Biochimica et Biophysica Acta, 596 (1980) 445—450
© Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71434

PARTITION OF RAT ERYTHROCYTES IN AQUEOUS POLYMER TWO-PHASE SYSTEMS

FRANK D. RAYMOND and DEREK FISHER*

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London, 8 Hunter Street, London WC1N 1BP (U.K.)

(Received September 11th, 1979)

Key words: Erythrocyte; Partition; Poly(ethylene glycol)

Summary

The partition of rat erythrocytes between the top phase and interface of aqueous poly(ethylene glycol)-dextran two-phase systems containing 0.15 M NaCl and 0.01 M sodium phosphate depends on the association of the cells with microscopic globules of dextran that persist in the poly(ethylene glycol)-rich top phase after the horizontal interface between the two phases has formed.

When aqueous solutions of poly(ethylene glycol) and of dextran are mixed above certain critical concentrations, two immiscible liquid phases are obtained; a poly(ethylene glycol)-rich top phase and a dextran-rich bottom phase [1]. Cells partition between the two phases and the horizontal interface between them [1]. The partitioning of cells can be affected by the concentration of the polymers, the pH of the buffer in which the polymers are dissolved and the presence of certain salts in the buffer (for review, see Ref. 2). Salts that partition unevenly between the phases e.g., phosphate, produce an electrical potential between the phases [3] (charged phase system) and the partition of cells is determined predominantly by the cell surface charge [2].

In contrast, in phases containing ions that distribute evenly between the phases e.g., NaCl, a negligible potential exists (non-charged phase systems) and cells from most sources collect at the interface [4]. However, for phases close to the critical point, the concentration of components below which phase separation does not occur, partition of cells into the top phase has been

*To whom correspondence should be addressed.

observed [4]. It has been suggested that factors other than membrane surface charge must be involved in cell partition under these circumstances and in particular membrane lipid composition appears to be important. Of the erythrocytes studied [4], rat erythrocytes showed the highest partition coefficient and in addition showed a sizeable partition coefficient in a phase system away from the critical point.

We have examined the partition behaviour of rat erythrocytes in phases without potential differences (non-charged phases) that are both close to and at some distance from the critical point. Our results stress the time-dependency of the values of partition obtained in these phases and indicate that the predominant interaction determining the partition of cells into the top phase is between the cells and microscopic globules of dextran that persist in the poly(ethylene glycol)-rich top phase for some considerable time after the main horizontal interface between the two phases has formed, rather than a specific interaction of the cells with poly(ethylene glycol), the major polymer present in the top phase.

Rat erythrocytes, collected into a citrate anticoagulant solution [5] from the severed carotid artery, were washed three times with 0.15 M NaCl and once with 0.15 M NaCl/0.01 M sodium phosphate buffer, pH 6.8. Dextran T500 (Lot Nos. 9863 and 4094) was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Poly(ethylene glycol) 6000 was obtained from B.D.H. Ltd., Poole, U.K. All other reagents were of analytical grade.

Phases were prepared from stock solutions of 20% (w/w) Dextran T500, standardised by polarimetry and of 20% (w/w) poly(ethylene glycol) 6000 in 0.15 M NaCl containing 0.01 M sodium phosphate buffer, pH 6.8, by mixing known weights of these solutions with the saline buffer and centrifuging the mixture for 1 h at 2000 × *g* to separate the layers. Phase systems were prepared in 5 × 1 cm glass tubes by mixing 1 g each of top and bottom phases (2 g phase system). Phase systems were prepared containing 5% (w/w) dextran and 3.5% (w/w) poly(ethylene glycol); 5% (w/w) dextran and 4% (w/w) poly(ethylene glycol); 5% (w/w) dextran and 4.5% (w/w) poly(ethylene glycol); 5% (w/w) dextran and 5% (w/w) poly(ethylene glycol).

The partition of rat erythrocytes was measured by adding 10 μl of packed cells to the system, which was then well shaken. A portion (50 μl) was immediately removed from each tube and the total number of cells present measured after suitable dilution with Isoton (Coulter Electronics Ltd., Harpenden, Herts., U.K.) in a Coulter Electronic Particle Counter Model F (Coulter Electronics Ltd., Harpenden, Herts., U.K.). After the tubes had stood for various times at room temperature (20°C), duplicate samples (50 μl) were removed from the top and bottom phases and the number of cells present measured. The number of cells present at the horizontal interface was obtained as the difference between the number of cells added to the system and the number present in both phases. The number of cells present in the top and bottom phases and at the interface was expressed as a percentage of the cells added.

To measure the concentration of dextran in the top phase at the same time as the partition was measured, phase systems of 5 g of top phase and 5 g of bottom phase (10 g system) were prepared. After cells had been added

and the system mixed, 4 ml of the top phase was removed, diluted with 6 ml of isotonic saline to break the phase system and centrifuged to sediment the cells. The concentration of dextran present in the supernatant was measured polarimetrically after suitable dilution.

Samples of the phase system were viewed on glass slides with a phase contrast microscope (Carl Zeiss, Degenhardt and Co. Ltd., London, U.K.) or were viewed directly in the tubes held vertically in a Vickers M26 inverted microscope arranged horizontally.

The partition of rat erythrocytes in the non-charged phase systems studied (Fig. 1) was predominantly between the top phase and the horizontal interface between the two phases. However, the partition was very time-dependent and although considerable partition into the top phase was observed for some time after mixing, the final position of the cells appeared to be at the horizontal interface. Thus, the behaviour of the cells in the different phases differed essentially only in the speed with which this final position was obtained.

Examination by phase contrast microscopy of samples of the top phase of cells partitioned in a 5% dextran:4% poly(ethylene glycol) system showed that almost all of the cells present in the top phase were associated with the outer surface of globules of the bottom phase (dextran) that persisted in the top phase for some time after the horizontal interface had formed (Fig. 2). When freshly-mixed phase systems were examined directly in glass tubes, the kinetics of cell partition could be followed. Rat erythrocytes appeared to associate immediately with the polymer interfaces formed on mixing the phases. The globules with cells attached were seen moving downwards until they fused with the horizontal interface. Their contents (dextran) were discharged into the bottom phase but the cells remained associated with the

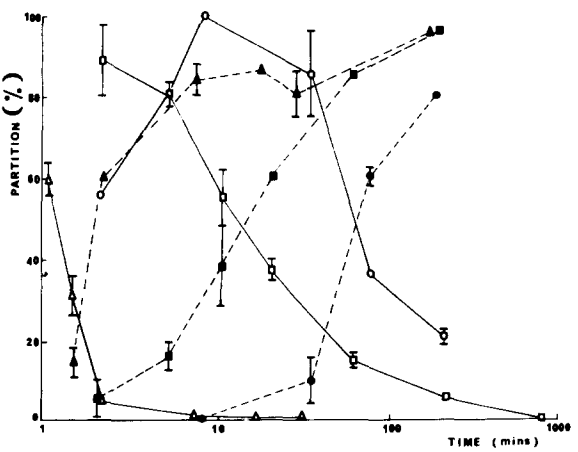

Fig. 1. The variation of the partition of rat erythrocytes with time. The partition of rat cells between the top phase (○—○, □—□, △—△) and horizontal interface (●- - -●, ■- - -■, ▲- - -▲) in three different phase systems; 5% dextran:3.5% poly(ethylene glycol) (○, ●), 5% dextran: 4% poly(ethylene glycol) (□, ■) and 5% dextran:5% poly(ethylene glycol) (△, ▲) was measured as described in the text at intervals following the addition of cells to the phase systems and mixing. Each point represents the mean (± S.D.) of three separate partitions, each sampled and counted in duplicate. There was little partition into the lower phase (< 10%) and these results are not shown.

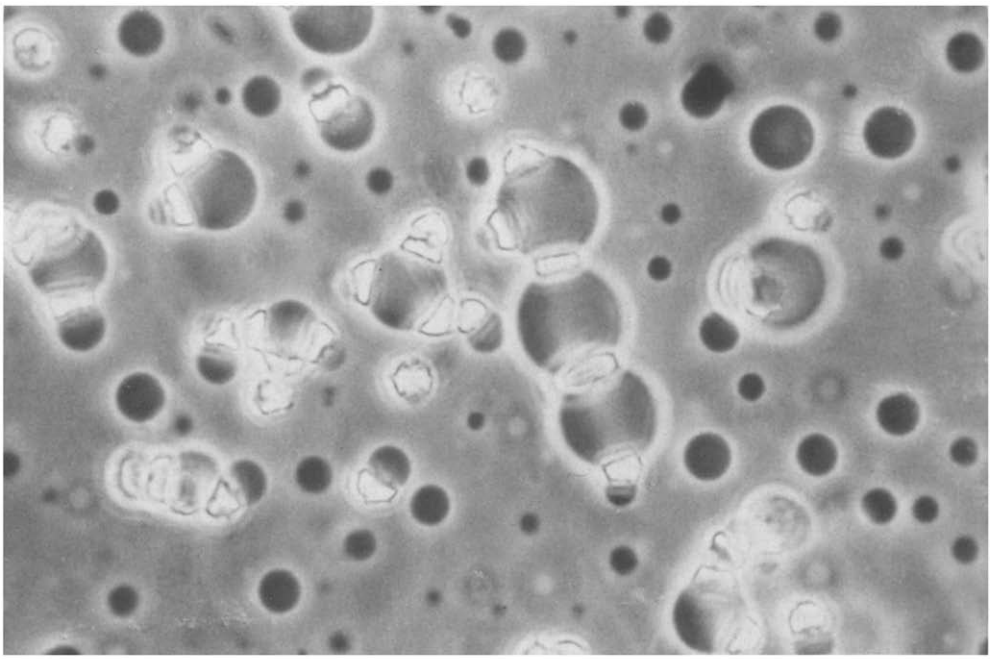

Fig. 2. Light micrograph (phase contrast × 800) of rat erythrocytes attached to outer surfaces of dextran globules present in the top phase of a 5% dextran:4% poly(ethylene glycol) system.

horizontal interface, where they formed a body of agglutinated cells. In phase systems closer to the critical point (5% dextran:3.5% poly(ethylene glycol)) or further from the critical point (5% dextran:5% poly(ethylene glycol)) similar observations were made, although the cell-loaded globules reached the horizontal interface more slowly in the 5% dextran:3.5% poly(ethylene glycol) system and faster in the 5% dextran:5% poly(ethylene glycol) system than in the 5% dextran:4% poly(ethylene glycol) system.

Although complex interfaces and globules of poly(ethylene glycol) were observed in the bottom phase, relatively few cells (< 10% of the total) associated with these structures and showed partition into the bottom phase.

These observations demonstrate that in these non-charged phase systems cells are predominantly attached to the interfaces between solutions of the two polymers. Initially, the cells adhered to the polymer interfaces formed at the surface of the dextran globules present in the developing top phase, which can be considered as microphases, and were carried on these globules to the horizontal interface, their final position. That globules were the vehicle by which cells reached the horizontal interface received additional support from the following observations. Firstly, cells added to top phase, prepared free of dextran globules by centrifugation, sedimented considerably more slowly than cells in the top phase of the mixed phase system sedimented to the horizontal interface. For example, in a 5% dextran:5% poly(ethylene glycol) phase, the proportion of cells (mean ± S.D.) remaining unsedimented after 30 min in the isolated top phase was 93.1% ± 4.7 in 4 separate partitions, whereas in the mixed phase system only 2.3% ± 0.5 remained in the

top phase and had not sedimented to the interface. Secondly, a quantitative measure of the removal of the dextran from the top phase as the phases separated was obtained from polarimetric measurements of the dextran content (Fig. 3). In the presence of rat erythrocytes, dextran was cleared more rapidly than in the cell-free system, indicating that the erythrocyte-loaded globules sedimented more rapidly than unloaded globules. Furthermore, the time-dependencies of the clearing of dextran and of the partition of cells were similar and showed a strong correlation ($r = 0.99$, $n = 5$), consistent with an association of the cells with the dextran globules.

These microscopic observations afford an explanation of the variation of partition with time and polymer concentration as shown in Fig. 1. Clearly, the rate at which cell partition into the top phase falls with time is determined by the rate at which cells reach the horizontal interface, which will depend on the rate of clearance of dextran from the top phase,i.e., the rate of phase separation. Since phase separation occurs more rapidly for phases that are further from the critical point [1], the partition into the top phase will fall more rapidly as the composition becomes more distant from the critical point in the order 5% dextran:5% poly(ethylene glycol) > 5% dextran:4%

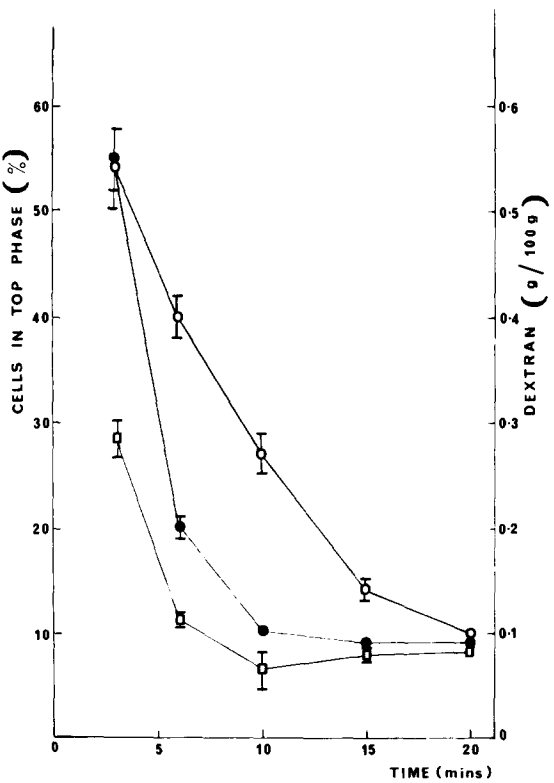

Fig. 3. The clearance of rat erythrocytes and dextran from the top phase. Rat erythrocytes were partitioned in 5% dextran:4.5% poly(ethylene glycol) system (10 g). The distribution of erythrocytes in the top phase (□) and the dextran content of the top phase in the presence (●) and absence (○) of cells were measured at intervals after the addition of cells and mixing of the phases. Each point represents the mean (± S.D.) of 7—9 separate partitions, each sampled and counted in duplicate.

poly(ethylene glycol) > 5% dextran:3.5% poly(ethylene glycol), as shown in Fig. 1. The marked time-dependency of the partition values that can occur in these phase systems emphasises the necessity for strict control of experimental conditions when comparative studies are being performed.

We consider that the interactions of cells with polymer microphases are very important in understanding the behaviour of cells in these phase systems. Thus, although human, pig and chicken erythrocytes showed associations with the microphases that were qualitatively similar to those described in the present study for rat erythrocytes, they showed quantitative differences in the kinetics of cell partition and dextran clearance (Raymond and Fisher, unpublished results) that gave rise to the species-dependent differences in partition previously reported [4]. In addition, the ability of small quantities (< 0.0001%, w/w) of poly(ethylene glycol)-palmitate to increase the partition of cells into the top phase [6] appears to result from its decreasing the association of cells with the dextran globules [7], causing the cells to reach the horizontal interface more slowly. Furthermore, when the NaCl is replaced by phosphate, changing the non-charged phase system to a charged phase system, we have observed that the interactions of cells with the polymer interfaces are decreased, so that partition was less dependent on the degree of phase separation.

Finally, our results indicate that the presence of cells in the top phase of the phase systems studied does not necessarily imply that the cells are present as single cells in a homogenous solution. Moreover, our observations that cells can show significant partition into the top phase yet be predominantly associated with polymer interfaces, suggests a mechanism for phase partition that should be considered in addition to, or possibly as an alternative to, the suggestion [4] that the partition of rat erythrocytes into the top phase of these non-charged phase systems arises from the interactions of the cell membranes with poly(ethylene glycol), the polymer predominant in the top phase.

We thank Professor J.A. Lucy, Dr. Harry Walter, Dr. Donald Brooks and Dr. Ian Sutherland for their advice and interest. This research was supported in part by a grant from the Peter Samuel Royal Free Fund to D.F. F.D.R. is a Junior Research Fellow of the Royal Free Hospital School of Medicine.

References

1 Albertsson, P.A. (1971) Partition of Cell Particles and Macromolecules, Wiley-Interscience, New York, NY
2 Walter, H. (1977) in Methods in Cell Separation (Catsimpoolas, N., ed.), Vol. 1, pp. 307—354, Plenum Publishing Corporation, New York, NY
3 Reitherman, R., Flanagan, S.D. and Barondes, S.H. (1973) Biochim. Biophys. Acta 297, 193—202
4 Walter, H., Krob, E.J. and Brooks, D.E. (1976) Biochemistry 15, 2959—2964
5 De Gowin, E.L., Hardin, R.C. and Alsever, J.B. (1949) Blood Transfusion, p 330, Saunders, Philadelphia, PA
6 Eriksson, E., Albertsson, P.A. and Johansson, G. (1976) Mol. Cell Biochem. 10, 123—128
7 Raymond, F.D. and Fisher, D. (1980) Biochem. Soc. Trans., 8, 118—119