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Influence of the Ester Carbonyl Oxygens of Lecithin on the Permeability Properties of Mixed Lecithin–Cholesterol Bilayers†

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ABSTRACT: The passive diffusion of Na^+, Cl^-, and glucose across the bilayer membranes of single-shelled vesicles of diester-lecithin (1,2-dioctadecenoyl-*sn*-glycerophosphocholine), diether-lecithin (*rac*-1,2-dioctadecenylglycero-3-phosphocholine), and 1-ether-2-ester-lecithin (*rac*-1-octadecenyl-2-octadecenoylglycero-3-phosphocholine) with and without cholesterol has been measured at 4 °C. The passive diffusion of Na^+ across pure membranes of diether-lecithin was found to be much slower as compared to membranes of diester-lecithin, but the opposite effect was observed with Cl^- and glucose. Mixed membranes of diester-lecithin and 30 mol % cholesterol showed strongly reduced diffusion rates for Na^+, Cl^-, and glucose; with mixed diether-lecithin–cholesterol vesicles, however, no significant reduction was observed. On the other hand, 1-ether-2-ester-lecithin showed a similar reduction of the glucose and Na^+ permeability upon addition of 30 mol % cholesterol as did the diester-lecithin. From these data it is concluded that the ester carbonyl oxygens of lecithin are strongly involved in the interaction between lecithin and cholesterol and that the diffusion of cations, on the one hand, and anions and uncharged solutes, on the other hand, is dictated by different mechanisms. Electron spin resonance (ESR) spectra of nitroxide-labeled stearic acid incorporated into multilamellar aqueous dispersions of lecithin and cholesterol have been recorded. With increasing amounts of cholesterol the order parameter S_3 calculated from the ESR spectra increased in the same way for both diester- and diether-lecithin. For diether-lecithin there is no apparent correlation between the ESR data and the results from the diffusion experiments, since the fluidity of the bilayer membrane is reduced by cholesterol while the diffusion rate remains unchanged.

Phospholipids and cholesterol are important components of many biological membranes. Their ordered arrangement as multilamellar bilayers and single-shelled vesicles in aqueous dispersions made it possible to use these systems as models for biological membranes and to study the chemical and physical properties of these membranes (Chapman, 1975; Phillips, 1972). The interaction between the phospholipid molecules as well as between phospholipids and steroids, mainly cholesterol, the nature of the forces between these molecules, and the molecular arrangement in biological and model systems have been studied intensively mainly by x-ray diffraction, differential scanning calorimetry, nuclear magnetic resonance, and electron spin resonance.

The incorporation of cholesterol into lipid membranes has been of special interest, since it affects drastically the physicochemical properties of these membranes. It causes a reduction of the fluidity of the lipid bilayer above the gel to liquid crystalline phase transition (Oldfield and Chapman, 1972) while it liquefies these membranes below the phase transition temperature.

The permeability for Na^+, K^+, Cl^-, and sugars is reduced if cholesterol is present (Demel et al., 1968; Papahadjopoulos et al., 1972, 1973). Based on these data several possible models for the molecular interaction between cholesterol and lecithin have been established. De Kruyff et al. (1973) concluded from their monolayer and DSC studies that not any specific part of the lecithin molecules was necessary for the cholesterol–lecithin interaction, while it turned out from NMR studies (Yeagle et al., 1975) that the 3β-OH group of cholesterol was interacting with the ester carbonyl oxygens of lecithin. The interaction between the OH group of cholesterol and one oxygen of the phosphate group of lecithin has also been proposed (Darke et al., 1972; Forslind and Kjellander, 1975).

To clarify these problems, we initiated a comparative study of the physicochemical properties in bilayer systems of dioleoyllecithin (diester-lecithin) and of the two analogues dioleyllecithin (diether-lecithin) and 1-oleyl-2-oleoyllecithin (ether-ester-lecithin), the two latter lacking two and one carbonyl oxygens, respectively. The advantage of using these analogues is that differences in the physicochemical properties

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and in the interaction with cholesterol between the ether-lecithins and the diester-lecithin may be directly related to the absence of the carbonyl oxygens.

In a previous paper (Schwarz et al., 1976), we could show that the apparent partial specific volume and the x-ray long spacing of multilamellar mixtures of lecithin and cholesterol yielded significant differences between diester- and diether-lecithin. We concluded that the ester bonds in lecithins were important for the lecithin–sterol interaction.

In this paper, we present the results of the influence of cholesterol on the diffusion of Na^+, Cl^-, and glucose across bilayer membranes of single-shelled vesicles prepared of diether- and diester-lecithin and of ether-ester-lecithin. The results confirm the model of the hydrogen binding between the OH group of cholesterol and the ester carbonyl oxygens of lecithin.

Materials and Methods

Dioleoyllecithin and dioleyllecithin were synthesized as published previously (Schwarz et al., 1976) and purified by preparative thin-layer chromatography. *rac*-1-Oleyl-2-oleoyllecithin was synthesized as described by Paltauf (1972). Cholesterol was purchased from Merck (Darmstadt, Germany) and recrystallized several times from absolute ethanol. Thin-layer chromatography of the purified cholesterol yielded single spots with chloroform–diethyl ether (9:1) and benzene–diethyl ether (1:9) as developing solvents.

Preparation of Vesicles for Na^+ Diffusion. Dry lipid (20 mg) (lecithin or lecithin–cholesterol) was dispersed in 3 mL of 0.145 M NaCl + 0.02% NaN_3 + 13 μCi of $^{22}Na^+$/mL. The milky suspension was sonicated with a Braun-Sonic 300 S oscillator for 20 min (at 20 kHz and 140-W input power) in a round-bottomed glass tube (1.1-cm diameter, 10-cm length) with the tip of the soniprobe (0.95-cm diameter) immersed to about half of the height of the dispersion. During the sonication, the glass tube was cooled with an ice bath and the dispersion was flushed with nitrogen. Under these conditions no chemical degradation was observed as checked by thin-layer chromatography.

Measurement of Na^+ Diffusion. Two methods were employed: (1) After sonication, particles from the tip of the soniprobe were removed by short centrifugation and the supernatant was fractionated with a Sepharose 2B column (2.5 × 40 cm, eluting solution 0.145 M NaCl + 0.02% NaN_3) in order to separate the single-shelled vesicles (fraction II) from the multilamellar aggregates (fraction I). Fraction II was pooled and at different times aliquots were removed and filtered through a Sartorius pressure filter (Type SM 12136, pore size 50 Å, applied pressure 4 atm). Filtrate (0.1 mL) containing the $^{22}Na^+$ that had permeated through the bilayer membranes was counted in Bray's solution in a Beckman liquid scintillator. The time when the sonication was finished was taken as $t = 0$ for the diffusion process. Method 1 is based on the procedure described by Huang (1969).

(2) After sonication, the multilamellar aggregates were removed by ultracentrifugation at 110 000g for 60 min with a Sorvall OTD 2 ultracentrifuge. Under these conditions, the supernatant contained only the single-shelled vesicles which were separated from the external radioactive label by passage over a Sephadex G-50 column (1.5 × 8 cm). The vesicles eluting with the void volume were collected. At different times 0.4-mL aliquots were removed and fractionated with a Sephadex G-50 column (1 × 5 cm). The first peak eluting at the void volume and containing the vesicles was collected directly into a counting vial containing Bray's solution. The second peak containing the $^{22}Na^+$ that had permeated through the vesicle membranes into the external solution at that time was similarily collected and radioactivity was measured.

Measurement of $^{36}Cl^-$ and [^{14}C]Glucose Diffusion. The lipid (10 mg) was dispersed in 3 mL of 0.1 M Tris + 0.1 M KCl + 0.02% NaN_3, pH 7.18 (final concentration of Cl^- 0.19 mol/L) for diffusion of $^{36}Cl^-$ or in 3 mL of 0.145 M NaCl + 0.02% NaN_3 for [^{14}C]glucose diffusion. Sonication and ultracentrifugation were performed as described above. At time $t = 0$, 8 μCi of $^{36}Cl^-$ or [^{14}C]glucose per mL was added to the removed supernatant containing the vesicles. The mixture was shaken mechanically at 4 °C. At different times, 0.2-mL aliquots were removed and the lipid vesicles separated from the external medium by rapid passage over a Sephadex G-50 column (1 × 5 cm). The eluted vesicles were collected directly into a counting vial containing Bray's solution and the radioactivity of the solutes that had penetrated into the vesicles was counted.

ESR[1] spectra were recorded on a Varian ESR spectrometer (Model E 104 A, 9.5 GHz). Dry lipid (4 mg) (lecithin + corresponding amounts of cholesterol) together with the stearic acid spin labels at a concentration of 1 mol of label per 100 mol of lipid were dispersed by short sonication in 0.4 mL of buffer solution (0.1 M Tris + 0.1 M KCl, pH 7.4). The milky suspension was transferred to the cuvette and the spectrum was recorded. The order parameters S_3 of the spin labels were computed from eq 1

$$S_3 = \frac{A_\parallel - A_\perp}{A_{zz} - A_{xx}} - \frac{a_N}{a'_N} \quad (1)$$

where, according to the treatment of Seelig (1976), $A_{zz} = 30.8$ G, $A_{xx} = 5.8$ G, and $a_N = 13.1$ G. $A_\parallel$ and $A_\perp$ are the hyperfine splittings parallel and perpendicular to the stearic acid long axis, a'_N is determined by

$$a'_N = \frac{1}{3}(A_\parallel + 2A_\perp) \quad (2)$$

Results

From the internal volume of the vesicles per mole of lipid, the vesicle radii were computed assuming a constant cross-sectional area of the lipid molecules on the inner and outer layer of the vesicle membranes (Finer et al., 1972; Stockton et al., 1976) and a given bilayer thickness. Hauser and Irons (1972) calculated a value of 46 Å for the bilayer thickness of EYL vesicles, while De Kruijff et al. (1975) found a value of 35 Å for EYL vesicles and Newman and Huang (1975) estimated 52 Å. From these data and from our results based on x-ray diffraction (Schwarz et al., 1976), we chose a value of 42 Å for diether- and diester-lecithin. With these data, the vesicle radii were calculated from

$$\left(\frac{N_{lec}}{V_i}\right) A_{lec} - r^3 - 6r^2 - 6dr - 3d^2 = 0$$

where N_{lec} and V_i are the number of lecithin molecules and the trapped internal volume, respectively, both per unit volume of a vesicle suspension, r is the inner radius of a vesicle, d is the bilayer thickness, and A_{lec} is the cross-sectional area per lecithin molecule. The values of the outer vesicle radii R ($R = r$

[1] Abbreviations used are: ESR, electron spin resonance; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; A_{lec}, cross-sectional area per lecithin molecule; A_{ves}, outer area of a vesicle; $A_\parallel$, $A_\perp$, hyperfine splittings; d, bilayer thickness; k_1, first-order rate constant; N_{lec}, number of lecithin molecules per unit volume; P, permeability coefficient; r, inner-vesicle radius; R, outer-vesicle radius; S_3, order parameter; V_i, trapped internal vesicle volume per unit volume of a vesicle suspension; V_{ves}^i, internal volume of a vesicle.

TABLE I: Mean Values of V_{ves}^{i}, the Inner Volume per Vesicle, and A_{ves}, the Outer Area per Vesicle, for Diether- and Diester-lecithin with and without Cholesterol.[a]

	Diester-lecithin	Diester-lecithin + 30 mol % cholesterol	Diether-lecithin	Diether-lecithin + 30 mol % cholesterol
V_{ves}^{i} (Å^3)	1.5×10^6	1.6×10^6	1.5×10^6	1.5×10^6
A_{ves} (Å^2)	1.6×10^5	1.7×10^5	1.7×10^5	1.7×10^5

[a] Accuracy ±5%.

+ d) were then 113 ± 6 and 117 ± 6 Å for diester- and diether-lecithin, respectively. The percentage of the number of lecithin molecules in the inner layer of the vesicles was 30 ± 3% of the total number of lecithin molecules in both layers. This agrees well with the result of Finer et al. (1972) who found a ratio of 28% (Table I).

In the presence of 30 mol % cholesterol, no significant change of the elution volume of fraction II on Sepharose 2B both for diester- and diether-lecithin was observed. A similar observation was recently reported (De Kruijff et al., 1976) for several diester-lecithins.

In order to check whether cholesterol was incorporated into the vesicles, [^{3}H]cholesterol was added and it was found that the molar concentration of 30 mol % cholesterol was maintained in all cases. From these data we conclude that the vesicles are single shelled and that they may be used as models for passive diffusion.

Diffusion Parameters. The passive diffusion across the vesicle bilayer membranes was treated as a first-order reaction (Hauser et al., 1973; Toyoshima and Thompson, 1975). The first-order reaction constant k_1 was calculated from the slopes of the linear plots ln $N_0/(N_0 - N_t)$ vs. t, where N_0 and N_t are the concentrations of the radioactive labels within the vesicles at the beginning of the experiment ($t = 0$) and at time t, respectively.[2] The values of k_1 are listed in Table II.

It is evident from results shown in Table II that cholesterol does not reduce the velocity of the influx of $^{36}Cl^-$ and [^{14}C]-glucose in the case of diether-lecithin, while there is a significant reduction in the case of diester-lecithin. With pure alkylacyllecithin vesicles, the influx is slightly increased for [^{14}C]glucose and decreased for $^{22}Na^+$ as compared to diester-lecithin; in the presence of 30 mol % cholesterol the permeability is reduced to a similar degree, as in the case of diester-lecithin (Table II).

The order parameters S_3 calculated from the ESR spectra of 5- and 12-nitroxide stearic acid incorporated into multilamellar bilayers of diether- and diester-lecithin are increased in the same way if cholesterol is added (up to 50 mol %). The values of the 5-NS label are 0.64 and 0.72 for 0 and 50 mol % cholesterol, respectively; for the 12-NS label they range from 0.33 to 0.55 for 0 and 50 mol % cholesterol, respectively. These results are supported by 90 MHz ^{1}H NMR spectra of sonicated vesicles of diether- and diester-lecithin in the presence of 30 mol % cholesterol which show the very same line-broadening effect of cholesterol upon the $(CH_2)_n$ signal of the two lecithins.[3]

Discussion

It is now generally accepted (Demel et al., 1972; De Kruyff et al., 1972; Papahadjopoulos et al., 1972) that one of the effects of cholesterol on phospholipid membranes is to reduce their permeability to cations, anions, and uncharged solutes. This effect was also observed with ester-lecithins in the present study as it is shown in Table II. The permeability of phospholipid membranes is dependent on the degree of unsaturation and on the length of the fatty acid chains of the lipid molecules (De Gier et al., 1968) and with mixed phospholipid–sterol bilayers it also depends on the structure of the sterol. From these results, Brockerhoff (1974) deduced a model for the interaction between lecithin and cholesterol. He postulated a hydrogen bond between the 3β-OH group of cholesterol and the ester carbonyl oxygens of lecithin. Recently this assumption was confirmed by NMR experiments of Yeagle et al. (1975) and by theoretical considerations of Huang (1976). The diffusion experiments presented in this study lend support to this model for the following reasons. Since the diether-lecithin is lacking the two carbonyl oxygens, significant differences in its interaction with cholesterol as compared to the diester-lecithin should be observed if the OH group of cholesterol was hydrogen binding to the carbonyl oxygens. As demonstrated in Table II, such differences can indeed be observed, since the permeability coefficients remain almost invariant upon addition of

TABLE II: Summary of the Diffusion Parameters for Diester-, Diether- and Ether-ester-lecithin.[a]

		k_1 (s^{-1})	P^c (cm s^{-1})
Diester-lecithin	Na^+	1.4×10^{-7}	1.3×10^{-14}
	Cl^-	2.2×10^{-4}	2.1×10^{-11}
	glucose	2.1×10^{-5}	4.3×10^{-12}
Diester-lecithin + 30 mol % cholesterol	Na^+	6.7×10^{-8}	6.4×10^{-15}
	Cl^-	1.5×10^{-4}	1.4×10^{-11}
	glucose[b]		
Diether-lecithin	Na^+	6.1×10^{-8}	5.3×10^{-15}
	Cl^-	2.7×10^{-3}	2.3×10^{-10}
	glucose	8.6×10^{-4}	7.4×10^{-11}
Diether-lecithin + 30 mol % cholesterol	Na^+	5.3×10^{-8}	4.7×10^{-15}
	Cl^-	2.6×10^{-3}	2.3×10^{-10}
	glucose	7.8×10^{-4}	6.5×10^{-11}
Ether-ester-lecithin	Na^+	2.5×10^{-7}	
	glucose	8.7×10^{-5}	
Ether-ester-lecithin + 30 mol % cholesterol	Na^+	1.0×10^{-7}	
	glucose	3.7×10^{-5}	

[a] The correlation coefficient r^2 for the first-order rate constants k_1 calculated from the least-squares fittings of the curves ln $[N_0/(N_0 - N_t)]$ vs. t varied between 0.94 and 0.97. N_0 and N_t are the radioactivities within the vesicles at equilibrium and at time t, respectively (for efflux measurements see Methods). All measurements were performed at 4 °C. [b] The reducing effect of cholesterol on the permeability of glucose is known from the literature (cf. Demel et al., 1972). [c] The permeability coefficient P was calculated from $P = k_1 V_{ves}^{i}/A_{ves}$, where V_{ves}^{i} and A_{ves} are the inner volume and the outer area per vesicle, respectively, which are shown in Table I.

[2] If the influx is measured, N_0 is the radioactivity at equilibrium.

[3] These spectroscopic results will be published in detail by Hauser, H., Laggner, P., Paltauf, F., and Schwarz, F. T.

30 mol % cholesterol to the diether-lecithin bilayer membrane. Monolayer investigations (Paltauf et al., 1971) and x-ray diffraction (Schwarz et al., 1976) yielded similar parameters for the structural organization of diester- and diether-lecithin in membranes and differences of the diffusion rates that might arise from different bilayer structures can therefore be ruled out.

Furthermore, the lecithin analogue containing one alkyl and one acyl moiety behaved as the diester-lecithin with respect to reduction of glucose and Na^+ diffusion upon addition of 30 mol % cholesterol. This means that introduction of only one carbonyl oxygen is sufficient to reduce glucose and Na^+ diffusion in the presence of cholesterol. These diffusion experiments therefore clearly demonstrate that the carbonyl oxygens are necessary for the lecithin–cholesterol interaction.

Brockerhoff (1974) postulated that membrane condensation upon addition of a sterol was not identical with membrane closure, i.e., the reduction of the permeability. This picture is strongly supported by our ESR and NMR studies. The order parameters S_3 of the spin labels incorporated into diether-lecithin bilayers increase upon addition of cholesterol in the same way as with diester-lecithin. This may be attributed to a condensation of the lipid bilayer (Butler et al., 1970; Hubbell and McConnell, 1971; Marsh and Smith, 1973; Suckling and Boyd, 1976) and to an increased viscosity of the hydrocarbon moiety (Cogan et al., 1973). Similarly, the broadening of the $(CH_2)_n$ signal in ^1H NMR spectra of mixed diester-lecithin–cholesterol vesicles has been attributed to a restricted motion and to a condensation of the acyl chains (Chapman and Penkett, 1966). Diester- and diether-lecithin showed the same effect in the presence of 30 mol % cholesterol and no difference of the extent of the line broadening between the two lecithins was observed (see footnote 3). It is therefore most remarkable that, while in the presence of cholesterol membranes of diether-lecithin are condensed in the same way as diester-lecithin, the diffusion rates of Na^+, Cl^-, and glucose across membranes of diether-lecithin are not affected by cholesterol.

Results in Table II also show that Na^+ diffuses much slower across bilayer membranes of pure diether-lecithin as compared to pure diester-lecithin membranes, while the opposite effect is observed with Cl^- and glucose. This suggests that the fluidity of the hydrocarbon moiety of the bilayer is not the only parameter determining the bilayer permeability.

Brockerhoff (1974) supposed that binding of cations and glucose to the carbonyl groups probably was necessary for the permeation across the bilayer membrane. Our results support this model only in the case of cations, since $^{22}Na^+$ diffuses slower across the diether-lecithin membrane than across the diester-lecithin membrane. As a consequence, cholesterol, if it is hydrogen binding to the carbonyl groups should not influence the permeation of $^{22}Na^+$ across mixed diether-lecithin–cholesterol membranes, which is also consistent with our results. The carbonyl groups then would act as a barrier for Cl^- and glucose, since these two species diffuse much slower across diester-lecithin membranes.

In this connection it is interesting to consider the influence of cholesterol upon the activation energies of the diffusion of Na^+, K^+, Cl^-, and glucose across lipid bilayer membranes. Papahadjopoulos et al. (1972) found that the energy of activation for the diffusion process was enhanced for Cl^- and glucose if cholesterol was present, while it was reduced for Na^+ and K^+. Clearly, it has to be kept in mind that the energy of activation might be temperature dependent, as it has also been pointed out by these authors, but the qualitative change of sign of the activation energy under the influence of cholesterol between cations, on the one hand, and anions and uncharged solutes, on the other, seems to be remarkable in the light of our results. Nevertheless, at this time we are not able to give an exhaustive explanation of our results on a molecular basis. Therefore, further experiments involving mainly spectroscopic techniques are in progress to throw more light upon membrane permeability and phospholipid–sterol interaction.

Biological Implications. The phospholipids of some halophilic bacteria consist almost entirely of diether-lipids (Joo and Kates, 1969). The reduced permeability of Na^+ across membranes of diether-lecithin, as it was found in our experiments, might indicate one possible function of the ether bonds, i.e., a protection of the bacterial cell against the extremely high concentration of NaCl in the surrounding medium. In this respect also plasmalogens containing an alk-1-enyl group are of interest. Following the argumentation of Brockerhoff (1974), mixed membranes of these phospholipids and cholesterol should exhibit minimum permeability. As it is shown in Table II, our results do not support this idea, since mixed ether-ester-lecithin–cholesterol membranes yield the same reduced permeability for Na^+ and glucose, as the diester-lecithin–cholesterol membranes, and it cannot be concluded that mixed plasmalogen–cholesterol membranes should be less permeable than diester-lecithin–cholesterol membranes. Therefore, the biological role of plasmalogens cannot be explained merely by their permeability-reducing abilities.

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Characterization of Mechanisms for Transfer of Cholesterol between Human Erythrocytes and Plasma†

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ABSTRACT: The removal from human erythrocytes of cholesterol (mass) and of [^{3}H]cholesterol which had been introduced into the erythrocyte by exchange was studied. Removal was accomplished by incubating erythrocytes in plasma, the free cholesterol content of which had been lowered by the action of lecithin:cholesterol acyltransferase. It was shown that the exchange of cholesterol between erythrocytes and plasma and the net movement of cholesterol out of the membrane into plasma are characterized by the same rate constant and are driven by cholesterol to phospholipid ratios in cells and plasma. The apparent limitation on cholesterol depletion of erythrocytes observed in experiments of this type is explicable as the result of equilibrium between cholesterol in the membrane and in the plasma, an equilibrium reached when there is still cholesterol left in the cells. It is concluded from this study that all the exchangeable cholesterol in human erythrocytes is available for removal from the membrane.

Recently, great progress has been made in the understanding of the molecular organization of the erythrocyte membrane. It has been established that phospholipids (Bretscher, 1972; Verkleij et al., 1973; Gordesky and Marinetti, 1973; Whiteley and Berg, 1974), proteins (Steck, 1972; Juliano, 1973; Whiteley and Berg, 1974; Amar et al., 1974), and carbohydrates (Nicholson and Singer, 1971; Juliano, 1973) are asymmetrically distributed between the two halves of the membrane bilayer. However, very little is known about the localization of cholesterol in the membrane and its interaction with other membrane constituents. One approach to this question has been through studies of cholesterol exchange between erythrocytes and plasma lipoproteins. Unfortunately, the results of such studies are divided between those that indicate that only a portion of erythrocyte cholesterol is exchangeable with plasma cholesterol (Bell and Schwartz, 1971; d'Hollander and Chevallier, 1972) and those that indicate that all of the cell cholesterol is available for exchange (Basford et al., 1964; Quarfordt and Hilderman, 1970). The presence of two classes of cholesterol in erythrocytes also has been suggested on the basis of permeability studies of cells having altered cholesterol content (Grunze and Deuticke, 1974) and from the results of a study of the correlation between erythrocyte cholesterol content and plasma cholesterol content in abnormal erythrocytes (Cooper et al., 1972).

In a recent paper (Gottlieb, 1976) the extent to which erythrocyte cholesterol could be depleted by incubation with plasma of lowered cholesterol content was studied. In these experiments, a maximum of 35% of membrane cholesterol could be removed. This finding was interpreted as indicating that the remaining 65% could not be removed because it was in a distinct, more firmly bound pool.

In this paper, we show that, in fact, all of the exchangeable cholesterol in the human erythrocyte membrane is available for depletion. This conclusion is based on a study of the removal from erythrocytes of cholesterol (mass) and [^{3}H]cholesterol which had been introduced into the membrane by exchange. We demonstrate that depletion and exchange are the same process, describable by a single rate constant. The limitation on cholesterol depletion observed when erythrocytes are incubated with plasma of lowered cholesterol content is because equilibrium between cholesterol in the membrane and in the plasma lipoproteins has been reached.

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