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Erythrocyte membranes—effect of increased cholesterol content on permeability

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SUMMARY

The permeability to several non-electrolytes and to Na^+ of guinea pig erythrocytes containing different amounts of cholesterol was measured. A marked decrease of the permeability of the cholesterol-loaded erythrocytes to both hydrophilic and amphiphilic non-electrolytes and to both the active and the passive component of Na^+ efflux was observed. We suggest that cholesterol loading causes a tighter packing of the lipid components which in turn decreases the mobility of the permeants within the membrane matrix.

The cholesterol content increases up to 2-fold in erythrocyte membranes from guinea pigs fed diets supplemented with 1% cholesterol while the phospholipid content and the fatty acid composition of these membranes remains essentially unchanged[1-4]. The increase in cholesterol content is associated with (a) the appearance of prominent abnormal spicules on the surface of the cell, (b) an increase in the internal viscosity of the lipid phases of the membrane, and (c) the eventual decrease in the viability of the erythrocytes (after 8–16 weeks on the diet). We have hypothesized that the abnormal morphology and the decrease in viability of the cholesterol-loaded erythrocytes may be related to permeability changes of the membrane. Several reports have previously shown that increases in the cholesterol content of both natural and synthetic membranes leads to a decrease in the permeability of the membranes[5,6]. However, these reports have dealt with relative cholesterol contents much lower than that present in erythrocytes from cholesterol-fed guinea pigs. We have investigated the permeability of erythrocytes from control and cholesterol-fed guinea pigs to several non-electrolytes and to Na^+. Our results demonstrate a marked decrease in the permeability of the cholesterol-loaded erythrocytes to all permeants investigated.

The cholesterol content of erythrocytes was altered *in vivo* by the addition of 1% cholesterol to the diet of male guinea pigs for 4–6 weeks[2]. *In vitro* alterations of the cholesterol content were accomplished by incubating cells from control animals at 37° for

6 h in plasma from cholesterol-fed guinea pigs (hematocrit = 30%). The cholesterol loading of the cells was reversed *in vivo* by feeding the control diet for 4 weeks following 4–6 weeks feeding of the cholesterol-supplemented diet.

Erythrocytes were isolated from heparinized blood and suspended in an isotonic buffer (pH 7.4, 300 mosM, NaCl 135 mM, KCl 3.5 mM, $MgCl_2$ 1 mM, glucose 4 mM, Na_2HPO_4 7 mM, KH_2PO_4 1.5 mM, penicillin 100 units/ml and streptomycin 100 μg/ml). The lipid content of the cells was determined as previously described[2]. The permeability of erythrocytes to Na^+ was estimated from the efflux of ^{22}Na (New England Nuclear) in 1 h at 37° with and without the addition of 0.1 mM ouabain[7]. The number of Na^+ pumping sites was estimated from the number of binding sites of [^{3}H] ouabain (New England Nuclear) per cell[8]. The relative permeability of the cells to erythritol was estimated from the half-time of equilibration of the cells with [^{14}C] erythritol[9] (Amersham Searle). The relative permeability of thiourea and monoacetin was estimated by determining the time required for 50% hemolysis of a dilute suspension of erythrocytes in isotonic permeant. The hemolysis was followed by measuring the decrease in the turbidity of the suspension at 623 nm as the cells lysed in a temperature controlled cuvette. The hemolysis time (t) is related to the relative permeability (p) according to the relationship: $p_1/p_2 = t_2/t_1$ (ref. 10).

The permeability of the cholesterol-loaded erythrocytes decreased to both hydrophilic non-electrolytes (erythritol and thiourea) and to an amphiphilic non-electrolyte (monoacetin) (Table I). The decrease in permeability was observed for cells loaded with cholesterol both *in vivo* and *in vitro* and was reversible *in vivo.* The cholesterol loading did not affect the activation energy of hemolysis with either thiourea or monoacetin. This suggests that the added cholesterol lowered the permeability without affecting the threshold energy of the permeation process. De Gier *et al.*[11] also found that cholesterol decreased the permeability of mycoplasma and synthetic membranes without affecting the activation energy. These authors suggested that the activation energy of this process is due to the dehydration of non-electrolytes prior to penetrating the membrane. On this basis cholesterol loading of membranes would not be expected to affect the activation energy since cholesterol is located within the membrane matrix while the dehydration process probably takes place at the membrane surface. The rate of penetration of the fully dehydrated molecules through the membrane, however, could be affected by its cholesterol content.

Both the active (ouabain-sensitive) and the passive (ouabain-insensitive) components of Na^+ efflux decreased in the cholesterol-loaded erythrocytes (Table II). However, the binding of [^{3}H] ouabain to the cells was approximately the same. These results suggest that the decrease in Na^+ permeability of the cholesterol-loaded cells was not due to a decrease in the number of Na^+ pump sites. Fox[12] has found a similar effect of lipids on the lactose transport system of *E. coli.* The kinetics of the transport was dependent on the fatty acid composition of the membranes, which could be altered by the fatty acid content of the growth media. Thus, some transport systems in membranes appear to be located in the lipid phases of the membranes.

It is remarkable that the increase in the content of cholesterol in guinea pig erythrocytes affects the movement of several permeants with such widely differing physical and chemical characteristics as erythritol, thiourea, monoacetin and ionic sodium.

TABLE I

EFFECTS OF CHOLESTEROL CONTENT ON PERMEABILITY OF ERYTHROCYTES TO NON-ELECTROLYTES

$t_{1/2}$ for erythritol refers to the half-time of equilibration of [^{14}C] erythritol between cells and supernatant at 37°; $t_{1/2}$ for thiourea and monoacetin refers to half-time of hemolysis of cells in isotonic thiourea and monoacetin at 22°. The energy of activation, E_a, for the hemolysis times were calculated from Arrhenius plots. Means ± S.E. from 3 animals per diet are shown*.

*Erythrocytes***	*Cholesterol content (mg/100 ml cells) (number of animals)*	*Erythritol $t_{1/2}$ (min)*	*Thiourea*		*Monoacetin*	
			$t_{1/2}$ (sec)	*E_a (kcal/mole)*	*$t_{1/2}$ (sec)*	*E_a (kcal/mole)*
Control	122 (5)	13.2 ± 0.5	84 ± 3.0	11.3 ± 0.9	11.8 ± 0.6	12.9 ± 0.6
Control in cholesterol-fed plasma	168 (5)	–	103 ± 1.6	–	14.6 ± 0.1	–
Cholesterol-fed	186 (7)	27.2 ± 1.5	100 ± 4.7	11.8 ± 1.0	14.5 ± 0.6	12.1 ± 0.7
Cholesterol-fed*** returned to control	131 (7)	–	86 ± 2.7	–	12.1 ± 0.5	–

*The hemolysis times and cholesterol content of cells from control animals incubated in control plasma (not shown) were the same as those of non-incubated cells. **Control and cholesterol-fed erythrocytes (plasma) refers to erythrocytes (plasma) from guinea pigs fed a control or a 1% cholesterol-containing diet, respectively, for 4–6 weeks. ***Erythrocytes from guinea pigs returned to the control diet for 4 weeks following 4–6 weeks feeding of the cholesterol-containing diet.

TABLE II

EFFECTS OF CHOLESTEROL CONTENT ON PERMEABILITY OF ERYTHROCYTES TO Na^+

Means ± S.E. were calculated from erythrocytes from 3 animals per diet.

*Erythrocytes**	*Na^+ efflux (mequiv/l cells per h)*		*[^{3}H] Ouabain binding (molecules/cell) (number of animals)*
	– Ouabain	*+ Ouabain*	
Control	2.0 ± 0.07	1.1 ± 0.05	146 (4)
Cholesterol-fed	1.3 ± 0.10	0.8 ± 0.05	153 (3)

*Control and cholesterol-fed erythrocytes (plasma) refers to erythrocytes (plasma) from guinea pigs fed a control or a 1% cholesterol-containing diet, respectively, for 4–6 weeks.

Cholesterol loading must be affecting some general characteristic of the membrane matrix. We have recently shown by the use of spectral method that cholesterol loading increases the local viscosity of the lipid phases of the membranes without any apparent effect on their protein phases[4]. Both the spectral and the permeability results are consistent with the hypothesis that the primary effect of an increase in the cholesterol content of erythrocytes is an increase in the packing of lipid components in the membrane. This would lead to a decrease in the mobility of several permeants within the membrane matrix. Further work is needed to try to relate these changes to the changes in viability and morphology observed in the erythrocytes from cholesterol-fed guinea pigs.

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REFERENCES

1 R. Okey and V. Greaves, *J. Biol. Chem.*, 129 (1939) 111.
2 R. Ostwald and A. Shannon, *Biochem. J.*, 91 (1964) 146.
3 R. Ostwald, W. Yamanaka, M. Light and J. Kroes, *8th Int. Congr. Nutr. Prague, 1969*, Abstr. 0–14.
4 J. Kroes, A. Keith and R. Ostwald, submitted for publication.
5 R.N. McElhaney, J. De Gier and L.L.M. Van Deenen, *Biochim. Biophys. Acta*, 219 (1970) 245.
6 K.R. Burckdorfer, R.A. Demel, J. De Gier and L.L.M. Van Deenen, *Biochim. Biophys. Acta*, 183 (1969) 334..
7 O. Rettori, V. Rettori, J.V. Maloney and M.F. Villamil, *Am. J. Physiol.*, 217 (1969) 605.
8 P.F. Baker and J.S. Willis, *Biochim. Biophys. Acta*, 183 (1969) 646.
9 G. Gardos, J.F. Hoffman and H. Passow, in H. Passow and R. Stamfli, *Laboratory Techniques in Membrane Biophysics*, Springer Verlag, New York, 1969, p. 9.
10 R. Whittam, *Transport and Diffusion in Red Blood Cells*, Williams and Wilkins Co., Baltimore, Md., 1964, p. 154.
11 J. De Gier, J.G. Mandersloot, J.V. Hupkes, R.N. McElhaney and W.P. Van Beek, *Biochim. Biophys. Acta*, 233 (1971) 610.
12 C.F. Fox, *Fed. Proc.*, 30 (1971) 1032.