SHORT COMMUNICATIONS

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Cholesterol effect on hydraulic conductivity of red cell membranes

FINKELSTEIN AND CASS[1] have recently shown that the hydraulic conductivity of thin lipid membranes is critically dependent on the amount of free cholesterol in the membrane. The membrane composition was expressed in terms of the ratio by weight of cholesterol to egg lecithin in the membrane-forming solution. FINKELSTEIN AND CASS found that in egg lecithin films the hydraulic conductivity, L_p, fell from $0.314 \cdot 10^{-11}$ cm^3/dyne·sec in the absence of cholesterol to $0.056 \cdot 10^{-11}$ cm^3/dyne·sec at the highest cholesterol to lecithin ratio (8:1). They suggested that their observation might have physiological significance because of the ubiquitous presence of cholesterol in biological membranes. The most striking effect was found in the range of ratios of 0:1 to 1:1. In the human red cell the cholesterol to lecithin ratio is about 0.6:1 on a mg/mg basis[2]. The present study was therefore undertaken to search for a correlation between the hydraulic conductivity of human red cell membrane and its free cholesterol content.

The hydraulic conductivity was measured by the stop-flow method described by SHA'AFI *et al.*[3]. For each separate measurement, L_p was determined by computer averaging and least square fitting of data from at least three runs and three controls. The red cell membrane cholesterol content could be altered by the technique used by MURPHY[4] who showed that incubating the plasma at 37° will result in a decrease in its free cholesterol content due to esterification. Since there is always an equilibrium of the free cholesterol between red cell membrane and the plasma the subsequent incubation of the red cells in previously incubated plasma will lead to a decrease in the free cholesterol content of the membrane. MURPHY's technique was used to

TABLE I

RELATION OF HYDRAULIC CONDUCTIVITY TO RED CELL MEMBRANE CHOLESTEROL CONTENT

	Free cholesterol content in membrane (mg/ml cell)	*Cholesterol content Expt./control*	L_p *(cm^3/dyne·sec* $\times 10^{11}$*)*	L_p *Expt./control*
Fresh cells	1.45 ± 0.02	0.57	0.8 ± 0.1	1.15
Experiment	0.84 ± 0.02		0.9 ± 0.1	
Fresh cells	1.39 ± 0.02	0.71	0.8 ± 0.1	1.30
Experiment	1.00 ± 0.02		1.1 ± 0.1	
Fresh cells	1.51 ± 0.02	1.24	0.9 ± 0.1	1.20
Experiment	1.87 ± 0.02		1.1 ± 0.1	
Average				1.2 ± 0.1

decrease human red cell membrane cholesterol by incubating the cells for 48 h at 37° in their native plasma, which had itself been previously incubated for 24 h at 37°. Conversely, the membrane cholesterol was increased by incubating red cells in their native plasma which had been saturated by cholesterol. No significant hemolysis was observed in the cells which had been incubated. MURPHY also showed that the incubation procedure does not affect the membrane phospholipid content.

In each experiment, the membrane cholesterol and L_p were initially determined in fresh blood. The experimental aliquot was incubated for 48 h at 37° with native plasma which had been pre-treated as described above. The results are given in Table I. As shown in the third column, the cholesterol content varied from 1.24 to 0.57 of the normal cell cholesterol, which is equivalent to a shift of the cholesterol to phospholipid ratio from 0.75:1 to 0.34:1. As can be seen from the fifth column of Table I the plasma incubation increased L_p uniformly by 20%, but there is no evidence that L_p is dependent on the cholesterol content. Over the same range of cholesterol to egg lecithin ratios FINKELSTEIN AND CASS found that L_p changed smoothly from $0.19 \cdot 10^{-11}$ to $0.24 \cdot 10^{-11}$ cm³/dyne·sec. This increase of L_p by about 30% may be contrasted with the evidence in human red cells in which no such change has been demonstrated; L_p appears to be independent of the membrane cholesterol content over the range that we have investigated. Thus it appears that the striking effects observed in lipid bilayers are not reproduced in human red cells. This may reflect the fact that the hydraulic conductance goes by a different path, so that L_p for human red cells need not be dependent on the properties of the membrane lipid. In this connection RICH *et al.*[5] have shown that the hydraulic conductivity of human red cells is controlled by a thin layer on the outer face of the membrane. The fact that the measured values for L_p in the human red cells are greater by a factor of three than those characteristic of the lipid bilayers (as given by FINKELSTEIN AND CASS[1]) is also consonant with this interpretation.

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