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RATES OF CHOLESTEROL EXCHANGE BETWEEN HUMAN ERYTHROCYTES AND PLASMA LIPOPROTEINS

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Summary

Rates of exchange of labelled cholesterol between human erythrocytes and three plasma lipoprotein species, LDL, HDL_2 and HDL_3, were measured over a range of lipoprotein concentrations and temperatures. The exchange rates reached limiting, concentration-independent values, which were the same for the three lipoproteins. The temperature dependencies correspond to activation energies of 12 kcal in the limiting rate region, and at lower lipoprotein concentrations to activation energies of 11 to 22 kcal. Calculations based on simple collision theory indicate that energetic barriers to the exchange are far smaller than indicated by these activation energies and that no particular orientation of lipoprotein molecules is required for the exchange. The occurrence of a limiting rate may be a result of the adsorption of lipoprotein molecules onto a limited number of sites on the cell surface, or of a slow process occurring in the membrane, possibly the diffusion of cholesterol. Present data do not permit a choice between these models.

Introduction

The blood circulation transports insoluble lipids to and from body tissue cells as parts of lipid-protein complexes, the plasma lipoproteins [1]. Several types of cells take up lipids through endocytosis which follows the binding of a

Abbreviations: LDL, low density lipoprotein (plasma lipoprotein with buoyant density 1.006—1.063); HDL, high density lipoprotein (plasma lipoprotein with buoyant density 1.063—1.210); HDL_2, HDL_3, high density lipoprotein sub-species, with buoyant densities in ranges 1.063—1.125, and 1.125—1.210 respectively.

particular lipoprotein, LDL, to high affinity receptor sites on the cell membrane [2]. In contrast to this specialized mechanism, a number of cell types are known to take up cholesterol and some phospholipids through a passive partitioning of these lipids between the cell membrane and lipoproteins [3]. This type of process probably plays a role in the regulation of lipids of all cells.

The partitioning of lipids between cell membranes and lipoproteins has been most extensively studied for the case of cholesterol and erythrocyte membranes. These studies have largely concerned determination of the fraction of the membrane cholesterol which participates in the partitioning. Recent work indicates that all of the erythrocyte membrane cholesterol is available for exchange with lipoprotein cholesterol [4], and at least 80% can be removed by lipoproteins of reduced cholesterol content [5].

The molecular mechanisms of the exchange of cholesterol between erythrocytes and lipoproteins are not known. Since the low aqueous solubility of cholesterol seemingly rules out its transfer through dissociation into the aqueous phase, direct contact of lipoprotein molecules with the membrane is apparently required *. The exchange could conceivably occur during transient collisions between lipoprotein molecules and cells, but also might require longer contacts, such as are involved in adsorption. It is also possible that the exchange rate is controlled by the movement of cholesterol within either the lipoprotein molecules or the membrane.

The literature contains little information on the effects on the exchange rates of variables such as lipoprotein concentration, and temperature, which could provide a basis for establishing the mechanism. While a number of isolated exchange rate measurements have been reported, the only systematic study is that of Quarfordt and Hilderman [6] whose measurements were limited to a single temperature, 37°C. These workers showed that all of the cell membrane cholesterol exchanges with the same rate constant, but their data for different lipoprotein concentrations do not permit conclusions regarding the effects of this variable. The only study of temperature effects is that of Bruckdorfer and Green [7], who found a small temperature coefficient for cholesterol exchange between human HDL and rat erythrocyte ghosts.

A study of the rates of the exchange of cholesterol between human erythrocytes and three species of plasma lipoproteins, LDL, HDL_2 and HDL_3, over a range of temperature and lipoprotein concentrations is reported on here.

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* This was confirmed in an experiment by Quarfordt and Hilderman [6], in which no cholesterol transfer between erythrocytes and lipoproteins took place when these were separated by a dialysis membrane. I have found previously (unpublished observations) that even where solubility conditions permit, no removal of cholesterol from (human) erythrocytes by dissolution in aqueous solution occurs. In this experiment, 0.10 ml of spun erythrocytes were shaken for 1 h at 25°C with three successive 100 ml portions of buffer (phosphate buffered saline: 0.15 M NaCl, 0.002 M sodium phosphate, pH 7.4). Since the solubility of cholesterol in water at 25°C is 180 μg/100 ml [8], and the cholesterol content of 0.10 ml of spun erythrocytes is about 130 μg [9], all of the cholesterol could have been removed from the erythrocytes in each extraction. However, no loss of cholesterol from the cells was found. In contrast, 30% of erythrocyte cholesterol is transferred to cholesterol depleted lipoproteins in three hours [5].

Experimental Procedure

Lipoprotein fractions were isolated from less than two week old human plasma, using standard density ultracentrifugation methods (NaBr solutions) [10]. Each density fraction was ultracentrifugally 'washed' at its upper density limit, and dialyzed for 24—48 h against buffer (phosphate buffered saline: 0.15 M NaCl, 0.002 M sodium phosphate, pH 7.4) at 5°C. Erythrocytes were used within 1 day after collection (into ACD), and washed 3 times with buffer with complete removal of the buffy coat.

Lipoproteins were assayed for protein by the method of Lowry et al. [11], cholesterol and cholesteryl esters by the cholesterol oxidase method [12] with and without cholesteryl esterase, phospholipid by the method of Rouser [13] and acylglycerols using the Beckman Instrument Co. (Carlsbad, CA) test kit. Erythrocytes were extracted with $CHCl_3$-isopropanol [14] prior to analysis for cholesterol by the *o*-phthalaldehyde method [15] and for phospholipid [13]. Radioactivity was counted by standard scintillation counting techniques, using 1 ml of aqueous solution with 10 ml of Aquasol (New England Nuclear, Boston, MA) and counting for at least 20 000 counts. Hemoglobin was estimated from absorption at 420 nm.

The actual rates measured were for the movement of radioactively labelled cholesterol (4-C^{14} labelled, Amersham Corp.) from erythrocytes to lipoproteins. The labelled cholesterol was incorporated into the erythrocytes by a procedure, described earlier [16], which involved a 24 h incubation of erythrocytes with a solution of HDL_3 containing labelled cholesterol (Chol*-HDL_3); the HDL_3 in turn had been loaded with labelled cholesterol by incubation with labelled cholesterol dispersed on celite [17,18]. It has been established [16] that the behavior of the labelled cholesterol incorporated in this manner is the same as that of native erythrocyte cholesterol. Under the conditions used, 40—50% of the native erythrocyte cholesterol was replaced by labelled cholesterol with the total cholesterol content being either unchanged or reduced by a small amount, up to 3%, from that of the original cells.

Exchange rates were measured in rooms in which the temperature was maintained within ±1°C of the value given. In a typical experiment, cells were washed three times with 15 volumes of buffer after completion of the incubation with Chol*-HDL_3, made up to an approx. 50% v/v suspension in buffer, and kept (along with the lipoprotein solutions) at the appropriate temperature for 1 h before initiating the experiment. Then 0.40 ml of the labelled erythrocyte suspension (typically containing about 35 μg cholesterol per ml) were added to 6.0 ml of the lipoprotein solution in buffer (which also contained 0.5% glucose, 0.1% adenosine and 1% v/v garamycin (Schering) antibiotic). Aliquots of 1.5 ml were taken as soon as possible and at appropriate time intervals; the remaining erythrocyte-lipoprotein suspensions were slowly stirred between withdrawal of the next aliquots. The aliquots were centrifuged at $4000 \times g$ for 5 min and the supernatants collected for assay. The sedimented cells from the last aliquot were washed three times with 0.9% NaCl, and their phospholipid and cholesterol contents determined. The cholesterol/phospholipid ratios of the cells were found to be unchanged in all instances.

Since several runs were usually carried out simultaneously, the first aliquots

could not be centrifuged until up to about 8 min after the mixing of lipoprotein and cells. Exchange intervals were timed from the start of the centrifugations to eliminate uncertainties regarding exactly when the cells were completely separated from the solution.

The supernatant solutions were assayed for hemoglobin, to assess hemolysis, and then for radioactivity. The occurrence of hemolysis required corrections both for the reduction of the efficiency of the scintillation counter by light absorption by hemoglobin, which was found to be about 1.5% each 1% of cell hemolysis, and also for the presence of ghosts of the hemolyzed cells in the supernatants. The duration of the experiments were set to limit hemolysis to less than 2%, and for most time points hemolysis was less than 1%. In practice the 2% restriction on hemolysis restricted experiments at 37°C to 2 h, and at 23°C and 4°C to 4 h, thus limiting the maximum extents of exchange to about 25%. In experiments in which buffer replaced the lipoprotein solutions, only small amounts of labelled cholesterol, which could be accounted for as unsedimented ghosts resulting from hemolysis, were found in the supernatants.

Results

The general time courses for the movements of labelled cholesterol into the lipoprotein solutions were the same in all cases. Fig. 1 shows the appearance of labelled cholesterol as a function of time, plotted according to the general expression for two compartment diffusion with a constant specific rate:

$$\frac{\log\left[1 - \left(\frac{C_e + C_l}{C_l}\right)\alpha\right]}{\frac{1}{C_e} + \frac{1}{C_l}} = kt \qquad (1)$$

Here C_e and C_l are the total cholesterol contents of the erythrocytes and lipoprotein solutions respectively, α is the fraction of the labelled cholesterol in the erythrocyte solution at the time, t, and k is the specific rate constant. As Fig. 1 shows, the exchange followed Eqn. 1 after the first time point, thus indicating that it takes place with a constant specific rate after this point. However, the time course line does not extrapolate back to the origin, but has a positive y-intercept. This anomaly is not due to trivial experimental factors, i.e. (a) it is not a result of incomplete removal of the Chol*-HDL_3 solution used to label the cells, since it was unaffected by increasing the number of cell washes following labelling, and (b) it is too large to be the result of an error from using the start of centrifugation to approximate the time of separation of cells from solution. The anomalous initial rate may signify either that a small sub-population of erythrocytes exists with particularly labile cholesterol, or that about 2% of the cholesterol of each erythrocyte exchanges at a greater rate than does the remainder. Lange and D'Alessandro [4] have reported that about 10% of the erythrocyte cholesterol is more rapidly exchangeable than the remainder. A possible explanation for the initial high rate, in terms of the intrinsic exchange mechanism, will be suggested in Discussion. Otherwise, the rapid exchange ocurring during the first few minutes will be ignored, and only the subsequent, constant exchange rates will be considered.

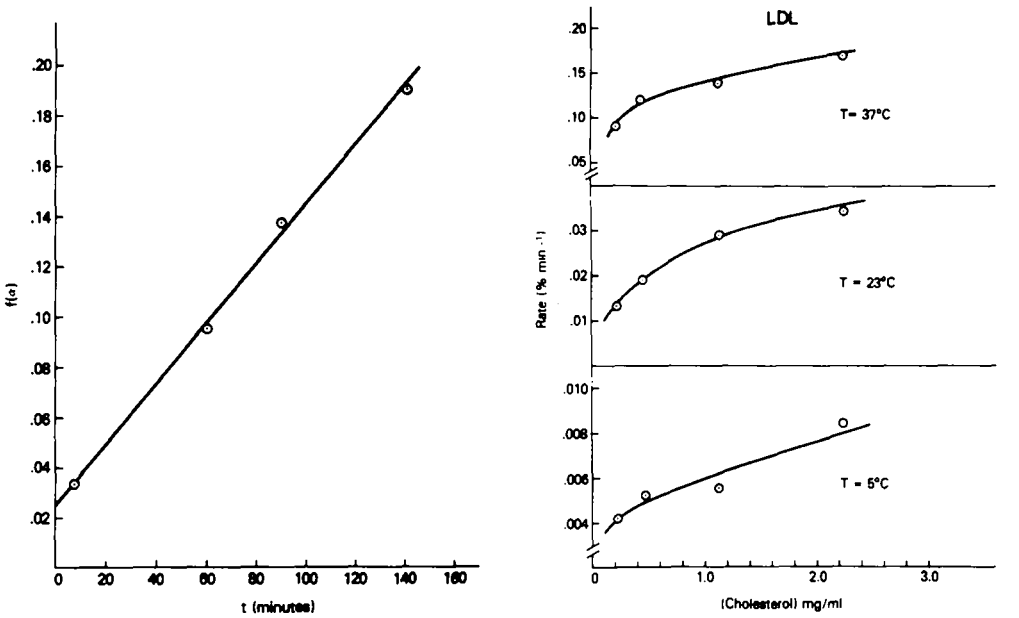

Fig. 1. Typical time course for the appearance of labelled cholesterol in the lipoprotein solution; $f(\alpha)$ is the l.h.s. of Eqn. 1. This particular plot is for HDL_3 at 37°C, 34 μg/ml lipoprotein cholesterol and 46 μg/ml erythrocyte cholesterol.

Fig. 2. Rates of cholesterol exchange (i.e. steady-state slopes of $f(\alpha)$-time plots) as a function of cholesterol concentration in LDL solutions at various temperatures. Composition of LDL as mg/mg protein: cholesterol 0.32, cholesteryl ester 1.07, phospholipid 0.97, triacylglycerol 0.35.

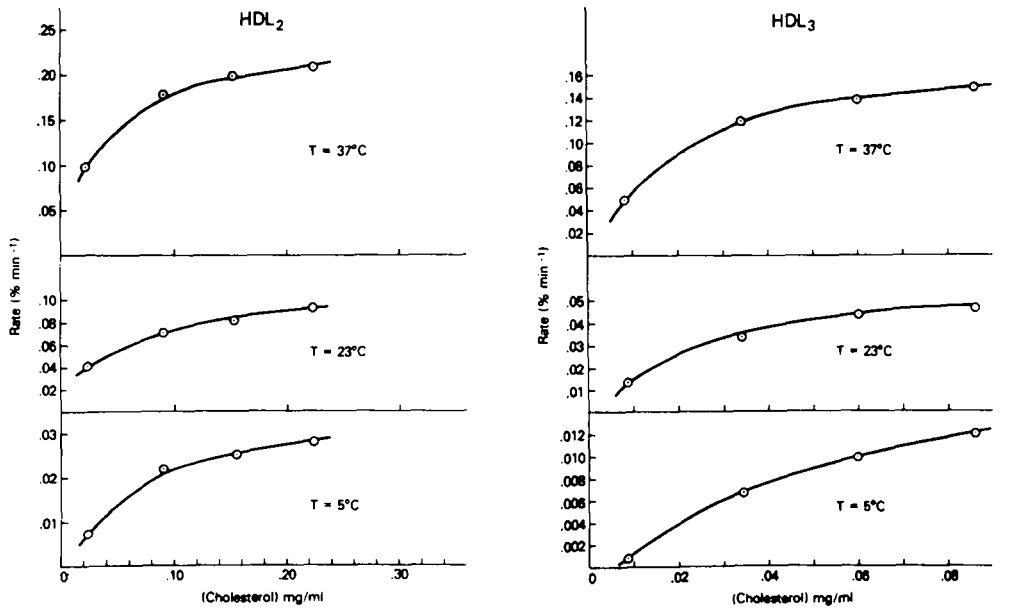

Fig. 3. Rates of cholesterol exchange for HDL_2 solutions. Composition of HDL_2 as mg/mg protein: cholesterol 0.10, cholesteryl ester 0.42, phospholipid 0.75, triacylglycerol 0.10.

Fig. 4. Rates of cholesterol exchange for HDL_3 solutions. Composition of HDL_3 as mg/mg protein: cholesterol 0.038, cholesteryl ester 0.18, phospholipid 0.28, triacylglycerol 0.10.

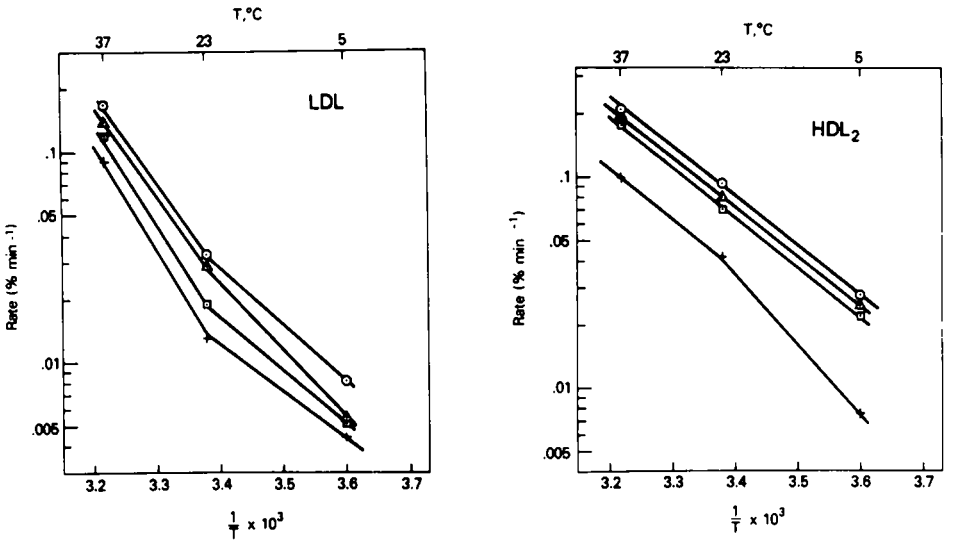

Fig. 5. Data of Fig. 2 replotted according to Arrhenius equation. ○, △, □, + indicate solutions of decreasing lipoprotein concentrations.

Fig. 6. Data of Fig. 3 replotted according to Arrhenius equation. Symbols have same significance as in Fig. 5.

The rates of exchange for the three lipoprotein species, over a range of concentrations and temperatures are shown in Figs. 2—4. Two significant points are common to the three lipoprotein species: (a) the exchange rates, at all temperatures, increase far less than proportionately to solution concentration, and (with the exception of LDL at 5°C) in fact appear to approach limiting values, and (b) the rates have a marked temperature dependence, which is similar for all the lipoproteins. Also, while the results with the various lipoproteins are not strictly comparable in these experiments, since different preparations of erythrocytes were used with each lipoprotein, it is clear that a much higher lipoprotein concentration, in terms of cholesterol content, is required for a given rate of exchange with LDL than with HDL_2 and HDL_3.

Figs. 5—7 show the data from Figs. 2—4 plotted for each lipoprotein solution according to the Arrhenius equation:

$$\Delta H_a = 2.3R \frac{\mathrm{d} \log k}{\mathrm{d}\left(\frac{1}{T}\right)} \tag{2}$$

where ΔH_a is the activation energy for the process, R the gas constant, k the rate of the process at the absolute temperature, T. With HDL_2 and HDL_3 essentially parallel straight lines are obtained with all solutions (excepting the lowest concentration at 5°C). These data indicate similar activation energies for the two higher density lipoprotein species, 11 kcal for HDL_2 and 14 kcal for HDL_3. The data for LDL solutions at the three temperatures do not fall on straight lines, the apparent activation energy from 23 to 37°C being about 22

TABLE I

EXCHANGE RATES AT HIGH LIPOPROTEIN CONCENTRATIONS

The concentrations are given in mg lipoprotein cholesterol per ml. The exchange rates are given in % · min^{-1} of erythrocyte cholesterol. Lipoprotein compositions as mg/mg protein. LDL: cholesterol 0.36, cholesteryl ester 1.07, phospholipid 1.03, triacylglycerol 0.42; HDL_2: cholesterol 0.15, cholesteryl ester 0.50, phospholipid 0.68, triacylglycerol 0.08; HDL_3: cholesterol 0.044, cholesteryl ester 0.26, phospholipid 0.31, triacylglyccerol 0.10.

T	LDL		HDL_2		HDL_3	
	Concn.	Rate	Concn.	Rate	Concn.	Rate
37°C	1.9	0.19	0.80	0.20	0.40	0.20
	2.6	0.22	1.1	0.21	0.60	0.20
23°C	1.9	0.089	0.80	0.091	0.40	0.088
	2.6	0.091	1.1	0.093	0.60	0.090
5°C	1.9	0.022	0.80	0.028	0.40	0.023
	2.6	0.025	1.1	0.023	0.60	0.021

kcal and that between 5°C and 23°C about 12 kcal.

The next series of experiments examined whether a limiting, concentration independent, exchange rate is reached at high lipoprotein concentrations. To permit comparison among the lipoproteins the same preparation of labelled erythrocytes was used throughout. As shown in Table I, a limiting rate is reached with all three lipoproteins at all three temperatures. Moreover, at each

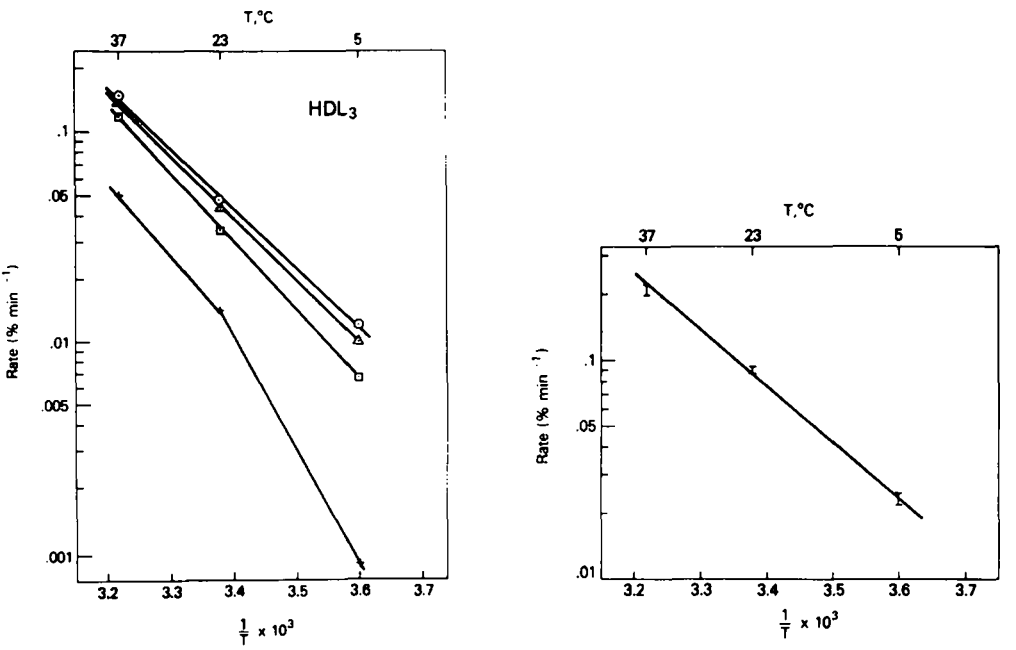

Fig. 7. Data of Fig. 4 replotted according to Arrhenius equation. Symbols have same significance as in Fig. 5.

Fig. 8. Arrhenius plot of maximal rates at various temperatures, from Table I.

TABLE II

CHOLESTEROL EXCHANGE RATES WITH MIXTURES OF LIPOPROTEINS (T = 37°C)

Solution *	Exchange rate ($\% \cdot min^{-1}$)
LDL (2.4 mg/ml)	0.18
HDL_2 (1.0 mg/ml)	0.20
HDL_3 (0.55 mg/ml)	0.21
LDL (2.4 mg/ml + HDL_2 (1.0 mg/ml)	0.22
LDL (2.4 mg/ml) + HDL_3 (0.55 mg/ml)	0.23
HDL_2 (1.0 mg/ml) + HDL_3 (0.55 mg/ml)	0.23

* Concentrations in terms of mg lipoprotein cholesterol per ml.

temperature, this limiting rate is independent of the lipoprotein species. In Fig. 8, the limiting rate is plotted according to the Arrhenius equation, giving an activation energy of about 12 kcal.

The final experiments examined whether the limiting exchange rates are affected by the presence of a second lipoprotein species. As shown in Table II, for all binary combinations of the lipoproteins, addition of an amount of a second species at a concentration great enough to give a limiting exchange rate by itself, had no effect on the rate of exchange. The data of Table II also agree with those of Table I, even though different lipoprotein and erythrocyte preparations were used for the two series of experiments.

Discussion

The experimental results can be summarized as follows:

(a) With all three lipoprotein species, and at all temperatures, the exchange rates first increased less than linearly with lipoprotein concentration, and then became independent of concentration at higher concentrations.

(b) In the region of concentration dependence, the concentrations of HDL_2 and HDL_3 (expressed in terms of solution cholesterol content) required for a given exchange rate were similar, while that for LDL was about 10-fold greater. Since the molecular weight of LDL is also about 10 times greater than those of the two higher density species, the exchange rates at equal molar concentrations of the three lipoproteins are similar.

(c) The limiting, concentration-independent, exchange rates were the same for the three lipoproteins at each temperatures.

(d) Both the concentration-dependent and limiting exchange rates varied with temperature, the apparent activation energies ranging from 12 to 22 kcal.

The magnitudes of the exchange rates of this present work are consistent with those reported by Quarfordt and Hilderman [6], and in fact a limiting exchange rate, similar to that reported here, is suggested by their results. In their experiments with LDL (37°C), one series showed a limiting rate of $0.25\% \cdot min^{-1}$, in agreement with the value at 37°C of about $0.20\% \cdot min^{-1}$ in Tables I and II of the present work; a second series indicated a limiting rate of $0.14\% \cdot min^{-1}$, and a third series did not show a limiting rate, but the rate at the highest concentration was $0.23\% \cdot min^{-1}$. In their single pair of experiments

with HDL, Quarfordt and Hilderman found a higher exchange rate at the higher HDL concentration, this rate being 0.29% · min^{-1}. Limiting exchange rates have also been found for related systems. The exchange of cholesterol between erythrocytes and phospholipid vesicles reaches a well defined limiting rate of 0.18% · min^{-1} [19]. Cholesterol exchange between adipose cells and HDL also exhibits a limiting rate [20]. However, the exchange between adipose cells and LDL is much faster than with HDL, and increases continuously with concentration of this lipoprotein [20].

The temperature effects reported here, corresponding to activation energies of 12 kcal and greater, are also similar to those for related systems. Thus the activation energy for cholesterol exchange between bovine HDL and single bilayer phospholipid vesicles is 17 ± 3 kcal [21], and for exchange between phospholipid vesicles and human erythrocyte ghosts the activation energy is 10 or 20 kcal, depending on the vesicle cholesterol content [22]. No explanation is offered for the finding by earlier workers [7] of only a small temperature dependence, which correspond to an activation energy of 2.6 kcal, in the human LDL-rat erythrocyte ghost system.

The possibly slow movement [23—25] of cholesterol from the inner to the outer half of the membrane bilayer cannot be a significant determining factor for the exchange rates considered in this paper, since these rates apply down to a few percent of the membrane cholesterol exchanged (cf. Fig. 1), while about half of the membrane cholesterol is located in the outer bilayer [3]. For convenience in discussion it will be assumed that the concentration dependence of the exchange rate can be characterized by two limiting regions, one in dilute solutions, where the rate would be expected to be proportional the rate of collisions of lipoprotein molecules with the cell surface, and hence to the lipoprotein concentration, and a second region at high lipoprotein concentrations, where the exchange rate is independent of concentration. Rates at the lowest lipoprotein concentrations experimentally studied here, where the rates vary less than linearly with concentration, are assumed to be under mixed control.

It is instructive to compare the measured rates to those computed on the basis of simple collision theory [26], for the dilute solution region. According to simple collision theory, the rate of cholesterol transfer, r, would be given by:

$$r = nJ \exp - \Delta\overline{H}_a/RT \tag{3}$$

where n is the number of cholesterol molecules transferred per collision, J is the rate of collisions of lipoprotein molecules with the cell surface and the quantity $\exp - \Delta\overline{H}_a/RT$ represents the fraction of these molecules having enough energy to overcome the activation energy barrier, $\Delta\overline{H}_a$ (R and T have their usual significance as in Eqn. 2).

In an ideal system, $\Delta\overline{H}_a$ would be given by ΔH_a, the activation energy computed from the variation of rate with temperature according to Eqn. 2. In real systems these quantities may be very different, because of large entropies of activation [26] or because of temperature dependent changes in the conformations of the reactants. The quantity n can be between 1 and the total cholesterol contents of the lipoprotein molecules, which are computed to be about 600, 40, and 10 cholesterol molecules for LDL, HDL_2 and HDL_3, respectively, based on cholesterol contents of 8%, 4%, and 2% and molecular weights of

$3 \cdot 10^6$, $4 \cdot 10^5$ and $2 \cdot 10^5$ [27]. The quantity J can be computed by assuming that the erythrocyte can be treated as a flat disc, for which according to Berg and Purcell [28] the flux would be given by

$$J = 8bDc \tag{4}$$

where b is the radius of the disc, which can be assumed to be about $3 \cdot 10^{-4}$ cm, D the diffusion coefficient of the lipoproteins, about $5 \cdot 10^{-7}$ $cm^2 \cdot s^{-1}$ [29] and c the lipoprotein concentration in mol $\cdot$ cm^{-3}.

Consider, for example, the lowest concentration HDL_2 solution at which measurements were made, where the lipoprotein concentration was 0.02 mg cholesterol per ml and the exchange rate was 0.10% $\cdot$ min^{-1} at 37°C (Fig. 3). This concentration corresponds to approx. $5 \cdot 10^{14}$ HDL_2 mol per ml, and since there are about $2 \cdot 10^8$ cholesterol molecules in an erythrocyte [9], the experimental exchange rate corresponds to $3.3 \cdot 10^3$ mol $\cdot$ $cell^{-1} \cdot s^{-1}$. From Eqn. 4, the number of lipoprotein molecules colliding with a cell each second is about $6 \cdot 10^5$, and therefore from Eqn. 3 the cholesterol exchange rate in the absence of energetic barriers is computed to be between $6 \cdot 10^5$ and $240 \cdot 10^5$ mol $\cdot$ $cell^{-1} \cdot s^{-1}$, depending on the number of molecules transferred in each collision. Thus the fraction of collisions successfully transferring cholesterol is between $1.5 \cdot 10^{-4}$ and $6 \cdot 10^{-3}$. (The probability that the rates are not solely under collision control at the concentration considered makes these fractions underestimates.) These collision efficiencies are quite high and indicate values for $\Delta\bar{H}_a$ of only 3—7 kcal according to Eqn. 3. In contrast, the lowest of the activation energies indicated by the temperature effects, 11 kcal, would indicate that only 1 collision in about 10^8 sucessfully transfers cholesterol. Similar considerations apply to the data for the other lipoproteins and at the other temperatures. Thus, despite the large apparent activation energy indicated by the temperature dependence of the rates, there are no large energetic barriers or orientational requirements for the transfer of cholesterol on collision of lipoprotein molecules with erythrocytes. The discrepancies between $\Delta\bar{H}_a$ and ΔH_a may be results of temperature dependent conformational changes in either the erythrocytes or the lipoproteins. Alternatively, following transition state kinetic theory, the discrepancies may indicate a large positive activation entropy [26], i.e. that the formation of the lipoprotein-erythrocyte activated complex is favored by entropic factors.

Turning now to the high lipoprotein concentration region, the occurrence of a limiting exchange rate indicates that the rate is not controlled by the frequency of collisions of lipoprotein molecules with the cell surface. One possible explanation for the limiting rate is that the lipoprotein molecules must adsorb onto particular sites at the cell surface before cholesterol exchange can take place, and that these sites become saturated at high lipoprotein concentrations. On this basis, the fact that the limiting rates are the same for the three lipoprotein species, and for their mixtures, would require both that the same adsorption sites are involved for the three lipoproteins, and that cholesterol moves at the same rate between each adsorbed species and the cell membrane. This seems unlikely in view of the large differences between LDL and the two higher density lipoproteins with respect to molecular weight, lipid composition and type and amount of apoprotein [27]. However, Hui and Harmony [30]

have observed the adsorption of LDL onto erythrocyte membranes, and the inhibition of this adsorption by HDL_2 and HDL_3.

An alternative explanation for the limiting rate being the same for the three lipoprotein species is that this rate is controlled by a process taking place within the membrane. The simplest such process would be the diffusion of cholesterol molecules to membrane sites at which transfer to lipoprotein can occur. Such diffusion control would be consistent with the observed activation energies, since the activation energy for diffusion of lipid molecules in (rat) erythrocyte membranes is about 12 kcal (Webb, W. and Bloom, J., personal communication). This mechanism also provides a possible explanation for the anomalously high initial exchange rates reported in an earlier section, since those cholesterol molecules which are close to the transfer sites would exchange more rapidly than the remainder.

A limiting rate controlled by the diffusion of cholesterol within the membrane implicitly requires that there be only a limited number of potential transfer sites at the cell surface, since otherwise the diffusion distances would be indefinitely small. The number of such sites required by the observed limiting rates and diffusion coefficient of lipids in erythrocyte membranes can be estimated by assuming that these sites are uniformly distributed on the membrane surface.

Thus for a membrane of area A, with m sites separated by the distance, l:

$$A = ml^2 \tag{5}$$

The average time, τ, for a cholesterol molecule to diffuse the distance $0.5\,l$ is approximately:

$$\tau = \frac{(0.5\,l)^2}{3D} \tag{6}$$

where D is the diffusion coefficient of cholesterol in the membrane. Since the reciprocal of τ gives the rate at which cholesterol molecules reach each site, r, the rate at which cholesterol molecules leave the membrane, is given by:

$$r = \frac{m}{\tau} \tag{7}$$

Combining Eqns. 5, 6, and 7 gives for m:

$$m = \left(\frac{Ar}{12D}\right)^{1/2} \tag{8}$$

The area of an erythrocyte membrane, A, is $160 \cdot 10^{-8}$ cm^2 [3] and $D \simeq 10^{-8}$ cm$^2 \cdot$ s^{-1} at 37°C [32]; since there are about $2 \cdot 10^8$ cholesterol molecules in an erythrocyte membrane, the limiting rate of $0.20\% \cdot$ min^{-1} at 37°C corresponds to a value for r of about 7000 mol $\cdot$ cell$^{-1} \cdot$ s^{-1}. Therefore from Eqn. 8, $m \simeq 300$ and from Eqn. 5, $l \simeq 7000$ Å. While these values are obviously very crude estimates, the computation indicates that for the rate to be limited by the diffusion of cholesterol within the membrane, the number of transfer sites on the membrane would have to be small, and the sites would have to be separated by distances which are large on a molecular scale.

It is not now possible to make a choice between the adsorption and diffu-

sion-control mechanisms. Indeed since each contains requirements which seem a priori improbable, it may well be that other mechanisms are responsible for the limiting exchange rates. (A reviewer has suggested a slow diffusion of lipoprotein molecules through carbohydrate surrounding the membrane.) Additional information, particularly on the adsorption of the various lipoproteins onto erythrocytes, is required to resolve the question.

Note added in proof (Received May 16th, 1980)

Since submission of this manuscript, it has been learned (Harmony, J.A.K., personal communication) that human erythrocytes have about 200 absorption sites for radioactively labelled LDL. This number is in essential agreement with the value computed on the basis of a membrane-diffusion limited cholesterol exchange.

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