REVIEW ARTICLE

The separation of cells and organelles by partitioning in two-polymer aqueous phases

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When solutions of poly(ethylene glycol) and dextran are mixed above certain concentrations, they form two immiscible aqueous phases: a poly(ethylene glycol)-rich top phase and a dextran-rich bottom phase. These solutions can be buffered and made isotonic. Cells and organelles added to such systems will partition between the two phases and the horizontal interface between them. Depending on the composition and concentration of the polymers and of salts present, partitioning of the cells appears to depend either on membrane charge or on hydrophobic properties of the cell membrane. Furthermore, when a ligand is coupled chemically to either of the polymers, the partition may be affected predominantly by the affinity of membrane receptors for the ligand; this type of separation is termed affinity partition. It is thus possible, by a suitable choice of phase composition and conditions, for the partition of cells to be determined by one of three distinct surface properties of the cells. By measuring the distribution of cells between the phases and the interface in a phase system, an index of these properties can be obtained simply and rapidly. This may then be used to follow changes in the properties of the cells that may arise following treatments with drugs or during cellular differentiation. Cell partition can thus be a useful analytical procedure.

In addition, if two cell types differ markedly in their partitioning behaviour, they may readily be separated in a single partition, i.e. one type of cell may partition entirely into the top phase and the other completely into the bottom phase. It is generally necessary, however, to use multiple partitions, termed counter-current distribution, to separate the cells. Use of this technique has enabled preparative separations of cells and subcellular fractionations based on cell-surface properties to be achieved. Furthermore since the use of multiple partitions is a more sensitive method for detecting differences in cell surface properties than is a single partition, the use of counter-current distribution can give information on very subtle changes in the cell surface. Both theoretical considerations and experimental observations have demonstrated that the

partitioning of cells is exponentially related to the properties that determine partition; this contributes to the sensitivity of the technique (Albertsson 1958, 1971; Walter, 1977; Zaslavsky *et al.*, 1978*a*, 1979; Ballard *et al.*, 1979*b*; Gerson, 1980).

The first part of this article comprises a survey of a number of recent applications of two-phase separations in various fields of biochemistry. Earlier work on the partition of cells and organelles has been reviewed by Albertsson (1970, 1971, 1977*a,b*, 1978), Eriksson & Johansson (1979), Larsson & Andersson (1979), Sherbet (1978) and Walter (1969, 1975, 1977, 1978).

If the technique is to be exploited fully as a preparative and analytical tool, it is clearly important to understand the mechanisms involved and the possible effects on the behaviour of cells and organelles of changes in the various parameters involved in the separation process. The second part of the present review is therefore concerned with a consideration of the principles underlying the method. In the earlier literature the monograph by Albertsson (1971) provides a firm basis for understanding the properties of aqueous phase systems and their potential for the separation of cells and macromolecules, while the review by Walter (1977) provides not only a clear account of the partition behaviour of various cell types but also of the factors affecting cell partition.

Properties of phase systems

A detailed description of the properties of aqueous polymer phase systems is given by Albertsson (1971). Phase separation is a common property of mixed polymer solutions and a range of polymer systems has been described by Albertsson (1958, 1971). The salient features of the dextran/poly(ethylene glycol) two-phase system, which has been most used in partitioning studies on cells and organelles, are as follows.

1. Separation into two phases occurs only above certain concentrations of the two polymers, and the curve relating these concentration in a phase diagram is called the binodial (Fig. 1). The position

Fig. 1. Phase diagram of poly(ethylene glycol)

This figure shows the behaviour of poly(ethylene glycol) 6000 (BDH batch 367827) and dextran T500 (Pharmacia batch 11648) in 0.15 M-NaCl/0.01 M-sodium phosphate, pH 6.8 at 25°C (P. S. Gascoine, I. Shelton & D. Fisher, unpublished work).

of the binodal is influenced by the molecular weights of the polymers—the higher their molecular weights the lower are the concentrations required to form two phases, and the larger the difference in the molecular size of the polymers, the more asymmetrical is the binodal (Albertsson, 1971). A mixture of total composition A_1 will separate into two phases. The composition of the top phase is given by point T_1 ; this phase is poly(ethylene glycol)-rich and relatively hydrophobic (Albertsson, 1970, 1971). The composition of the bottom phase, which is dextran-rich, is given by point B_1 . The line T_1B_1 is called a tie line. Any systems that have an overall composition that fall on this tie line will separate into two phases with compositions defined by T_1 and B_1 . The relative volumes of the top and bottom phases will differ, however, since the ratio of the weights (approximately equal to the volumes), of the top to bottom phases is given by the ratio of the lengths of A_1B_1 to A_1T_1 . As the overall composition is moved in the direction $A_1 \rightarrow A_2 \rightarrow A_3$, the phases that separate have increasingly similar compositions. Point O, the critical point, describes a system having a vanishingly small tie line that just separates into two phases of equal volume and composition. Systems described by A_1 , A_2 and A_3 are of increasing distance from the critical point and they fall on tie lines of increasing length.

2. Both phases are highly hydrated, and they can be buffered and made iso-osmotic with salts or sucrose, etc. The interfacial tension is extremely low.

Close to the critical point [5% (w/w) dextran T500/3.5% (w/w) poly(ethylene glycol) 6000] the interfacial tension is 4.6×10^{-4} dynes/cm (4.6×10^{-9} N/cm) but it increases logarithmically with increasing length of the tie line as the composition of the system is moved away from the critical point (Ryden & Albertsson, 1971).

3. The viscosities of the two phases can be markedly different, particularly for systems away from the critical point; the dextran-rich bottom phase is more viscous than the poly(ethylene glycol)-rich top phase. The rate of separation of the two phases is slow for systems close to the critical point, but it increases as the concentration of either phase is increased, although the consequent increasing viscosity may ultimately slow down phase separation.

4. Some anions and cations have different affinities for the poly(ethylene glycol)-rich top phase and the dextran-rich bottom phase (Johansson, 1970a). This unequal partitioning gives rise to an electrostatic potential difference (an interfacial potential) between the phases, which can affect the distribution of charged molecules such as proteins and cells (Johansson, 1970a, 1974a,b). For example, since phosphate favours the dextran phase whereas chloride partitions almost evenly, a phase system of poly(ethylene glycol) and dextran containing 0.11 M-phosphate is described as a 'charged' phase system, and this has a top phase that is positively charged with respect to the bottom phase (potential difference of approx. 2.7 mV) (Reitherman *et al.*, 1973). On replacing the phosphate with NaCl, but maintaining the system iso-osmotic, the potential decreases virtually to zero, as in a system containing 0.01 M-sodium phosphate/0.15 M-NaCl (a 'non-charged' phase system). It is thus possible to obtain 'charged' phase systems with a range of potential differences by varying the ratio of these salts and also by varying the anions and cations used. The interfacial potential increases as a system is moved further from the critical point (Johansson, 1974b).

5. Modified forms of dextran and poly(ethylene glycol) distribute in a similar, though not necessarily identical, manner to the unmodified polymer if their partition is principally determined by the polymer moiety. Thus the positively charged trimethylamine/poly(ethylene glycol) and the negatively charged poly(ethylene glycol)/sulphonate favour the top phase (Johansson, 1970b) as does the non-charged monopalmitate derivative of poly(ethylene glycol) (Walter & Krob, 1976). However DEAE-dextran, which is highly substituted with positive charges, shows a partition behaviour at relatively low concentrations that is determined by the electrostatic potential between the phases (Walter *et al.*, 1968).

Partition behaviour of cells and particles

The partitioning behaviour of cells and particles between the two aqueous phases and the interface is readily characterized. The number of cells in each phase may be measured and those present at the interface determined by difference from the total number in the system. In the poly(ethylene glycol) 6000/dextran T500 phases described by Walter (1977), cells are usually distributed between the poly(ethylene glycol)-rich top phase and the horizontal interface, whereas in a phase of poly(ethylene glycol) 6000/dextran T40 cells can, under certain conditions, be found exclusively in the lower dextran phase. In the Ficoll/dextran systems described by Zaslavsky *et al.* (1978*a,b*, 1979, 1980) conditions can be obtained under which cells partition into either aqueous phase as well as the interface. However, results of partition studies are generally expressed in terms of a partition coefficient (K) presented as the quantity of cells in the top phase as a percentage of the total cells added (Walter, 1977). Partition behaviour can also be described by a partition ratio (G):

$$G = \frac{\text{Amount in upper phase}}{(\text{Total amount added} - \text{amount in upper phase})}$$

(Larsson & Anderson, 1979), and this is particularly useful in counter-current distribution since the difference between values of $\log G$ is a measure of separating capacity (Eriksson & Johansson, 1979).

When multiple partitions are carried out on a cell population by counter-current distribution in the apparatus described by Albertsson (1965), cells with the highest affinity for the top phase (high K) are transferred to the right of the counter-current train and they appear in the fractions of highest number (see Fig. 2), whereas cells having the lowest affinity for the top phase remain in the fractions of lowest number to the left of the train. Counter-current distribution with a limited number of transfers can be carried out manually relatively easily, and Eriksson & Johansson (1979) describe such a multistage partition with nine transfers.

Applications of phase partition to cells, micro-organisms and organelles

Phase partition has been used to give information on the charge and hydrophobic properties of the surfaces of a wide range of cells, micro-organisms and cell organelles, and has also been employed to obtain separations and fractionations based on these properties. Generally, only aspects of these applications to cells that have developed since Walter's review (1977) are considered here.

Erythrocytes. Walter (1977) summarizes studies that have used phase partition to detect differences in erythrocytes of different species, differences in the

Fig. 2. Summary of a counter-current distribution separation of human peripheral blood lymphocytes

The average distribution curves of separated cells after 119 transfers are shown. The areas in which lymphocytes were present with distinctive markers and mediating various functional activities were recovered are indicated below the curve for surface immunoglobulin positive cells (SmIg⁺), T lymphocytes detected by E rosetting (E⁺) and α -naphthyl acetate esterase (ANAE⁺) staining, low affinity (EA⁺ 1:16) and high affinity (EA⁺ 1:64) membrane receptors for Fc of IgG, cells stimulated by the mitogens phytohaemagglutinin (PHA) and concanavalin A (Con A), antibody-dependent cellular cytotoxicity (ADCC) and natural killer activity against K 562 cells (NK-K562) and fibroblasts (NK-fib). ----, 50–100% and —, above 100% of the proportion of activity of unseparated lymphocytes. Re-drawn from Malmström *et al.* (1980b).

membranes of erythrocyte ghosts and inside-out and right-side-out vesicles derived from them, surface changes in reticulocytes and erythrocytes as a function of maturation and aging and produced severe anaemia, and the consequences of modifying erythrocyte membranes by neuraminidase and trypsin and glutaraldehyde. Recently, further studies on the effect of maturation and anaemia (Walter & Krob, 1978), and on enzymic modifications (Ballard *et al.*, 1979*a,b*) have been reported, and it has been shown that the enrichment or depletion of human erythrocytes of cholesterol modifies their partition behaviour (Walter *et al.*, 1979*a*). Surface

properties of sheep erythrocytes that are involved in their use as an immunological reagent have also been examined, and it has been observed that sheep erythrocytes that had been reacted with anti-(sheep erythrocyte) antibody only showed altered partition behaviour when complement was added subsequently (Walter & Krob, 1979).

Platelets. Species-specific differences in the partition behaviour of platelets have been reported (Walter *et al.*, 1969b). It appears that EDTA-induced increases in platelet surface charge, which are associated with a loss of aggregability, also increase cell partitioning as well as the partition electrophoretic mobility of cells (Grant & Zucker, 1978).

Leucocytes. Different partition coefficients for leucocytes of different species have been observed (Walter *et al.*, 1969b), and separations by counter-current distribution of leucocytes from red cells have been reported (Walter *et al.*, 1969a,b). Guinea pig polymorphonuclear leucocytes incubated with liposomes showed modified surface properties which were associated with a reduced capacity to phagocytose bacteria, and were detected as decreased negative surface charge and decreased surface hydrophobicity (Dahlgren *et al.*, 1977). Fractionation of human peripheral-blood monocytes in a charged phase system by counter-current distribution has shown that, with increasing K (presumably higher surface charge), the proportion of monocytes that phagocytosed latex particles increased (Walter *et al.*, 1980a). Furthermore, it was found that the charge-associated properties of the monocytes changed as a consequence of phagocytosis.

Lymphoid cells. A number of studies on the separation of lymphoid cells has been carried out in charged phase systems; heat-inactivated foetal calf serum (5%, w/w) was included in the later studies. Lymphocytes were seen to remain viable after the separation, and functionally active subsets of lymphocytes have thus been obtained. Partial or complete separations have been reported for plaque-forming and colony-forming cells from mouse spleen (Brunette *et al.*, 1968), for human tonsillar lymphoid cells that form rosettes with sheep erythrocytes (E^+) and non-rosetting cells (Walter & Nagaya, 1975), and for rat leucocytes of the spleen, lymph nodes and thoracic duct (Malström *et al.*, 1978; Nelson *et al.*, 1978). Surface-immunoglobulin-positive ($SmIg^+$) lymphocytes were obtained in a restricted area of the distribution, having the lowest partition coefficient, and a separation of subsets of thymus derived lymphocytes was obtained. Lymphocytes mediating antibody-dependent cellular toxicity and natural killer activity to adherent cell lines were also detected in a restricted area of the distribution that was separate from the majority of cells. These effector

cells were recovered in fractions separate from those containing lymphocytes mediating selective toxicity to colon carcinoma cells and to allogenic fibroblasts. Recently, fractionations of human peripheral blood lymphocytes have been performed and the properties of the separated cells detected by a battery of cell markers. A summary of the results of Malstrom and his colleagues (1980a,b) is shown in Fig. 2. Further separation of the lymphocytes in peak IV was obtained by carrying out counter-current distribution in the presence of sheep red blood cells for which human T cells have affinity, a technique previously described by Walter *et al.* (1978b). Over 50% of the lymphocytes of peak III and IV were redistributed to peak I. Lymphocytes mediating natural killing to adherent target cells were not redistributed by this procedure, indicating that they lacked high-affinity receptors for sheep erythrocytes. The authors comment, however, that such an affinity counter-current distribution could be obtained if an antibody against a chosen cell-surface marker were attached to erythrocytes having a suitable partition behaviour, thus providing an antigen-specific separation. Similar studies by Walter *et al.* (1979b,c, 1980b) showed a broad and skewed distribution pattern indicating a highly heterogeneous lymphocyte population. The bulk of complement-receptor bearing cells (B-cells) distributed to the left of the separation train (lowest partition coefficient), cells that rosetted with sheep erythrocytes (T-cells) were found in the centre of the distribution, whereas cells bearing Fc receptors were distributed to the right (highest partition coefficient). Walter *et al.* (1980b) suggest that it should be possible to obtain populations enriched with respect to these latter cells by a simple one- or two-step extraction process. Whilst cells with the lowest partition coefficient (K) had the lowest electrophoretic mobilities, cells with an intermediate value of K had the highest mobilities and those cells with the highest value of K had intermediate mobilities (Walter *et al.*, 1980b). These results indicate that the charge measured by cell electrophoresis and by partitioning are not necessarily the same. A population of large lymphocytes was found among the lymphocytes of high partition coefficient (Walter *et al.*, 1979c). By use of a less charged phase system the bulk of the cells was shifted to the left, reflecting decreased partition coefficient, but the large lymphocytes persisted to the right of the counter-current-distribution train and this population had essentially no mitogen responsiveness and did not form E-rosettes.

Plasma membranes and subcellular fractions from animal cells. Plasma membranes that have been isolated rapidly by using two-phase systems include the surface membranes of L cells (Brunette & Till, 1971), membrane from rat kidney brush-

border (Glossmann & Gips, 1974), plasma membranes of rat liver (Lesko *et al.*, 1973), mouse sarcoma (Miller *et al.*, 1974), and crayfish nerves (Uehara & Uyemura, 1979), and the use of phase partition as a general method for preparing plasma membranes has been discussed by Evans (1978).

Nicotinic receptor-rich membranes have been separated from those containing acetylcholinesterase in a single partition step by using a charged polymer (Hartman & Heilbronn, 1978). Membranes obtained from *Acholeplasma laidlawii* are homogeneous with respect to buoyant density but can be separated on the basis of their surface properties (charge and hydrophilic/hydrophobic properties) into at least two subpopulations by counter-current distribution in two-phase systems (Weislander *et al.*, 1979). Studies on the fatty acid and cholesterol contents of the membranes indicated that the resolution of different subpopulations was due to a critical ratio of monoglucosyldiglyceride to diglucosyldiglyceride; a high ratio favoured the dextran phase. Microsomes have been subfractionated on the basis of hydrophobic differences in their membrane surface (Ohlson *et al.*, 1978), and sub-fractionation of the rat liver Golgi apparatus has been reported (Hino *et al.*, 1978a,b). The application of phase partition to the resolution of fragile organelles such as lysosomes and peroxisomes has also been explored by Morris & Peters (1980) using a small-volume counter-current distribution apparatus described by Pritchard *et al.* (1975).

Drug-cell interactions. As part of a study to characterize interactions between antitumour agents and murine leukaemia cells in culture, Kessel and his colleagues have used cell partition, as well as changes in membrane permeability, to identify drug-cell interactions that directly affect the cell surface, as contrasted with interactions primarily leading to inhibition of nucleic acid or protein biosynthesis. The antitumour agents studied have been amphotericin (Kessel, 1976), *S*-trityl-L-cysteine and analogues (Kessel *et al.*, 1976), acronycine (Kessel, 1977b), lymphosarcin (Kessel, 1978), dithiocarbamylates (Kessel & McElhinney, 1978), fluorouracil (Kessel, 1980), photoactivated porphyrins (Kessel, 1977a; Kohn & Kessel, 1979) and adriamycin (Kessel, 1979). A role for cell surface glycosylation in determining cell partition behaviour has been indicated by Kessel's recent studies on the effect of the anthracycline antitumour drug adriamycin (Kessel, 1979). A drug-induced increase in the production of electronegative and hydrophilic membrane glycoproteins was observed to be associated with a decreased partition coefficient of the cells in a non-charged phase system containing poly(ethylene glycol) palmitate, indicating that cell surface hydrophobicity, as measured by

cell partition, decreased as cell surface glycosylation increased.

Bacteria. Differences in surface properties between *Escherichia coli* cells that are susceptible and resistant to erythromycin (Wayne & Walter, 1974) and between various mutants of *E. coli* that are resistant to streptomycin (Pestka *et al.*, 1977) have been detected, and the surface charge and hydrophobicity of *Neisseria gonorrhoea* have been studied by phase partition (Magnusson *et al.*, 1979a,b). Gerson & Akit (1980) have shown that the order of hydrophobicity of the surfaces of several bacteria is *Serratia marcescens* ~ *Saccharomycopsis lypolytica* > *Staphylococcus epidermidis* > *Micrococcus luteus*. The surface hydrophobicity of *Corynebacterium lepus* changed continuously through its growth cycle, due to the combined effects of its liquid-hydrocarbon growth substrate and the production of surface-active material.

Properties of bacteria that determine their tendency to attach to and to interact with mammalian cells are reflected by their partition behaviour. For example Stendahl *et al.* (1973) found that the liability of a series of mutants of *Salmonella typhimurium* to be phagocytosed by polymorphonuclear leucocytes correlated with their behaviour in a dextran/poly(ethylene glycol) two-phase system. Smooth (S) bacteria tended to escape phagocytes and to prefer the poly(ethylene glycol)-rich phase whereas rough (R) mutants, which have an incomplete lipopolysaccharide that lacks S-specific side chains, were more liable to phagocytosis and preferred the dextran phase. Opsonization of S bacteria with IgG increased their partition into the dextran-rich phase with a concomitant increase in phagocytosis (Stendahl *et al.*, 1974). The role of hydrophobicity and charge of the bacterial surface in determining its susceptibility to phagocytosis has been extensively studied by partition techniques for *Salmonella* (Magnusson & Johansson 1977; Magnusson *et al.*, 1977, 1979c; Stendahl *et al.*, 1977a,b; Stjernström *et al.*, 1977), for *E. coli* (Edebo *et al.*, 1975) and in relation to the inhibition of phagocytosis by capsular polysaccharides (Kozel *et al.*, 1980).

It has been found that the clearance of liposomes from blood to the liver and spleen following intravenous injection into mice correlates with the partition behaviour of the liposomes in phase systems; liposomes with a high affinity for the dextran-rich phase were more readily cleared than were liposomes of different composition which had low partition for the dextran phase (Tagesson *et al.*, 1977). The hydrophobicity and charge of certain enterobacteria measured by partition behaviour also appears to determine their association with intestinal mucosa (Perers *et al.*, 1977).

Plant cells, membrane preparations and sub-

cellular fractions. The application of phase partition to separation of chloroplasts, chloroplast elements and mitochondria has recently been reviewed by Larsson & Andersson (1979), who have given practical guidance for obtaining suitable partitions and separations. Cell walls have been isolated from the algae *Scenedesmus obliquus* and from *Chlorella* (Burczyk, 1973, 1979) and the properties of *Chlorella* have been studied by phase partition (Burczyk, 1975; Burczyk *et al.*, 1976) with particular reference to its life cycle (Walter *et al.*, 1973).

Recent technical developments. The separation of cells by partition has recently been accomplished using a non-synchronous flow-through, coil planet, centrifuge which makes possible higher resolution separations (up to 1000 counter-current-distribution transfers) (Sutherland & Ito, 1980). Matsumoto & Shibusawa (1980) have investigated the possibility that phase partition could be carried out in a column procedure if one of the polymer phases were held immobile (stationary phase) and a solution of the other polymer run over it (mobile phase). By the use of a column packed with chemically-bonded poly(ethylene glycol) as the stationary phase and a phosphate-buffered saline solution of dextran as the mobile phase, they have obtained a fractionation of peripheral blood cells from human and rabbit.

Membrane properties involved in partition

Phase partition can give information on cell surface charge, hydrophobicity and on surface receptors; the extent to which each of these factors contributes to the observed value of partition coefficient will depend on the choice of the phase system.

Properties associated with membrane charge. The importance of cell surface charge in partition behaviour was established from extensive studies in 'charged' phase systems (reviewed by Walter, 1977), by single-tube partition, and by examining the charge properties of cells fractionated by counter-current distribution. In charged phase systems such as those having relatively high concentrations of phosphate, cell partition into the poly(ethylene glycol)-rich top phase increases as the concentration of phosphate increases and the electrochemical potential of the top phase becomes more positive. Partition tends to increase with increasing cell surface charge but the membrane charge detected by phase partition is not necessarily the same as that measured by cell electrophoresis. A body of evidence indicates that 'partition measures membrane charge deeper into the cell membrane than does electrophoresis, which reflects only the charge at the shear plane' (Walter, 1977). The correlation of partition in charged phases with electrophoretic behaviour has also been examined by Ballard *et al.* (1979a,b) with a view to using phase partition to

detect changes in the surface charge of indicator cells which have been registered by electrokinetic measurements and have been used to detect some lymphokines released from lymphocytes sensitized by specific antigen stimulation (Smith & Dickinson, 1976). Clearly, the partition of cells and organelles can be influenced markedly by the types of salts present in the phases owing to their effects upon the interfacial potential. This 'steering effect' increases with the electrical charge of the particle (Eriksson & Johansson, 1979) and it is a useful procedure by which to obtain a suitable partitioning of cells in counter-current distribution.

Inclusion of charged derivatives of the polymers in the phase system also influences the partition of negatively charged particles; poly(ethylene glycol) sulphonate decreases partition into the top phase whereas trimethylamino-poly(ethylene glycol) increases it (Johansson, 1973). When these poly(ethylene glycol) derivatives are being used, the concentration of buffer must be kept low (10 mM or less) lest the effect of the charged poly(ethylene glycol) be diminished by electrostatic shielding. Sucrose may be included to keep the system iso-osmotic.

If a particle is partitioned in two phases containing different salts or charged polymer ligands that influence interfacial potential, a plot of $\log K$ versus pH will produce two lines which intersect at a pH identified as the isoelectric point, since the partition of an uncharged molecule or particle is almost independent of the electrostatic potential between the phases. Such so-called 'cross partitions' have been used to determine the isoelectric point of mitochondria, of submitochondrial particles (Lundberg & Ericson, 1975), of peroxisomes (Horie *et al.*, 1979), and of spinach thylakoid membranes (Akerlund *et al.*, 1979).

Non-charged membrane components. For phase systems in which the interfacial potential is very low, the partition of cells would appear to depend upon membrane factors other than charge (non-charged membrane components) which qualitatively might be referred to as cell-surface hydrophobicity (Gerson, 1980). Walter *et al.* (1976a) found that, in 'non-charged', low-phosphate/high-NaCl phase systems close to the critical point, some species of erythrocytes (e.g. rat and mouse) showed a high partition into the top phase whereas for other species, e.g. pig, partition into this phase was low. A correlation was found between the partition coefficient and the ratio of polyunsaturated/mono-unsaturated fatty acids in the membranes of the nine species of erythrocytes studied, and the authors suggested that an increased affinity for the poly(ethylene glycol)-rich top phase arises from an increasing ability of poly(ethylene glycol) to interact with the cell membranes when the unsaturation of the membrane lipid chains is

increased. In non-charged phase systems away from the critical point, most cells are adsorbed to the horizontal interface or go to the bottom phase, but this tendency can be dependent upon the type of cells (Albertsson & Baird, 1962; Walter, 1977). Fig. 3 shows our own results for pig and rat erythrocytes partitioned in non-charged systems of increasing distance from the critical point. It should be noted that the greatest change of partition from the interface to the top phase occurs over a very narrow range of polymer concentration [approx. 0.25% w/w poly(ethylene glycol)] as has been observed for chloroplast lamellae (Larsson & Andersson, 1979) and *Chlorella* (Albertsson, 1958). Partition studies on liposomes as model systems to determine the contribution of lipids to the partition of membrane particles indicate that the polar head group plays a dominant role, while the degree of unsaturation is less important (Eriksson & Albertsson, 1978).

When a small amount of poly(ethylene glycol) monopalmitate is included in a non-charged phase system away from the critical point, erythrocytes partition into the top phase (Walter *et al.*, 1976b) in a species-dependent manner which is generally similar to that observed for a non-charged phase system that is close to the critical point (Walter *et al.*, 1976a). Since such a molecule will partition into the top phase because it is predominantly 'PEG-like', it is possible to consider (Walter, 1977) that cells which interact with the hydrophobic palmitoyl moiety will be pulled out of the interface into the top phase (hydrophobic affinity partition). To explain the species-dependent effects of poly(ethylene glycol) palmitate upon partition of erythrocytes, Eriksson *et al.* (1976) suggested that the ability of poly(ethylene glycol) palmitate to bind to erythrocytes may be species-dependent.

Partition in non-charged phases in the absence and presence of poly(ethylene glycol) palmitate are thus explained by hydrophobic interactions with poly(ethylene glycol) or poly(ethylene glycol) palmitate respectively. Either system might be used to detect surface hydrophobicity effects.

Specific membrane receptors. When a ligand for a specific membrane receptor is coupled chemically to poly(ethylene glycol) and incorporated into a poly(ethylene glycol)/dextran phase system, only cells bearing the receptor will associate with the poly(ethylene glycol)-ligand and show enhanced partition into the top poly(ethylene glycol) phase. This technique (biospecific affinity partition) has been used to purify the cholinergic receptor of *Torpedo californica* electric organs (Flanagan *et al.*, 1975, 1976).

Relative contributions of surface charge and hydrophobicity. The ability of charge and hydrophobic effects to alter partitioning behaviour can

readily be understood from the following simple explanation given by Larsson & Andersson (1979). The partition ratio, G , for a particle is described by $G = G_0 G_z$ where G_z is a function of particle charge and phase interfacial potential, and G_0 is a function of other surface properties and of polymer concentrations ($G = G_0$ at the isoelectric point of the membrane or when the interfacial potential is zero). Thus the effects of changing salt composition and polymer concentration are due to changes in G_z and G_0 respectively. Charged polymers, by giving a higher interfacial potential, will influence G_z whereas G_0 will be affected by polymers carrying uncharged ligands such as poly(ethylene glycol) palmitate. Gerson (1980) has shown that the partition coefficient (K) of a cell that distributes between two phases can be described by the equation

$$-\log K = \alpha \Delta\gamma + \delta \Delta\psi + \beta$$

where $\Delta\gamma$ is the difference in surface free energy of the cells in the two phases, $\Delta\psi$ is the difference in electrical potential between the phases and α , β and δ are constants containing parameters such as surface area (α), surface charge (δ), activity coefficients (β) and standard chemical potential (β). This equation predicts a linear relationship between $\log K$ and either the difference in interfacial free energy or the potential difference between the phases or a linear combination of both. In a phase system of constant potential difference, but with differing values of $\Delta\gamma$ (constant salt composition but variable polymer concentration), $\log K$ will be proportional to $\Delta\gamma$ whereas for phases with constant $\Delta\gamma$ (constant polymer composition) but varying potential $\log K$ will be proportional to $\Delta\psi$. By obtaining differences in surface free energy from contact angle measurements for cells in different phase systems of a constant salt composition, Gerson has shown that $\log K$ is proportional to surface free energy differences for several transformed lymphocytic cell lines (Gerson, 1980) and for a variety of bacterial cells (Gerson & Akit, 1980). Since surface free energy changes are a quantitative measure of the van der Waals' forces at the cell surface (a measure of cell surface hydrophobicity), he concludes that phase partition can be used as a direct measure of cell surface hydrophobicity, and that the partition coefficient is directly related to the differences in the surface energy of the partitioned cell between the two phases. Partition in aqueous biphasic polymer mixtures can clearly be very sensitive to small differences in the hydrophobicity of particles.

Considerations of the relative contributions of charge and cell surface hydrophobicity in determining cell partition have also been undertaken by Zaslavsky and his colleagues (Zaslavsky *et al.*, 1978a,b, 1979, 1980; Miheeva *et al.*, 1978). They found that the published data on the effects of the

ionic composition of phosphate/saline buffers on the partition of different species of erythrocytes in dextran/poly(ethylene glycol) phases (Walter, 1977) showed a linear relationship when $\log K$ was plotted against the ionic strength (I) of the systems. These workers have developed a general equation for the partition of cells, primarily based on their studies in Ficoll/dextran two-phase systems, that:

$$-\ln K = A + BI$$

where A and B are constants, specific for the cell type being partitioned, and I is the ionic strength. The term A is a measure of the relative cell surface hydrophobicity, and it can be expressed in terms of the number of real and equivalent $-\text{CH}_2-$ groups on the cell surface. The term B reflects the overall effect of the ionic strength on all the ionogenic groups present on the cell surface. Interestingly it is suggested that the membrane surface charge is the determinant of cell partition only as a factor affecting the relative hydrophobicity of the cell surface.

Our recent studies on the partitioning of erythrocytes from the rat (Raymond & Fisher 1980a,b), and from chicken, pig, turkey, ox, mouse and man (F. D. Raymond & D. Fisher, unpublished work), on Raji lymphocytes (Raymond & Fisher, 1980b) and on L1210 and P388 murine leukaemia cells (F. D. Raymond, D. Fisher & D. Kessel, unpublished work) in 'non-charged' phases of dextran and poly(ethylene glycol) have indicated that the interface has a very important role in the partition process. Cells adhere to the interfaces at the surfaces of globules and streams (microphases) of dextran in the developing top phase and of poly(ethylene glycol) in the developing bottom phase as phase separation proceeds (Raymond & Fisher, 1980a). With time, the cells are delivered, on the surfaces of these microphases, to the horizontal interface that forms between the two bulk phases. This interface has been found to comprise the equilibrium position of all the cells studied in our experiments. The affinity of cells for the micro-interfaces, and consequently for the bulk interface, increases as the composition of the system is moved away from the critical point, presumably because of the increased interfacial tension. The affinity for the interfaces also varies with the cell. Mouse and rat erythrocytes have much less affinity than pig erythrocytes for the interfaces, and in systems close to the critical point (low interfacial tension), they can be observed as free cells (not associated with an interface) early in partition-time. However, later in partition these cells adhere to the relatively small slow-moving globules of dextran that are present in the top phase during the terminal stage of the slow separation that occurs in these phases. The association of cells with the microphases can, furthermore, influence the rate of

Fig. 3. Partition of rat (●) and pig (○) erythrocytes in a two-phase system

The system comprised 5% (w/w) dextran T500 and various concentrations of poly(ethylene glycol) 6000 in 0.15 M-NaCl/0.01 M-sodium phosphate, pH 6.8, measured 8 min after mixing of phases had ceased, at 21.5°C (F. D. Raymond & D. Fisher, unpublished observations).

phase separation and consequently the rate at which cells are delivered to the horizontal interface. Thus at any particular time during partition, a proportion of cells may be present in the top phase and will generally be associated with droplets of dextran.

Species-dependent differences in partition are observed, not only close to the critical point but at some distance from it. Poly(ethylene glycol) palmitate decreases the extent of interaction with the microphase droplets, and separate single cells are then observed in the top phase. Cells that adhere most weakly to the interfaces require less poly(ethylene glycol) palmitate to displace them and to cause an increased partition into the top phase. Partition into the top phase at a particular concentration of poly(ethylene glycol) palmitate for erythrocytes in our experiments was found to be rat > human > pig. The association of cells with microinterfaces was less in a charged-phase system. It could also be decreased by modifying cell surfaces with neuraminidase or with trypsin, after which an increased partition of single cells into the top phase was seen. A simple interpretation of the behaviour of the cells we have studied in these non-charged phases is that cells show an affinity for the interfaces between the polymer solutions that depends on the nature of the cell surfaces. By modifying the interfacial tension, the interfacial potential, or the nature of the cell surface, cells can be displaced from

the interfaces. This displacement probably occurs over a very narrow range of conditions, so that cells can be removed from the microinterfaces (and consequently from the horizontal interfaces between the bulk phases) to the top phase in a very sensitive way.

Conclusions

Phase partition of cells is a technique that can be carried out relatively easily, and the considerable contributions of Albertsson, Walter and Johansson have yielded a technique of great flexibility. Furthermore, if increasingly clear definitions of the features of cell surfaces that influence partition behaviour can be developed, it is likely that the use of cell partition in aqueous polymer two-phase systems may be more widely used as a procedure for the analysis of cell surfaces and for the fractionation of cell populations.

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