# PARTITION BEHAVIOR OF CELLS AND SOLUBLE SUBSTANCES IN TWO-POLYMER AQUEOUS PHASE SYSTEMS

## Comments on Zaslavsky's general rule

Harry WALTER and Janet L. ANDERSON

*Laboratory of Chemical Biology, Veterans Administration Medical Center, Long Beach, CA 90822* and *Department of Physiology and Biophysics, College of Medicine, University of California, Irvine, CA 92717, USA*

Received 24 June 1981

### 1. Introduction

Partitioning of cells (and membranes) in two-polymer aqueous phase systems depends, in an extremely sensitive manner, on the cells' surface properties and on the phase system's physical properties with which the cells interact [1,2]. The physical properties of the phase systems can, to a great extent, be manipulated by the choice of polymer and ionic composition and concentration [1,3]. In the case of dextran–poly(ethylene glycol) aqueous phases it has been found that by appropriate selection of phase composition one can effect separations based predominantly on charge-associated or lipid-related properties or on biospecific affinity [4–6].

A number of papers has appeared by the same group [7–11] claiming to have found a 'General rule of partition behaviour of cells and soluble substances in aqueous two-phase polymeric systems' [9] in which the partition coefficient of cells, in both dextran–poly(ethylene glycol) and dextran–ficoll aqueous phases, is dependent on the ionic strength of the phase system.

This communication is intended to show that:

(i) The 'General rule' is a special circumstance when using certain selected NaCl/phosphate ratios over limited concentrations of each;

(ii) The relation over the limited range over which the 'General rule' holds is a fortuitous consequence of offsetting alterations in the physical properties of the phases when the ratio of phosphate to NaCl is changed;

(iii) The limited validity of the 'General rule' is even further delimited by the polymer concentrations chosen;

(iv) The conclusion reached that the partition coefficients of cells in charged systems is merely a reflection of their hydrophobic properties [10] is without convincing basis.

### 2. Materials and methods

#### 2.1. *Human acetaldehyde-fixed erythrocytes*

Human blood was obtained by venipuncture using acid–citrate–dextrose (ACD) as anticoagulant. The cells were washed 3 times with 10 times their volume of isotonic aqueous sodium chloride solution and fixed with acetaldehyde as in [12].

#### 2.2. *Preparation of phase systems*

Four systems were used. They were prepared as in [1] and had the following compositions:

System A1 – 5% (w/w) dextran T500, lot 5556 (Pharmacia Fine Chemicals, Piscataway NJ), 4% (w/w) poly(ethylene glycol) 6000 (renamed '8000', Union Carbide, NY) and 0.11 M Na-phosphate buffer (pH 6.8);

System A2 – Same polymer concentrations but 0.20 M NaCl + 0.01 M Na-phosphate buffer (pH 6.8);

System B1 – Same polymer concentrations but 0.085 M Na-phosphate buffer (pH 6.8);

System B2 – Same polymer concentrations but 0.15 M NaCl + 0.01 M Na-phosphate buffer (pH 6.8);

Systems 'A' have, as calculated according to [9], an ionic strength of 0.22 and systems 'B' an ionic strength of 0.17.

*Published by Elsevier/North-Holland Biomedical Press*
00145793/81/0000–0000/$02.50 

2.3. *Partition of aldehyde-fixed erythrocytes*

Fixed erythrocytes were partitioned [12] in each of the above-indicated phase systems. After addition of a known aliquot of cells to a phase system, the latter was mixed and permitted to settle by the clock. A 20 min settling time was used.

2.4. *Determination of cells adhering to dextran droplets in the top phase*

At the time when cells were sampled for determination of the partition coefficient, a droplet of top phase was also placed on a slide and covered with a cover slip. Using phase optics, the number of cells adhering to dextran droplets as a percentage of total cells present was microscopically determined [13].

2.5. *Presentation of data*

The partition coefficient of cells is defined as [1]: Quantity of cells in the top phase (percent of total cells added).

## 3. Results and discussion

When aqueous solutions of different polymers (e.g., dextran, poly(ethylene glycol)) are mixed above certain concentrations they give rise to liquid immiscible two-phase systems with one of the phases rich in one of the polymers and the other phase rich in the second polymer. Such systems can be buffered and rendered isotonic and are suitable for the separation by partitioning of cells based on subtle differences in cells' surface properties [1,14]. At high polymer concentrations (i.e., high interfacial tensions) all cells tend to be adsorbed at the interface because it is there that they have their lowest energy. As the polymer concentrations are lowered cells tend to partition between the interface and one of the phases giving rise to characteristic partition coefficients which depend on the ionic composition and concentration of the phase system and on the surface properties of the cells. If phosphate is used to make the system isotonic, an electrostatic potential difference exists between the dextran–poly(ethylene glycol) phases under discussion [1,9] with the top phase positive, and cells will be pulled out of the interface when the interaction between the charge of the top phase and the negative surface charge of the cell can overcome the adherence of the cell to the interface. When NaCl is used to make the system isotonic, there is virtually no electrostatic potential difference between the phases and, at higher polymer concentrations (at which cells do partition in phosphate-containing systems), cells will remain in the interface.

If the interfacial tension of a dextran–poly(ethylene glycol) aqueous phase system containing NaCl is further reduced (by reducing the polymer concentrations), cells will partition into the top [poly(ethylene glycol)-rich] phase as a possible consequence of their interaction with the poly(ethylene glycol) itself. The partition coefficients obtained in this latter case have been shown to have, at least in the case of red blood cells from different species, an excellent correlation to the ratio of their membrane poly/monounsaturated fatty acids as well as to some other membrane lipid components [5].

A series of papers have appeared [7–11] in which a 'General rule' [9] has been promulgated that the partitioning of (red) cells in phases containing decreasing quantities of phosphate (from ionic strength 0.22) and increasing amounts of NaCl (to ionic strength 0.17) is a function of the phase system's ionic strength, as calculated in [9]. To demonstrate that ionic species (rather than ionic strength) is the prime determinant of cell partition coefficients (at constant polymer

Table 1

Partition coefficients of acetaldehyde-fixed human erythrocytes in dextran–poly(ethylene glycol) phase systems containing Na-phosphate or NaCl of similar ionic strength[a] and the extent of cell binding to dextran droplets in poly(ethylene glycol) phase

| System | | $K^b$ | % Cells bound |
|---|---|---|---|
| A1 | 5% D: 4% PEG cont. 0.11 M NaPB (pH 6.8) | 78 ± 5 (6) | 31 ± 10 (6) |
| A2 | 5% D: 4% PEG cont. 0.20 M NaCl + 0.01 M NaBP (pH 6.8) | 9 ± 3 (2) | 95 ± 1 (3) |
| B1 | 5% D: 4% PEG cont. 0.085 M NaPB (pH 6.8) | 94 ± 5 (4) | 2 ± 2 (4) |
| B2 | 5% D: 4% PEG cont. 0.085 M NaCl + 0.01 M NaBP (pH 6.8) | 9 ± 3 (2) | 95 ± 1 (3) |

[a] Calculated according to [9]; [b] $K$, the partition coefficient is the quantity of cells in the top phase as a percentage of total cells added. Cells were permitted to settle 20 min at room temperature (21–24°C)

composition and concentration at some distance from the critical point) we have undertaken the experiment depicted in table 1. Phase systems A1 and A2 have equivalent ionic strengths [9] of 0.22 while B1 and B2 have ionic strengths of 0.17. Acetaldehyde-fixed human erythrocytes were partitioned in these phase systems. It is apparent that the cells' partition coefficient is low in those systems in which NaCl predominates (A2,B2) and high in those in which phosphate is the main salt (A1,B1). The percentage of cells bound to droplets of dextran in the top phase in each of these systems at the time of sampling was also determined (see [13]) and further underlines the difference in partition coefficients found as a function of salt composition. In [9] a series of dextran–poly(ethylene glycol) phases were observed of which A1 and B2 represent the range of phosphate/NaCl ratios and never at phase systems in which the low ionic strength was due primarily to phosphate and the high ionic strength due to NaCl. Based on our simple experiment we conclude that ionic strength is not the basis for the changes observed in partition coefficients. Analogous results to those depicted in table 1 were also obtained using a dextran–ficoll system.

In [14] the partition coefficient of cells was shown to be higher in phosphate-containing phase systems and to decrease systematically with decreasing phosphate, increasing NaCl concentrations. Their data appear to be explained by the measured, concomitant decrease in the electrostatic potential difference between the phases as a function of replacing phosphate with NaCl [15]. Ionic species are a major determinant of the partition coefficients obtained [16].

Thus the relation forwarded [9], although dubbed a 'General rule', is one which holds only over a rather narrow range of ionic strengths and only in the case of selected phosphate/NaCl ratios. If one considers that phosphate-containing systems have an electrostatic potential difference between the phases under discussion [1,9], one would expect cells to have a higher partition coefficient in these phases when compared to a system of identical polymer concentration but containing NaCl. As the phosphate concentration is reduced and NaCl is substituted the potential difference drops as does the cell partition coefficient [1]. Hidden in this change of ratio (phosphate/NaCl) is the fact that phosphate concentration itself has an effect on the interfacial tension of the system [8] and probably on the potential difference [17]. As the phosphate is decreased so is the interfacial tension and, in the absence of NaCl, the partition coefficient of cells goes up [18]. NaCl has only a small measurable effect on the interfacial tension [19]. So we have with a decreasing ratio (phosphate/NaCl) not only a diminution of the overall electrostatic potential difference between the phases but also a decrease in the interfacial tension. The former would tend to decrease the particial coefficient while the latter tends to increase it. It is therefore clearly fortuitous that the relation between ionic strength and partition coefficient holds, a conclusion fortified by the fact that it holds only over a narrow range of selected phosphate/NaCl concentration ratios.

Furthermore, in dextran–poly(ethylene glycol) phases closer to the critical point (i.e., in systems of lower polymer concentrations) the partition coefficient of red blood cells from many species (e.g., dog, mouse, rat, rabbit) is the same in NaCl (ionic strength 0.17) and phosphate (ionic strength 0.22) containing systems [5] clearly indicating that the 'General rule' is not only confined to a selected range of ionic concentrations (and just of phosphate and NaCl at that), but is also restricted to a narrow range of polymer concentrations.

Based on experiments in which sodium alkyl sulfates of different chain lengths were partitioned, it was concluded [7] that in phase systems with >0.2 M ionic strength the partition coefficient of materials depends on their hydrophobic properties and not on their net charge. This conclusion, based on the partitioning behavior of these soluble compounds is said to be generally applicable not only to all soluble materials but also to cells [10]. We find this to be an improbable conclusion.

In the case of soluble materials, the isoelectric point of proteins can be obtained by partitioning them in phases of constant polymer concentration at different pH-values and containing one of two salts (i.e., phases having potential differences with the top phase positive in one set and negative in the other) [20]. The partition coefficients obtained when plotted against pH give two curves which cross the isoelectric point. Isoelectric points have been obtained in phases at all salt concentrations tested up to 0.5 M [21].

In the case of cells (and other particulate materials) all theories and 'rules' relating to their partitioning behavior must include reference to the interface since, as indicated above, they partition between one of the phases and the interface [1,14,16]. Thus parti-

tioning results obtained with selected soluble materials cannot be directly applied to an interpretation of surface properties involved in the partitioning of cells.

In conclusion we find that while in certain phase systems charge-associated or lipid-related surface properties may predominate in determining the cell partition coefficient [1,3], the interaction of cells with the physical properties of the phases is complex and, hence, the properties reflected by partitioning can neither be simply defined nor unequivocally stated.

## Acknowledgement

This work was supported by the Medical Research Service of the Veterans Administration.

## References

[1] Walter, H. (1977) in: Methods of Cell Separation (Catsimpoolas, N. ed) vol. 1, pp. 307–354, Plenum Press, New York.
[2] Fisher, D. (1981) Biochem. J. 196, 1–10.
[3] Walter, H. (1978) Trends Biochem. Sci. 2, 97–100.
[4] Brooks, D. E., Seaman, G. V. F. and Walter, H. (1971) Nature New Biol. 234, 61–62.
[5] Walter, H., Krob, E. J. and Brooks, D. E. (1976) Biochemistry 15, 2959–2964.
[6] Flanagan, S. D., Barondes, S. H. and Taylor, P. (1976) J. Biol. Chem. 251, 858–865.
[7] Zaslavsky, B. Yu., Miheeva, L. M. and Rogozhin, S. V. (1978) Biochim. Biophys. Acta 510, 160–167.
[8] Miheeva, L. M., Zaslavsky, B. Yu. and Rogozhin, S. V. (1978) Biochim. Biophys. Acta 542, 101–106.
[9] Zaslavsky, B. Yu., Miheeva, L. M., Mestechkina, N. M., Pogorelov, V. M. and Rogozhin, S. V. (1978) FEBS Lett. 94, 77–80.
[10] Zaslavsky, B. Yu., Miheeva, L. M. and Rogozhin, S. V. (1979) Biochim. Biophys. Acta 588, 89–101.
[11] Zaslavsky, B. Yu., Miheeva, L. M., Rogozhin, S. V., Borsova, L. V. and Kosinez, G. I. (1980) Biochim. Biophys. Acta 597, 53–63.
[12] Walter, H., Krob, E. J., Brooks, D. E. and Seaman, G. V. F. (1973) Exptl. Cell Res. 80, 415–424.
[13] Raymond, F. D. and Fisher, D. (1980) Biochim. Biophys. Acta 596, 445–450.
[14] Albertsson, P.-Å. and Baird, G. D. (1962) Exp. Cell Res. 28, 296–322.
[15] Reitherman, R., Flanagan, S. D. and Barondes, S. H. (1973) Biochim. Biophys. Acta 297, 193–202.
[16] Albertsson, P.-Å. (1971) Partition of Cell Particles and Macromolecules, 2nd edn, Wiley-Interscience, New York.
[17] Ballard, C. M., Dickinson, J. P. and Smith, J. J. (1979) Biochim. Biophys. Acta 582, 89–101.
[18] Walter, H., Krob, E. J. and Garza, R. (1968) Biochim. Biophys. Acta 165, 507–514.
[19] Brooks, D. E. (1981) personal communication.
[20] Albertsson, P.-Å., Sasakawa, S. and Walter, H. (1970) Nature 228, 1329–1330.
[21] Walter, H., Sasakawa, S. and Albertsson, P.-Å. (1972) Biochemistry 11, 3880–3883.