and a shaker speed of 45 cycle/min. The buffer for all incubations was 0.1 M Tris, pH 7.4. Glucose was added to each incubation flask to a final concentration of 2 mg/ml, and penicillin was added to a concentration of 100,000 Units/ml. Incubations were begun by adding either labeled red cells or labeled lipoprotein to the incubation media, which consisted of 0.1 M Tris buffer, pH 7.4, glucose, penicillin, and either the corresponding unlabeled red cells or lipoprotein. The total volume of the incubation mixtures was 10–20 ml. A zero-time sample was withdrawn at 1 min, and aliquots were taken from the incubation vessel serially to 24 hr. Red cells were isolated by centrifugation, as described above, and washed four times with 5 volumes of normal saline prior to the extraction of lipid. The supernate from the first red cell centrifugation was extracted directly when incubations involved a single lipoprotein and red cells. When both HDL and LDL were incubated with red cells, the lipoproteins were subsequently isolated from the supernate by a heparin–$MnCl_2$ precipitation technique (8). When incubations were performed in a high ionic strength media, the supernates were diluted to isotonicity with distilled water prior to precipitation of the lipoproteins. The presence of 0.15 M acetone in the buffer had no effect on the lipoprotein precipitation. The recoveries of free cholesterol from the isolated HDL and LDL in the studies with added acetone and salt were identical with the control recoveries. LDL was precipitated at 4°C with 0.05 ml of 1 M $MnCL_2$ and 0.04 ml heparin (6000 Units/ml) per ml of lipoprotein, and centrifuged at 1200 *g* for 30 min at 4°C. The supernate containing HDL was removed, and the lipids were extracted. The precipitate was washed twice with 5 volumes of heparin–$MnCl_2$ solution, and redissolved in an equal volume of 10% sodium citrate prior to extraction of lipid.

Chemical Determination

Lipids were extracted from the lipoproteins by the technique of Dole, Schwartz, Thorn, and Silver (9) and from the washed red blood cells by the method of Rose and Oklander (10) in which isopropanol–chloroform was used. The extracts were plated on Silica Gel G thin-layer plates, which had been washed in chloroform and activated at 100°C for 30 min. The plates were developed in petroleum ether–diethyl ether–glacial acetic acid 80:20:1. The cholesterol and cholesteryl ester bands were scraped off and eluted with chloroform; aliquots of the eluates were removed for the assay of radioactivity and for the determination of cholesterol concentrations. Cholesteryl ester was never found in the thin-layer chromatograms of the red blood cells, and the red cell extracts in later studies were not chromatographed.

Radioactivity was determined in a Packard liquid scintillation spectrometer using DPO (2,5-diphenyloxazole) 0.5% in toluene as the scintillation solvent. Quench corrections were made by the channels-ratio method and were found to be insignificant. Cholesterol determinations were made by a modification of the procedure of Abell, Levy, Brodie, and Kendall (11).

Computations

When cholesterol exchange between red cells and HDL or LDL (Fig. 1*a*), was studied, the fractional exchange rates were calculated from the exponentials and coefficients of the following biexponential tracer decay curve:

$$F_1(T) = C_1 e^{-\alpha_1 T} + C_2$$

$F_1(T)$ is the cholesterol specific activity in the initially labeled compartment as a function of time, i.e. red cell cholesterol in Fig. 2 and LDL cholesterol in Fig. 3. C_1 and C_2 are the intercepts of the two exponentials obtained by curve peeling, and α_1 the slope of the first exponential. The coefficient of the second or zero exponential term (C_2) was obtained at isotopic equilibrium, or could be calculated from the relative quantities of the lipoprotein and red cell free sterol, assuming no net cholesterol transfer and complete isotopic equilibration for the system. The first exponential (Fig. 2) was determined after curve peeling using a linear regression analysis program on an IBM 1130 computer to obtain the slope and intercept.[1] The two fractional exchange rates ($\lambda_{L/R}$, $\lambda_{R/L}$)[2] were calculated from the coefficients and exponentials by the following two equations:

$$\lambda_1 = C_1\alpha_1$$
$$\lambda_2 = C_2\alpha_1$$

[1] Courtesy of P. McHale, M.I.R. Unit, Duke University Medical Center, Durham, N.C. 27706.

[2] In the expressions for fractional turnover rate, λ, and flux, ρ: L, lipoproteins; R, red cells; α, HDL; β, LDL.

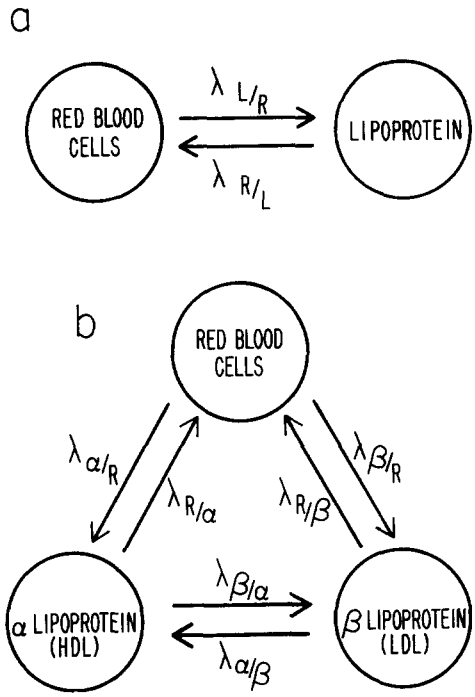

FIG. 1. (*a*) Model of the free cholesterol exchange between a single population of lipoproteins and red blood cells. $\lambda_{L/R}$, the fractional exchange rate of red blood cell cholesterol with lipoprotein cholesterol; $\lambda_{R/L}$, the fractional exchange rate of lipoprotein cholesterol with red blood cell cholesterol. (*b*) Model of the free cholesterol exchange between both HDL and LDL and red blood cells. $\lambda_{R/\alpha}$, the fractional exchange rate of HDL cholesterol with red blood cell cholesterol; $\lambda_{\alpha/R}$, the fractional exchange rate o red blood cell cholesterol with HDL cholesterol; $\lambda_{R/\beta}$, the fractional exchange rate of LDL cholesterol with red blood cell cholesterol; $\lambda_{\beta/R}$, the fractional exchange rate of red blood cell cholesterol with LDL cholesterol; $\lambda_{\beta/\alpha}$, the fractional exchange rate of HDL cholesterol with LDL cholesterol; $\lambda_{\alpha/\beta}$, the fractional exchange rate of LDL cholesterol with HDL cholesterol.

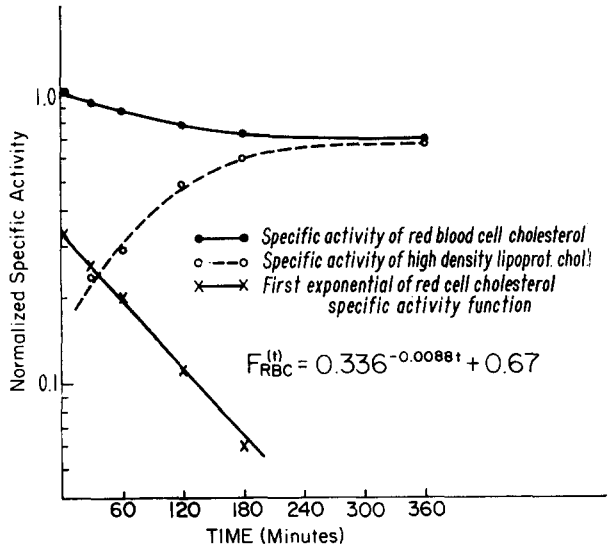

FIG. 2. Normalized specific (specific activity per specific activity of initially labeled component at time zero) of cholesterol-^{3}H in labeled red blood cells and HDL (originally unlabeled) as a function of time.

λ_1 and λ_2 are the fractional exchange rates of the initially labeled pool and the unlabeled pool, respectively.

When both HDL and LDL were incubated with red cells, a closed three-compartment model (Fig. 1*b*) was used for the analysis of the data. The fractional exchange rates for the three compartments were derived by the simulation-analysis and modeling technique of Berman, Shahn, and Weiss (12). The SAAM 23 program (13) was adapted to an IBM 360-75 computer for these studies. In order to solve this three-compartment system, it was necessary to determine independently the fractional exchange rates between HDL and LDL. The cholesterol exchange rates between these two lipoproteins were determined in independent incubations and calculated using the coefficients and exponents of the bi-exponential tracer curve as described above.

RESULTS

The fall in specific activity of cholesterol-labeled red blood cells when incubated with a single unlabeled lipoprotein species can be described by a biexponential function (Fig. 2). A biexponential function also describes the fall in specific activities when the lipoprotein contains the labeled sterol and when the red cells are unlabeled (Fig. 3). Complete isotopic equilibrium was observed in all 15 in vitro incubations of red cells and isolated LDL. Studies of isolated HDL and red cell sterol exchange similarly exhibited equal cholesterol specific activities for both red cells and lipoproteins at the conclusion of the incubation in four studies. However, in two HDL–red cell incubations there was not complete isotopic equilibration of sterol after sufficient incubation time (10 hr). When both HDL and LDL were incubated with red cells, complete exchange of cholesterol was observed in each of the nine incubations. No changes in lipoprotein or red cell cholesterol mass were seen in any of these in vitro incubations. Also, no significant increments in lipoprotein cholesteryl ester mass or radioactivity were observed in these studies.

Fractional cholesterol exchange rates for red cells and lipoproteins (Table 1, $\lambda_{L/R}$, $\lambda_{R/L}$) and the mass of sterol which exchanged between them (ρ_{Chol}) were different from experiment to experiment. The fractional red cell exchange rates ($\lambda_{L/R}$) were relatively similar within each study despite large differences in the concentrations of incubated lipoprotein. In experiment 15 (Table 1) when the LDL cholesterol was 1620 μg/ml the fractional red cell cholesterol exchange rate was 0.0023 per min, and the flux was 0.0013 μEq/min per ml of incubation media. At one-sixth the LDL cholesterol concentration (270 μg/ml), the fractional red cell sterol exchange rate decreased only to 0.0014 per min with a concomitant reduction in flux to 0.00080 μEq/ml per min. A decrease

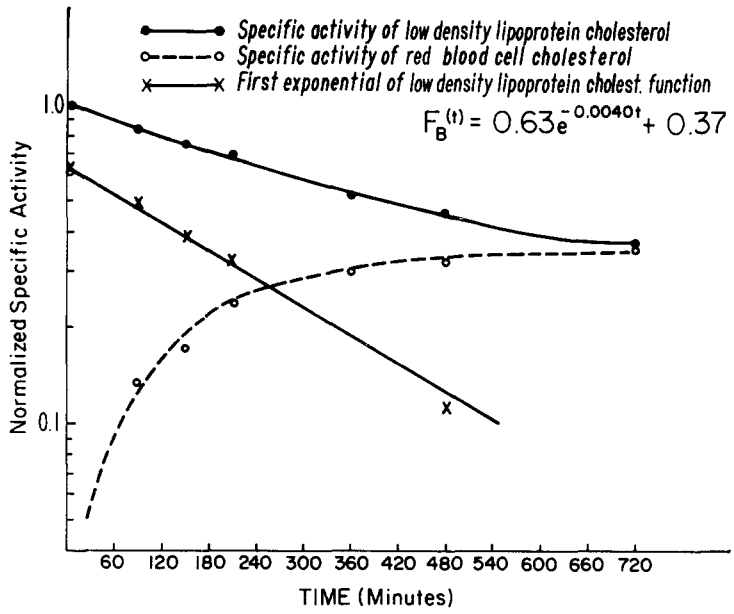

FIG. 3. Normalized specific activity (specific activity per specific activity of initially labeled component at time zero) of free cholesterol-^{3}H in labeled LDL and red cells (originally unlabeled) as a function of time.

in red cell fractional cholesterol exchange was also observed in study 8 (Table 1) when the lipoprotein content was reduced. However, the decreases of red cell fractional exchange rates and fluxes were considerably less than the reductions of the receptor lipoprotein concentration. In three separate studies when the concentration of lipoprotein was reduced to one-half of its initial value, no change occurred in the fractional red cell sterol exchange rate (Table 1: 8 A and B; 9 A and B; 15 B and C).

The exchange of free sterol was evaluated when both HDL and LDL, as well as red cells, were incubated together. The concentrations of cholesterol in HDL and LDL in these studies were similar to their lower limits of normal in blood. The hematocrits (33%) of the incubated mixtures were less than those found in normal blood. In each of these incubations HDL cholesterol approached isotopic equilibrium somewhat more rapidly than did the cholesterol in LDL (Fig. 4).

The specific activity data in these three-component

TABLE 1 EXCHANGE OF RED BLOOD CELL CHOLESTEROL WITH THE CHOLESTEROL OF HDL OR LDL*

	Expt. No.	RBC Cholesterol Concentration	Lipoprotein Cholesterol Concentration	$\lambda_{L/R}$†	$\lambda_{R/L}$†	ρ Chol †
		$\mu g/ml$	$\mu g/ml$	min^{-1}		$\mu Eq/ml\ min^{-1} \times 10^2$
LDL						
	8 A	340	270	0.0025	0.0033	0.22
	B	340	135	0.0025	0.0074	0.22
	C	340	67	0.0015	0.0077	0.13
	9 A	267	309	0.0013	0.0011	0.090
	B	267	155	0.0014	0.0025	0.096
	13 A	214	74	0.0014	0.0038	0.078
	15 A	220	1620	0.0023	0.0003	0.13
	B	220	1080	0.0018	0.0004	0.10
	C	220	540	0.0017	0.0007	0.096
	D	220	270	0.0014	0.0011	0.080
HDL						
	8 H	340	172	0.0029	0.0059	0.25
	12 B	250	112	0.0016	0.0026	0.10

* Incubations were performed in 0.1 M Tris buffer, pH 7.4.

† $\lambda_{L/R}$ is the fraction of red blood cell cholesterol exchanging with lipoprotein per min; $\lambda_{R/L}$ is the fraction of lipoprotein free cholesterol exchanging with red blood cells per min. ρ Chol is the μEq of cholesterol exchanging between red cells and lipoproteins per min per ml of incubation mixture.

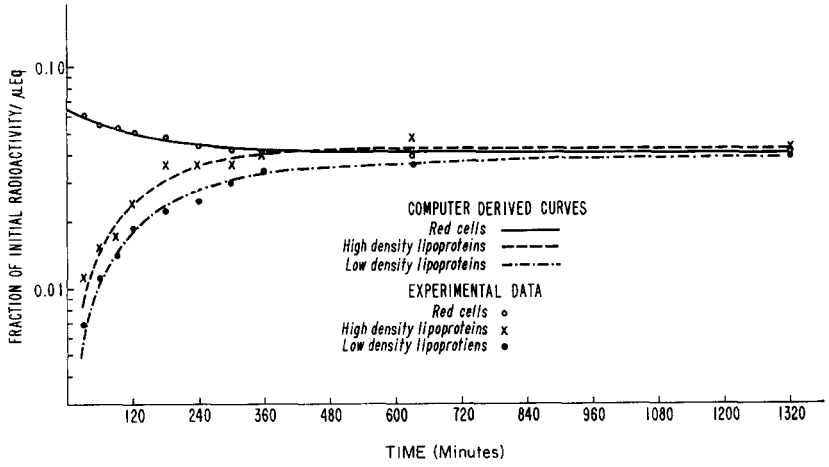

FIG. 4. Specific activity of free cholesterol in cholesterol-^{3}H-labeled red cells and originally unlabeled HDL and LDL as a function of time.

incubation studies were analyzed by a computer technique (12) using a closed three-compartment model (Fig. 1*b*). It was necessary to determine independently the exchange rates of cholesterol between HDL and LDL (i.e. $\lambda_{\alpha/\beta}$ and $\lambda_{\beta/\alpha}$)[2] in order to derive the other four fractional exchange rates of the system. The value of $\lambda_{\beta/\alpha}$ was 0.034–0.063 min^{-1}, and the value of $\lambda_{\alpha/\beta}$ was 0.0047–0.014 min^{-1} in four studies. Values on either end of the range of these two parameters were used for the computer solutions with little effect on the four computer-calculated parameters. Mean values of $\lambda_{\alpha/\beta}$ and $\lambda_{\beta/\alpha}$ of 0.01 and 0.05 min^{-1}, respectively, were used for the final computer solutions tabulated in Table 2.

The fraction of red cell sterol exchanging with LDL cholesterol per minute (Table 2, $\lambda_{\beta/R}$) in the three studies was two to three times greater than the fraction exchanging with HDL ($\lambda_{\alpha/R}$). Both values ($\lambda_{\beta/R}$, $\lambda_{\alpha/R}$) were fairly reproducible in all three studies; the highest percentage fractional standard deviation was less than 21%. The fractional exchange rates of LDL ($\lambda_{R/\beta}$) and HDL ($\lambda_{R/\alpha}$) sterol with red cells were less reproducible in these studies; the fractional standard deviation was 25–135%. It appeared, however, that the fractional exchange rates of HDL sterol were somewhat higher than the LDL cholesterol exchange rates. The exchange flux of cholesterol between LDL and red cells (Table 2, $\rho_{\beta/R}$) was about two to three times the flux between red cells and HDL ($\rho_{\alpha/R}$). The larger fractional sterol exchange rates between HDL and LDL ($\lambda_{\beta/\alpha}$ = 0.05 min^{-1}; $\lambda_{\alpha/\beta}$ = 0.01 min^{-1}) and the concomitant greater flux ($\rho_{\alpha/\beta}$ = 0.0032 $\mu Eq/ml/min^{-1}$) indicates a more facile exchange between the free sterol of lipoproteins than with the sterol of the red cell membrane.

The influence of increased ionic strength and pH on free sterol exchange was evaluated during incubations with isolated LDL and red cells (Table 3, Expt. 8). No change in sterol flux was observed when the ionic strength was increased by making the buffer 0.5 M with respect to NaCl, nor when the pH was increased to 9.0. Incubations at lower pH or ionic strength produced hemolysis of the red cells and denatured the lipoproteins, thus precluding study of sterol exchange. No difference in sterol exchange was produced by sodium glycocholate at concentrations of 200 μg and 1000 μg/ml (Table 3, Expt. 13). No changes in the free sterol content of the LDL or red cells were seen at the end of these 8-hr incubations with sodium glycocholate.

When acetone was added to the combined HDL–LDL–red cell incubations to a concentration of 0.15 M-cholesterol exchange proceeded more rapidly. The frac,

TABLE 2 CHOLESTEROL EXCHANGE BETWEEN RED CELLS, HDL, AND LDL*

Expt. No.	$\lambda_{\beta/R}$†	$\lambda_{\alpha/R}$	$\lambda_{R/\beta}$	$\lambda_{R/\alpha}$	$\rho_{\beta/R}$†	$\rho_{\alpha/R}$
		min^{-1}			$\mu Eq/ml\ min^{-1} \times 10^2$	
23	0.0016 (10)‡	0.00048 (20)	0.0029 (25)	0.0037 (135)	0.12 (10)	0.035 (20)
24	0.0016 (11)	0.00045 (19)	0.0032 (25)	0.0038 (87)	0.11 (11)	0.033 (19)
25	0.0015 (14)	0.00067 (21)	0.0028 (36)	0.01 (39)	0.10 (14)	0.048 (21)

* Incubations in 0.1 M Tris buffer, pH 7.4; cholesterol concentrations of red cells, HDL, and LDL were 340, 180, and 40 μg/ml of incubation mixture, respectively.

† λ, fractional exchange rates; ρ, exchange flux (subscripts defined in legend to Fig. 1*b*).

‡ The values in parentheses are percentage fractional standard deviations.

TABLE 3 INFLUENCE OF HIGH NaCl CONCENTRATION, HIGH pH, AND PRESENCE OF SODIUM GLYCOCHOLATE ON EXCHANGE OF RED CELL AND LDL CHOLESTEROL*

Expt. No.	Buffer	$\lambda_{R/L}$†	$\lambda_{L/R}$†	ρ_{Chol}†
		min^{-1}		$\mu Eq/ml\ min^{-1} \times 10^2$
8 B	0.1 M Tris, pH 7.4	0.0025	0.0074	0.22
D	0.1 M Tris, pH 7.4 plus, 0.4 M NaCL	0.0023	0.0069	0.20
E	0.1 M Tris, pH 9.0	0.0022	0.0065	0.19
13 A	0.1 M Tris, pH 7.4	0.0014	0.0038	0.078
B	0.1 M Tris, pH 7.4 plus 200 μg/ml Na glycocholate	0.0015	0.0043	0.083
C	0.1 M Tris, pH 7.4 plus 1000 μg/ml Na glycocholate	0.0012	0.0034	0.067

* Incubations were performed in 0.1 M Tris buffer, pH 7.4.

† $\lambda_{L/R}$ is the fraction of red blood cell cholesterol exchanging with lipoprotein per min; $\lambda_{R/L}$ is the fraction of lipoprotein free cholesterol exchanging with red blood cells per min. ρ_{Chol} is the μeq of cholesterol exchanging between red cells and lipoproteins per min/ml of incubation mixture.

tion of red cell cholesterol exchanging with LDL cholesterol per min ($\lambda_{\beta/R}$) doubled from a control value of 0.0015 min^{-1} to 0.0034 min^{-1} (Table 4). The fractional LDL exchange rate ($\lambda_{R/\beta}$) also increased from 0.0028 min^{-1} to 0.0071 min^{-1}. Similar increments in the fractional exchange rates between HDL and red cells ($\lambda_{\alpha/R}$, $\lambda_{R/\alpha}$) were not noted. Cholesterol flux between red cells and LDL doubled with acetone in the buffer, but no significant difference was observed in red cell–HDL cholesterol flux. Despite the acetone-induced increment in LDL–red cell cholesterol exchange, no change was seen in the cholesterol content of either, indicating no significant net transfer of sterol.

High salt concentrations were of little consequence to the sterol exchange between isolated LDL and red cells (Table 3, Expt. 8). However, incubations of HDL and LDL with red cells were significantly influenced by high salt concentrations. Under these conditions a larger fraction of red cell sterol exchanged with HDL, and less with LDL (Table 4, Expt. 27a and b). Although the distribution of sterol exchange between HDL and LDL was changed with high salt concentrations, the total flux of sterol between the red cells and the lipoproteins (Table 4, $\rho_{\beta/R} + \rho_{\alpha/R}$) was not substantially altered.

DISCUSSION

A substantial quantity of unesterified cholesterol of human red cells exchanges with HDL and LDL during in vitro incubations. If a similar amount of exchange occurs in vivo, as was suggested by previous investigations (3), the 24 hr exchange flux of red cell sterol in a 5 liter blood volume would be approximately equivalent to three times the daily cholesterol turnover of man (7). This estimate was determined using an average red cell cholesterol flux of 0.0015 μEq/ml min^{-1} (red cell flux $= \rho_{\beta/R} + \rho_{\alpha/R}$). The unesterified cholesterol exchanging between the HDL and LDL was about twice the red cell flux under these in vitro conditions. Therefore, the total free sterol exchange within a 5 liter blood volume would equal approximately nine times the total body cholesterol turnover. This value is probably an underestimate of the total exchange occurring in the blood since concentrations of free cholesterol in these incubations are somewhat less than those of the HDL and LDL and red cells in whole blood. Also, the exchange occurring with very low density lipoproteins was neglected in these estimates.

These fractional cholesterol exchange rates for human red cells, HDL, and LDL are considerably more rapid than those published for the exchange of cholesterol of spleen, lung, heart, muscle, kidney, and brain with serum cholesterol in the rat (14). A study by Eckles, Taylor, Campbell, and Gould (2) suggests that sterol exchange between the dog liver and plasma lipoproteins

TABLE 4 INFLUENCE OF ACETONE AND HIGH NaCl CONCENTRATION ON HDL AND LDL CHOLESTEROL EXCHANGE WITH RED CELLS*

Expt. No.	Buffer	$\lambda_{\beta/R}$†	$\lambda_{\alpha/R}$	$\lambda_{R/\beta}$	$\lambda_{\alpha/R}$	$\rho_{\beta/R}$†	$\rho_{\alpha/R}$
		min^{-1}				$\mu Eq/ml\ min^{-1} \times 10^2$	
25	0.1 M Tris, pH 7.4	0.0015 (14)‡	0.00067 (21)	0.0028 (36)	0.01 (39)	0.10 (14)	0.048 (20)
26 A	0.1 M Tris, pH 7.4 plus 0.15 M acetone	0.0034 (19)	0.00066 (56)	0.0071 (36)	0.01 (85)	0.25 (19)	0.048 (56)
B	Same as 26 A	0.0038 (18)	0.00054 (32)	0.0073 (42)	0.008 (68)	0.27 (18)	0.042 (32)
27 A	0.1 M Tris, pH 7.4 plus 0.5 M NaCl	0.00062 (22)	0.0011 (15)	0.0017 (54)	0.01 (43)	0.045 (22)	0.079 (15)
B	Same as 27 A	0.00055 (28)	0.0014 (18)	0.0016 (43)	0.013 (56)	0.043 (28)	0.081 (18)

* Incubations in 0.1 M Tris buffer, pH 7.4. Cholesterol concentrations in red cells, LDL and HDL were 340, 180, and 40 μg/ml of incubation mixture, respectively.

† λ, fractional exchange rates; ρ, exchange flux (subscripts defined in legend to Fig. 1*b*).

‡ The values in parentheses are percentage fractional standard deviations.

is even more rapid than red cell–lipoprotein exchange. When considered in the context of cholesterol kinetic studies in man (7), it is apparent that the free sterol of red cells, HDL, and LDL are part of the rapidly exchangeable cholesterol pool. This pool is usually defined by data obtained at daily intervals and most likely consists of a number of rapidly turning-over pools which could be defined by more frequent sampling during the first few days of the in vivo study. Some of these newly defined pools might have the same turnover characteristics as red cell or lipoprotein cholesterol. The turnover time for the total rapidly exchangeable pool is approximately 10 days, whereas, the turnover time for free sterol in the red cell membrane is about 8 hr, and for LDL and HDL cholesterol approximately 80 and 20 min, respectively.

These in vitro red cell–lipoprotein cholesterol exchange studies were performed under closed conditions, and their analogy to the open in vivo situation is uncertain. Exchange between blood and tissue cholesterol, cholesterol esterification, and fecal sterol loss might produce different in vivo lipoprotein red cell sterol exchange, if a maximum blood cholesterol flux existed. Although the various other processes might conceivably affect the kinetics of the in vivo system, they are, being relatively slow, unlikely to appreciably influence the amount of sterol moving between red cells and lipoproteins in vivo.

Despite a large range of lipoprotein concentrations, the flux of cholesterol was relatively constant between red cells and lipoprotein. This finding suggests that at these concentrations of lipoprotein neither the available cholesterol binding sites on the lipoprotein nor the number of red cell–lipoprotein "collisions" are limiting the interchange of sterol. Red cells depleted of cholesterol exchange sterol as readily with LDL as do normal cells (5). Both of these studies suggest that cholesterol exchange is not influenced by available receptor sites on either the lipoprotein aggregate or the red cell. The studies are not incompatible with the "collision complex" concept of cholesterol exchange proposed by Gurd (15) where the magnitude of exchange would be governed by the diffusion of cholesterol in the complex. Although it is probably unlikely that a saturated, water-soluble intermediate is responsible for the exchange of cholesterol, the data presented here are compatible with this consideration. However, when the red cells are separated from the lipoprotein by a semipermeable dialysis membrane, no exchange occurs;[3] this suggests that if such an intermediate existed it would be of a molecular size greater than hydrated cholesterol.

Changes in the buffer system have previously been observed to produce alterations in the cholesterol exchange between red cell ghosts and LDL. Increasing the ionic strength of the buffer was without significant effect on exchange in the red cell ghost–LDL incubations of Bruckdorfer and Green (16). This was also the case in our studies when exchange was evaluated between a single type of lipoprotein and intact red blood cells. However, when exchange was studied in incubations of both HDL and LDL with red blood cells, although the total free sterol flux was not significantly different in the high salt buffer, the distribution of this flux was substantially altered. Significantly less exchange was noted between red cells and LDL and considerably more with HDL. One explanation for this observation is that the contact of LDL and red cells, which is probably necessary for free sterol exchange, is more dependent on electrostatic interaction than is the red-cell–HDL association. This is somewhat unlikely considering that both LDL and red blood cells are anions at physiologic pH, and an increase of ionic strength should produce an increase in the frequency of association between the two and thus facilitate exchange. Another more plausible possibility for this observation is that under the usual conditions of pH and ionic strength the anionic charges of red cells and HDL would restrict their interaction. With an increase in the ionic strength of the media, this interaction would occur more readily, producing a greater sterol exchange between the red cell and HDL. If the two lipoproteins were competing for red cell sterol, less LDL exchange would result.

The increment in sterol exchange which occurred when acetone was added to the buffer was originally observed in incubations of red cell ghosts and LDL (16). Our data indicate that the stimulation of acetone on the system was fairly specific. A doubling of sterol flux between LDL and red cells was seen in the presence of acetone, with no significant change in the sterol flux of HDL and red cells. The explanations for the different effect of acetone on red cell free sterol exchange with HDL and LDL are speculative. The increase in flux of red cell and LDL cholesterol may reflect a decreased hydrophobic binding of this molecule in the red cell membrane and lipoprotein aggregate, producing a more rapid diffusion in the red cell lipoprotein collision complex. The lower dielectric constant resulting from the presence of acetone in the buffer possibly also increases the charge effect on red cell–HDL interactions making a collision event between the two less likely.

Although the magnitude of cholesterol exchange in blood is considerable, its physiologic significance is completely undefined. The similar cholesterol exchange of erythrocyte ghosts and normal red cells and the lack of correlation between red cell metabolic activity and cholesterol exchange (17) would suggest that the process is not intimately related to the transport function of the

[3] Quarfordt, S. Unpublished observations.

red cell membrane. The data of Basford, Glover, and Green (5) also indicate that this exchange process is not responsible for net incorporation of cholesterol into sterol-depleted red cells. The role of this exchange process in the structure and function of the lipoprotein is also unknown. The fact that free sterol is the most rapidly exchanging component of the lipoproteins suggests that cholesterol is possibly more easily accessible and more loosely bound than the other components of the lipoprotein aggregate.

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