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EFFECT OF MEMBRANE CHOLESTEROL ENRICHMENT OR DEPLETION ON THE PARTITION BEHAVIOR OF HUMAN ERYTHROCYTES IN DEXTRAN-POLY(ETHYLENE GLYCOL) AQUEOUS PHASES

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Summary

It has previously been shown that by appropriate manipulation of polymer concentrations and ionic composition and concentration one can select whether charge-associated or lipid-related membrane surface properties are reflected by cell partition in dextran-poly(ethylene glycol) aqueous two-phase systems (Walter, H. (1977) in Methods of Cell Separation ((Catsimpoolas, N., ed.), Vol. 1, pp. 307—354, Plenum Press, New York). In the current experiments we have studied the partition behavior of human erythrocytes and found that not only lipid-related but also charge-associated membrane properties are altered as a consequence of cholesterol-enrichment or -depletion. Results further indicate that, just as cell partition in charged phase systems reflects membrane charge-associated properties not readily measured by means other than partition (Brooks, D.E., Seaman, G.V.F. and Walter, H. (1971) Nat. New Biol. 234, 61—62; Walter, H., Tung, R., Jackson, L.J. and Seaman, G.V.F. (1972) Biochem. Biophys. Res. Commun. 48, 565—571), cell partition in uncharged phases reflects membrane lipid-related properties also not readily measured by other means.

Aqueous solutions of dextran and of poly(ethylene glycol), when mixed above certain concentrations form liquid, immiscible two-phase systems, with a poly(ethylene glycol)-rich top and a dextran-rich bottom, suitable for the partition of cells [1,2]. Depending on the polymer concentrations and on the ionic composition and concentration such phase systems have greatly differing physical properties [2] which interact with the membrane surface of added cells and yield partition coefficients that reflect the cells' membrane charge-

associated or lipid-related properties [2]. In the present communication we examine the effect of cholesterol enrichment or depletion of human erythrocytes on their partition coefficient in two phase systems, one of which reflects charge-associated and the other lipid-related membrane surface properties.

Even though both dextran and poly(ethylene glycol) are non-ionic polymers, some salts (e.g., phosphate, sulfate) have unequal affinities for the two phases [3], a phenomenon which gives rise to an electrostatic potential difference between them [4]. That is, the phases are actually charged with respect to each other (in the cases of phosphate or sulfate the top phase is positive [4,5]). The membrane charge of cells added to such systems will interact with the phase charge and their partition coefficients will, hence, be charge associated [2,6].

If a salt is used which has essentially equal affinity for the two phases (e.g. NaCl) there is no electrostatic potential difference and cells added to such a system will tend to be adsorbed at the interface (i.e., they will not partition). When, under these circumstances, the polymer concentrations are reduced, resulting in a concomitant reduction in interfacial tension [7], the interaction between poly(ethylene glycol) and the cell surface will be adequate to 'pull' cells out of the interface and into the top, poly(ethylene glycol)-rich, phase [1,5]. The partition coefficient of cells in an uncharged phase system near the critical point (i.e., low polymer concentrations) correlates extremely well, at least in the case of erythrocytes from different species, with the ratio of their membrane poly/monounsaturated fatty acids [5]. The probable basis for this correlation is that with higher ratios the lipid packing is less efficient [8] and poly(ethylene glycol) may be better able to intercalate with the membrane surface, changing the surface to one which is more 'poly(ethylene glycol)-like' and causing the cells to partition in favor of the upper phase [5].

Lipid dispersions to dipalmitoyl phosphatidylcholine (Sigma Chem. Co., St. Louis) with or without cholesterol (Sigma) were prepared according to Cooper et al. [9] with the exception that the sonication was carried out at 45°C for 39 min (as suggested by Cooper, private communication). Freshly obtained human erythrocytes, washed thrice in Hanks' balanced salt solution, (Grand Island Biological Co., Grand Island, N.Y.) were incubated for 16 h at 37°C in a 1 : 1 mixture of the lipid dispersion in heat-inactivated serum (from the same individual from whom the erythrocytes had been obtained) and Hank's balanced salt solution containing 100 units penicillin per ml (all in accordance with ref. 9). Adenosine (Sigma) (10 mM) was added to the cell suspensions 1 h before the end of the incubation. Cells were washed 3 times with balanced salt solution and aliquots were then analyzed for lipid or used in the countercurrent distribution experiments described below.

Total lipids in erythrocyte ghosts were extracted as described by Ways and Hanahan [10]. Aliquots of these extracts were analyzed for free cholesterol content by gas chromatography as described by Morin and Elms [11]. Phospholipids were quantitated by the colorimetric method of Bartlett [12]. Total red cell lipids were hydrolyzed and methylated in BF_3-methanol, and their fatty acid composition determined by gas chromatography in a Varian model 2700 gas chromatograph using flame ionization and a 10% EGGS-X column at 180°C.

TABLE I

CHOLESTEROL AND PHOSPHOLIPID QUANTITIES IN NORMAL, CHOLESTEROL-ENRICHED AND CHOLESTEROL-DEPLETED HUMAN ERYTHROCYTES

Data are given as $\mu g/10^8$ cells plus or minus the standard deviation with the number of experiments in parentheses.

Human erythrocytes	Cholesterol	Phospholipid
Normal	11.1 ± 0.6 (11)	28.6 ± 1.2 (14)
Enriched	25.9 ± 1.8 (4)	27.6 ± 0.6 (3)
Depleted	3.0 ± 0.4 (7)	28.1 ± 1.0 (7)

Two different phase systems were used. Phase system 1 reflects membrane charge-associated properties and is composed of 5% (w/w) dextran T500, lot no. 3936 (Pharmacia Fine Chemicals, Piscataway, N.J.), 4% (w/w) PEG 6000 (Union Carbide), and 0.11 M sodium-phosphate buffer, pH 6.8. Phase system 2 reflects membrane lipid-related properties and consists of 4.85% (w/w) dextran, 3.3% (w/w) poly(ethylene glycol), 0.15 M NaCl and 0.01 M sodium-phosphate buffer, pH 6.8. Countercurrent distribution of untreated, cholesterol-enriched or cholesterol-depleted erythrocytes was carried out on a thin-layer countercurrent distribution apparatus as described previously [2] using a shaking time of 25 s and a settling time of 7 min for phase system 1, and 8 min for phase system 2. Sixty transfers were completed at 4—5°C.

Table I shows the quantities of cholesterol and phospholipids in normal, cholesterol-enriched and cholesterol-depleted human erythrocyte membranes. Incubation of red cells in lipid dispersions containing phospholipid and cholesterol results in cholesterol-enrichment of the membrane; incubation of cells with disperions of phosphatidylcholine alone results in cholesterol depletion [9,13]. No changes in membrane phospholipid occur during the incubations, and no obvious alterations in fatty acid compositions as a consequence of the incubations were noticed. These results are in accord with previous findings [13] and are presented solely to show the cholesterol-phospholipid content of erythrocytes used in the countercurrent distribution experiments.

In Fig. 1 we show results obtained using phase system 1. Cholesterol enrichment reduces the partition coefficient of human erythrocytes (Fig. 1A) while cholesterol depletion increases the partition coefficient (Fig. 1B). Since the partition behavior in this phase system depends on charge-associated membrane properties [2,6] it appears that cholesterol enrichment and depletion have marked effects on the membrane charge or charge-associated properties available for interaction with phase charge. The basis for the altered partition coefficients in the charged phases is not known. Red cell area changes accompanying cholesterol enrichment or depletion [9] may cause the observed changes in partition behavior. The increased partition coefficient observed in cholesterol-depleted erythrocytes may also be analogous to the previously found increase in partition coefficient of aldehyde-fixed, lipid-extracted red cells [14]. In the latter case the great increase in partition coefficient is not reflected by an increase in electrophoretic mobility [14], again demonstrating that the charge measured by partition and by cell electrophoresis need not be the same [15].

Fig. 2 shows the distribution patterns in phase system 2. Both cholesterol

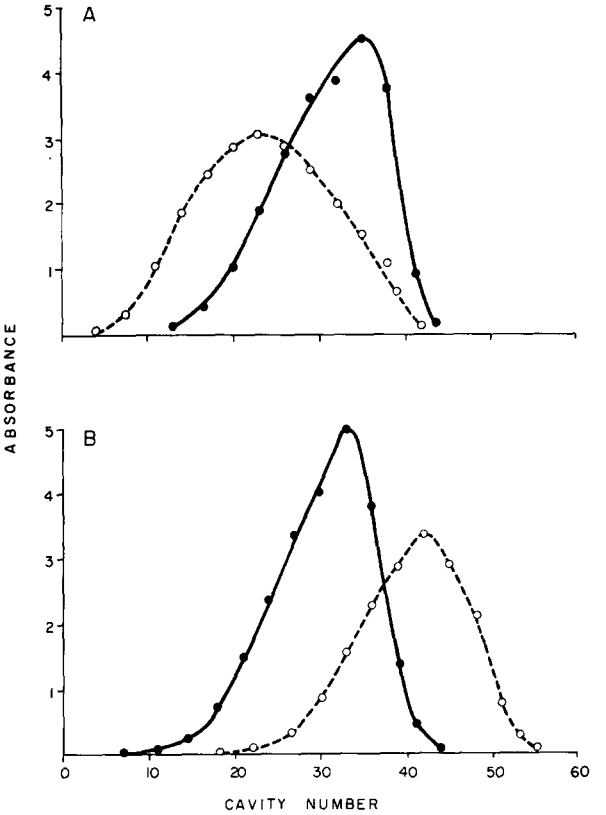

Fig. 1. Superimposed countercurrent distribution curves of human normal erythrocytes and cholesterol-enriched (A) or cholesterol-depleted (B) red cells from the same individual. •———•, normal red cells; ○———○, cholesterol-enriched (A) or -depleted (B) erythrocytes. Phase system contained 5% (w/w) dextran, 4% (w/w) poly(ethylene glycol) and 0.11 M sodium-phosphate buffer, pH 6.8, and measures surface-charge associated membrane properties. 60 transfers were completed at 4—5°C. For additional details see text.

enrichment and depletion increase the partition coefficient of human erythrocytes, as evidenced by displacement of the distribution curves to the right (Fig. 2, A and B). The partition behavior of erythrocytes from different species in an uncharged phase system near the critical point (i.e., low polymer concentrations). correlates very well with the ratio of their membrane poly-/mono-unsaturated fatty acids [5]. Correlations of increasing partition coefficient with increasing quantities of membrane phosphatidylcholine and decreasing quantities of sphingomyelin have also been found [5]. Since there is no appreciable change in phospholipid or fatty acid composition as a consequence of the cholesterol-enrichment or -depletion procedures, the alteration in distribution patterns must be due to the interaction of cholesterol with other membrane components. If one considers that the extent of interaction of poly(ethylene glycol) with the membrane surface depends on the degree of lipid packing [5], then the presence of cholesterol appears to decrease the efficiency of packing and permits greater surface interaction with poly(ethylene glycol) resulting in the observed increase in partition coefficient. Using various probes others have reported an increase in microviscosity (i.e., rigidity) of cholesterol-

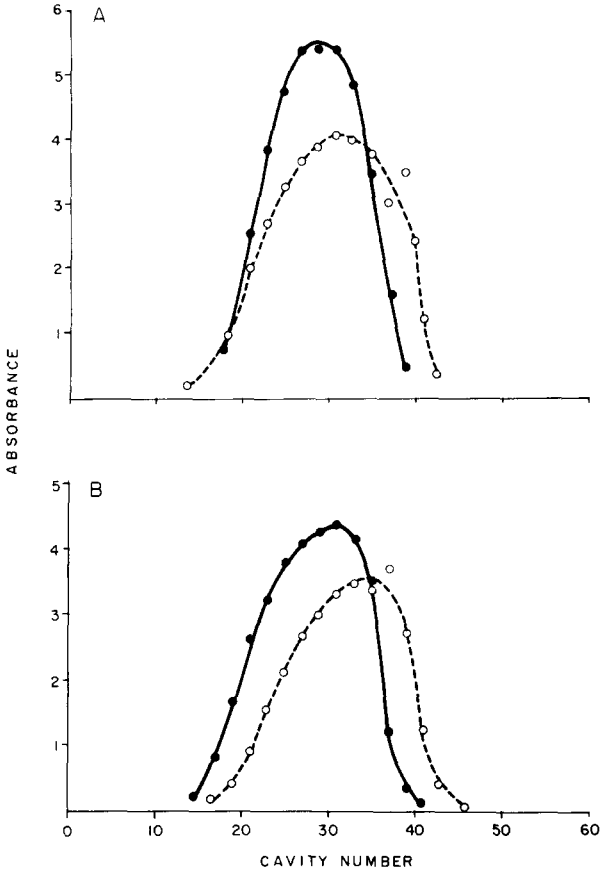

Fig. 2. Superimposed countercurrent distribution curves with symbols as in Fig. 1. Phase system contained 4.85% (w/w) dextran, 3.3% (w/w) poly(ethylene glycol), 0.15 M NaCl and 0.01 M sodium-phosphate buffer, pH 6.8, and reflects lipid-related membrane properties. See text for additional details.

enriched membranes [13,16–18]. Our results show that, at the membrane stratum at which the phases measure lipid-related properties of human erythrocytes, cholesterol enrichment causes increased interaction between cell surface and poly(ethylene glycol).

In contrast to the partition behavior of human erythrocytes cholesterol-enrichment of rat red cells causes them to have a decreased partition coefficient in an uncharged phase system reflecting species differences (see also ref. 19). These data also suggest caution before making general statements on cholesterol-membrane interactions based on results with one given cell type or population.

The results depicted in Figs. 1 and 2 in which cholesterol-enriched or -depleted human erythrocytes are compared to untreated erythrocytes from the same individual are highly reproducible. We have also examined, in a similar manner, the countercurrent distribution patterns of erythrocytes from some chronic alcoholic patients whose red cells have been reported to be cholesterol enriched [20], and compared these to the behavior of human normal red cells.

TABLE II

APPARENT PARTITION COEFFICIENT, G *, OF HUMAN ERTHROCYTES FROM CHRONIC ALCOHOLIC PATIENTS AND HEMATOLOGICALLY NORMAL INDIVIDUALS IN CHARGED ** (SYSTEM 1) AND UNCHARGED ** (SYSTEM 2) AQUEOUS PHASES

For details see text.

	Phase system	
	1	2
Normal	1.21 ± 0.15 (21) ***	1.08 ± 0.34 (22)
Chronic alcoholic patient	0.84 ± 0.17 (12)	1.32 ± 0.33 (15)

* G, the apparent partition coefficient, is obtained according to the following equation from the counter-current distribution curves: $G = r_{max}/(n - r_{max})$, where r_{max} is the number of the peak cavity of the distribution curve and n is the total number of transfers.

** Phase system 1 was composed of 5% (w/w) dextran, 4% (w/w) poly(ethylene glycol) and 0.11 M sodium-phosphate buffer, pH 6.8; phase system 2 contained 4.85% (w/w) dextran, 3.3% (w/w) poly(ethylene glycol), 0.15 M NaCl and 0.01 M sodium-phosphate buffer, pH 6.8. Counter-current distributions on which G values are based were run at 4—5°C.

*** Data are given as G values plus or minus the standard deviation with the number of experiments in parentheses.

Some tendency was found for erythrocytes from patients to have a lower partition in charged and a higher partition coefficient in uncharged phase systems than did normal erythrocytes (Table II). However, the range of partition coefficients obtained both for normal individuals and for chronic alcoholics in the uncharged phase system is so broad (Table II) that overlap between the two groups is appreciable. This variability (from individual to individual) may be a consequence of differences in membrane components in addition to cholesterol (e.g., variable phosphatidylcholine content).

In summary, we have found that both cholesterol enrichment and depletion of human erythrocytes affect the cells' partition behavior in charged as well as uncharged two-polymer aqueous phases. Cholesterol enrichment appears to decrease membrane charge-associated properties and increase membrane interaction with poly(ethylene glycol) in an uncharged phase system. Depletion results in increased charge-associated properties and also an increased membrane interaction with poly(ethylene glycol). It appears that just as cell partition in charged phase systems reflects membrane charge-associated properties not readily measured by means other than partition [6,15], cell behavior in uncharged phase systems reflects membrane lipid-related properties also not readily measured by other means.

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References

1 Albertsson, P.Å. and Baird, G.D. (1962) Exp. Cell Res. 28, 296–322
2 Walter, H. (1977) in Methods of Cell Separation (Catsimpoolas, N., ed.), Vol. 1, pp. 307–354, Plenum Press, New York
3 Johansson, G. (1970) Biochim. Biophys. Acta 221, 387–390
4 Reitherman, R., Flanagan, S.D. and Barondes, S.H. (1973) Biochim. Biophys. Acta 297, 193–202
5 Walter, H., Krob, E.J. and Brooks, D.E. (1976) Biochemistry 15, 2959–2964
6 Brooks, D.E., Seaman, G.V.F. and Walter, H. (1971) Nat. New Biol. 234, 61–62
7 Ryden, J. and Albertsson, P.Å. (1971) J. Colloid Interface Sci. 37, 219–222
8 van Deenen, L.L.M. and de Gier, J. (1974) in The Red Blood Cell (Surgenor, D.MacN., ed.), pp. 147–211, 2nd edn., Academic Press, New York
9 Cooper, R.A., Arner, E.C., Wiley, J.S. and Shattil, S.J. (1975) J. Clin. Invest. 55, 115–126
10 Ways, P. and Hanahan, D.J. (1964) J. Lipid Res. 5, 318–328
11 Morin, R.J. and Elms, N.J. (1975) Ann. Clin. Lab. Sci. 5, 52–56
12 Bartlett, G.R. (1969) J. Biol. Chem. 234, 468–471
13 Cooper, R.A., Leslie, M.H., Fischkoff, S., Shinitzky, M. and Shattil, S.J. (1978) Biochemistry 17, 327–331
14 Walter, H., Krob, E.J., Brooks, D.E. and Seaman, G.V.F. (1973) Exp. Cell Res. 80, 415–424
15 Walter, H., Tung, R., Jackson, L.J. and Seaman, G.V.F. (1972) Biochem. Biophys. Res. Commun. 48, 565–571
16 Chapman, D. (1973) in Biological Membranes (Chapman, D. and Wallach, D.F.H., eds.), Vol. 2, pp. 91–144, Academic Press, New York
17 Kroes, J., Ostwald, R. and Keith, A. (1972) Biochim. Biophys. Acta 274, 71–74
18 Vanderkooi, J., Fischkoff, S., Chance, B. and Cooper, R.A. (1974) Biochemistry 13, 1589–1595
19 Renooij, W. and Van Golde, L.M.G. (1977) Biochim. Biophys. Acta 470, 465–474
20 Cooper, R.A. (1977) New Engl. J. Med. 297, 371–377