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Erythrocyte partitioning in dextran–poly(ethylene glycol) aqueous phase systems

Events in phase and cell separation

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ABSTRACT

Early events in the partitioning process which involve characteristic kinetics of cell- and phase-specific interactions and phase separation have been described previously. This paper reports on red cell–phase droplet interactions pertaining at the time of usual phase sampling (*i.e.*, the time at which a clear bulk interface is first apparent) and beyond in cell partitioning and countercurrent distribution experiments. In non-charge-sensitive phase systems close to the critical point, cells can be free or attached to phase droplets. Cells that are free are virtually completely in the top phase, whereas different cell populations that show essentially complete binding to droplets can nevertheless have different partition ratios and be separated, thus reflecting the effects of the difference in the cells' avidity for the phase droplets during the early, elapsed events in partitioning. At higher polymer concentrations (*i.e.*, higher interfacial tensions), the cell populations, completely bound to phase droplets, partition completely to the interface, and consequently cannot be separated. When such systems are made charge-sensitive by the generation of a Donnan potential between the phases or made into affinity systems by the incorporation of PEG ligands (*e.g.*, PEG–palmitate), there is a decrease in the avidity of the cells for phase droplets. The resulting increase in the ratio of free to droplet-bound red cells in the top phase at the time of sampling correlates with an increase in the partition ratio, P, observed. The results show that a major difference (at the time of sampling) between non-charge-sensitive and charge-sensitive or affinity systems is that only in the former can cell populations be separated when they are almost completely bound to droplets. It was also found that although red cell–droplet interactions still pertain at the usual sampling times, there is a reduced involvement of kinetics at this late phase separation stage. This is operationally important as it provides leeway in selecting the sampling (settling) time.

INTRODUCTION

Partitioning in dextran–poly(ethylene glycol) aqueous phase systems is an established, sensitive method for the separation and fractionation of cell

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populations based on differences in their surface properties (for some recent reviews, see refs. 1–4). Unlike the partitioning of soluble materials, which occurs between the bulk phases and depends on the materials' relative solubility in them, the partitioning of cells generally take place between one of the bulk phases and the interface and is time dependent [1,2,5]. Early events in the partitioning process which involve characteristic kinetics of erythrocyte- and phase-specific interactions and phase separation have been described previously [5–7]. In this paper we report on the red cell–phase droplet interactions pertaining at the time of usual phase sampling (see Experimental) and beyond in cell partitioning and countercurrent distribution experiments. We thereby (a) contribute further to an appreciation of the partitioning mechanism, (b) put into perspective the early events which, to a great extent, determine cell partition ratios but are usually unobserved in an operational sense, and the later events, which are observed without appreciation of basis, and (c) show the reduced involvement of kinetics in these later stages of partitioning.

EXPERIMENTAL

Reagents

Dextran T500 (lots 4094, 5556 and 11648) were obtained from Pharmacia LKB (Piscataway, NJ, USA). Poly(ethylene glycol) 8000 (PEG, Carbowax 8000) was from Union Carbide (Long Beach, CA, USA) and PEG 6000 was from BDH (Poole, UK). All salts used were of analytical-reagent grade. The ester of PEG 6000 and palmitic acid (PEG 6000–palmitate) was synthesized by the method of Shanbhag and Johansson [8]. Analysis established that 38% of the hydroxyl groups were esterified.

Preparation of two-polymer aqueous phase systems

Dextran–PEG aqueous two-phase systems having the compositions specified in the text were made up as previously described [1] and are designated by the nomenclature established there. In short, the first number in the description of phase systems denotes the percentage concentration of dextran (w/w). This is followed by a colon and a number giving the percentage concentration of PEG (w/w) (PEG 8000 unless indicated otherwise). The salt content follows as:

#1 being 0.11 *M* sodium phosphate buffer (pH 6.8);

#5 being 0.01 *M* sodium phosphate (pH 6.8) + 0.15 *M* NaCl.

Systems in which phosphate predominates have an electrostatic potential difference ($\Delta\psi$) between the phases (top phase positive) and are deemed charge-sensitive [1]. Phases in which NaCl is the main salt have virtually no potential between the phases and are non-charge-sensitive [1]. The interfacial tension between the phases increases with increasing polymer concentration [1,2].

Collection of blood and washing of erythrocytes

Volumes of 10 ml of blood from human donors and from dogs were obtained by venipuncture and collected in 3 ml of the anticoagulant acid–citrate–dextrose (ACD). A similar volume of blood was obtained, by heart puncture, from male Sprague–Dawley rats which weighed 275–400 g. Rabbit blood, usually 5 ml in 1.5–2 ml of ACD, was from the ear marginal vein. Sheep erythrocytes, in Alsevier's solution, were purchased from Mission Laboratories (Rosemead, CA, USA). Chicken blood was taken from the bracheal vein by venipuncture and collected in Alsevier's solution. Aliquots of the erythrocyte populations were washed three times with at least ten times the cell volume of isotonic aqueous salt solution (saline) before being used in the experiments described below.

Partitioning of erythrocytes in aqueous two-phase systems

Erythrocyte partitioning was carried out as described previously [9]. Phase systems to be used, at 21–24°C, were mixed and 10 ml were poured into calibrated tubes (125 × 15 mm I.D.). The tubes were centrifuged to speed phase separation and the top and bottom phase volumes were adjusted to be equal. The top phase volumes were recorded. A 0.1-ml volume of washed, packed erythrocytes to be partitioned was added to each tube and the contents were well mixed. As phase separation (settling) times are, among other things, a function of phase column height [1,2,10] and increase markedly as one approaches the critical point (*i.e.*, the polymer concentrations below which no phase separation occurs), tubes were capped and permitted to settle

in a horizontal position when using low polymer concentrations (*i.e.*, 5:3.5) and in a vertical position when using higher polymer concentrations. Times for phase settling (indicated in the text and/or tables) were chosen such that the phase systems had just formed a sharp interface at the level of the initially determined interface. In a few experiments (see below) we also show, for comparative purposes, results at longer settling times. An aliquot of the top phase was withdrawn and the number of cells determined either by electronic counting (Celloscope; Particle Data, Elmhurst, IL, USA) or by lysing and measuring the absorbance at 540 nm. The number of cells initially added to the partition tubes was similarly determined. The partition ratio, P, of cells is given as the number of cells in the top phase as a percentage of total cells added [1,2]. The partition ratio was found to be independent of the number of cells added to the partition tubes over the range tested (0.01–0.5 ml packed erythrocytes).

Microscopic examination of erythrocyte–phase droplet interactions

Between 0.015 and 0.035 ml of packed erythrocytes were added to partition tubes containing equal top and bottom phase volumes as described above. The tubes were mixed and permitted to settle. An aliquot of top (or bottom) phase was then removed with a Pasteur pipet and placed on a slide, covered with a cover-slip and immediately examined on a phase contrast microscope (American Optical, Buffalo, NY, USA). The percentage of cells attached to phase droplets was determined three times by counting a minimum of 100 cells each time during a period of not more than 2 min to prevent the slide from warming (due to the microscope lamp).

RESULTS

Non-charge-sensitive phases: attachment of cells to droplet surfaces

Microscopic examination of top and bottom phases of non-charge-sensitive dextran–PEG 8000 (or 6000) phase systems containing erythrocytes from rat, human, dog or rabbit reveals that the cells are bound on the PEG side of phase droplets (Fig. 1 shows an example for human blood cells). The percentage of these cells bound to phase droplets present in the top phase (referred to as "dextran" droplets as they are composed of dextran-rich bottom phase) was determined at the same time as their partition ratios, P, were measured (Table I). In the phase system of lowest polymer concentration, 5:3.5 (lowest interfacial tension), the rat erythrocytes were completely in the top phase and were not bound to dextran droplets. Increasing the polymer concentrations (5:3.5 → 5:3.7 → 5:4 → 7:4.4), thereby increasing the interfacial tension, led to increased binding to droplets and decreased P values. In the 5:3.5 phase system the partition ratios were in the order rat > dog > rabbit > human as reported previously [9], indicating differences in the surface properties of these cells. However, the attachment

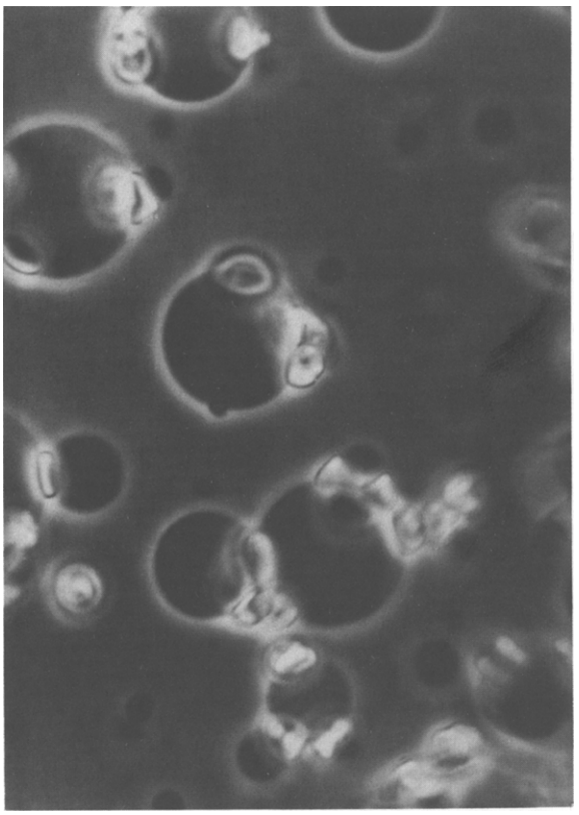

Fig. 1. Phase contrast light micrographs of human erythrocytes (× 585) which attach to the outer surfaces of droplets of the dextran-rich bottom phase suspended in the top phase of a non-charge-sensitive phase system containing 5% (w/w) dextran T500, 4.75% (w/w) PEG 6000, 0.15 *M* NaCl and 0.01 *M* sodium phosphate buffer (pH 6.8). See text for discussion.

TABLE I

PARTITION RATIO, *P*, AND THE PERCENTAGE OF ERYTHROCYTES, FROM DIFFERENT SPECIES, BOUND TO BOTTOM PHASE DROPLETS IN THE TOP PHASE OF NON-CHARGE-SENSITIVE PHASE SYSTEMS

See text for discussion.

Species	Phase system[a]							
	5:3.5 #5[b]		5:3.7 #5[c]		5:4 #5[d]		7:4.4 #5[e]	
	P	% bound	*P*	% bound	*P*	% bound	*P*	% bound
Rat	102 ± 3(3)	1 ± 1(3)	37 ± 8(18)	62 ± 15(16)	16 ± 2(3)	92 ± 3(3)	3 ± 3(3)	93 ± 3(3)
Dog	89 ± 5(9)	83 ± 7(9)	–	–	–	–	–	–
Rabbit	71 ± 6(18)	81 ± 14(16)	–	–	–	–	–	–
Human	55 ± 5(3)	96 ± 1(3)	–	–	<5	97 ± 3(3)	–	–

[a] Phase systems were composed of the indicated concentrations of dextran T500, poly(ethylene glycol) 8000 and 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer (pH 6.8). Data are presented ± S.D. with the number of experiments in parentheses.
[b] Settling time: 7 min horizontal + 1 min vertical.
[c] Settling time: 30 min vertical.
[d] Settling time: 20 min vertical.
[e] Settling time: 10 min vertical.

of cells to droplets was not as discriminatory as partitioning: erythrocytes of rat were unattached whereas those of dog, rabbit and human were predominantly attached (>80%), and rabbit and dog cells could not be distinguished by droplet binding. Further, cells could be virtually completely attached to droplets and still display different partitioning behavior, *e.g.*, the partition ratio of human erythrocytes decreased from 55 to <5% on increasing the polymer concentrations from 5:3.5 to 5:4, yet in both phases more than 95% of cells were attached to droplets.

Evidence that cells differ in their affinity for droplets was obtained by examining the sequence of detachment of the cells from phase droplets as the interfacial tension was reduced (Fig. 2): rat erythrocytes were virtually detached at all polymer concentrations, whereas the other species' cells, while attached in the 5:3.55 system, detached in the order dog > rabbit > human as the polymer concentrations were reduced.

Charge-sensitive phases: attachment of cells to droplet surfaces

Microscopic examination of the top and bottom phase of charge-sensitive phase systems of dextran T500–PEG 8000 (or 6000) containing erythrocytes from rat, human, dog or rabbit revealed that the cells are bound on the PEG side of dextran droplets present in the top phase and on the PEG side of PEG droplets in the bottom phase. The percentage of these cells bound to dextran droplets present in the top phase was determined at the same time as their partition ratios, *P*, were measured (Table II). In the phase system of lowest polymer concentrations, 5:3.5, the rat erythrocytes have a high *P* value and were not bound to dextran droplets. Increasing the polymer concentrations (to 5:5 and to 7:4.4) led to increased binding to droplets and was accompanied by decreased cell partition ratios. A similar trend was also seen for human erythrocytes when increasing the polymer concentration from 5:3.5 to 5:4. In the 5:4 system the *P* values decreased (from 94 to 5%) in the order rat > dog > human > rabbit as reported previously [9] and were associated with an increase (from 0.3% to 97%) in the percentage of cells bound to dextran droplets. Unlike the behavior of these cells in non-charge-sensitive phase systems (Table I), high *P* values in charge-sensitive systems were associated with low percentages of cells bound to droplets.

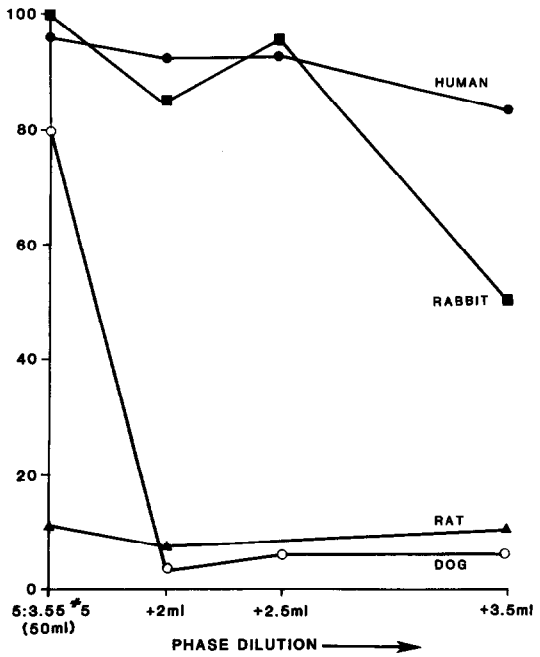

Fig. 2. Relative avidity of cells for bottom phase droplets suspended in the top phase as shown by the sequence of their detachment as the polymer concentrations of a non-charge-sensitive phase system [composed of 5% (w/w) dextran T500, 3.55% (w/w) PEG 8000, 0.15 *M* NaCl and 0.01 *M* sodium phosphate buffer (pH 6.8)] are reduced by titration with a solution containing 2% (w/w) PEG 8000, 0.15 *M* NaCl and 0.01 *M* sodium phosphate (pH 6.8). In the original system rat red blood cells are free, whereas dog, rabbit and human erythrocytes are bound. As the polymer concentrations are reduced, dog red blood cells become free first, followed by partial (50%) detachment of rabbit and (18%) human red cells. Human < rabbit < dog < rat is the sequence of these species' erythrocyte partition ratios in non-charge-sensitive phase systems [9]. See text for discussion.

Involvement of charge- or non-charge-related surface parameters in partitioning reflected by the extent of cell attachment to droplets

The *P* values of cells measured in charge-sensitive phase systems do not, in themselves, indicate the extent of involvement of charge- or non-charge-related parameters in effecting the observed partition ratio. The partition ratios and binding to droplets of dog and rabbit erythrocytes were examined in the non-charge-sensitive phase system 5:3.5 #5, the composition of which is close to the critical point, and its charge-sensitive counterpart, 5:3.5 #1 (Table III). While the *P* values of dog erythrocytes are equally high in both phase systems, the cells are predominantly attached to droplets in the non-charge-sensitive system and virtually completely free in the charge-sensitive system. Hence the electrostatic potential difference between the phases in the latter system resulted in the detachment of the cells and the resulting *P* value can be ascribed to the dog cells' surface charge. By contrast, rabbit red cells (which have a very low surface charge [9]) showed no decrease in attachment to phase droplets on switching from 5:3.5 #5 to 5:3.5 #1. Rather, there was a slight increase in attachment which, most likely, is a consequence of the higher interfacial tension associated with phase systems containing phosphate [11]. Rabbit erythrocytes had similar *P* values in both phases. Thus, even in the charge-sensitive phase system the partitioning behavior of rabbit red blood cells reflects their non-charge-related surface properties.

Phases with a polymer ligand: attachment of cells to droplet surfaces

The percentage of chicken erythrocytes bound to dextran droplets present in the top phase was determined at the same time as their partition ratios, *P*, were measured in two non-charge-sensitive phase systems: 5:4.5 #5 and 5:5 #5 (Table IV). In each instance the *P* value was zero and all the cells were bound to the droplets. Incorporation of the hydrophobic affinity ligand, PEG–palmitate, into the phase systems caused an increase in *P* associated with a decrease in the percentage of cells bound to the droplets. Higher concentrations of PEG–palmitate were required in the phase system with higher polymer concentration (*i.e.*, with 5:5) to produce increases in the *P* value and free (unbound) cells similar to those obtained in the 5:4.5 system.

Relation of cell partition ratio (and percentages of cells bound to droplets) to sampling time

The effect of doubling the usual sampling time (defined earlier) on the *P* value of human erythrocytes and their attachment to dextran droplets in the top phase was examined in both non-charge-sensitive and charge-sensitive phase systems (Table V). There was little change observed in either the *P* value or the percentage of cells attached to droplets. In experiments (not shown) using erythrocytes from other species and a number of different phase sys-

TABLE II

PARTITION RATIO, *P*, AND THE PERCENTAGE OF ERYTHROCYTES, FROM DIFFERENT SPECIES, BOUND TO BOTTOM PHASE DROPLETS IN THE TOP PHASE OF CHARGE-SENSITIVE PHASE SYSTEMS.

See text for discussion.

Species	Phase system[a]							
	5:3.5 #1[b]		5:4 #1[c]		5:5 #1[c]		7:4.4 #1[d]	
	P	% bound	*P*	% bound	*P*	% bound	*P*	% bound
Rat	93 ± 2(3)	0	94 ± 2(3)	0.3 ± 1(3)	22 ± 3(3)	91 ± 6(3)	16 ± 1(3)	87 ± 9(3)
Dog	–	–	95 ± 6(6)	2 ± 3(6)	–	–	–	–
Human	87 ± 0(3)	1 ± 2(3)	52 ± 7(6)	53 ± 13(6)	–	–	–	–
Rabbit	–	–	5 ± 3(3)	97 ± 2(3)	–	–	–	–

[a] Phase systems were composed of indicated concentrations of dextran T500, poly(ethylene glycol) 8000 and 0.11 *M* sodium phosphate buffer (pH 6.8). Data are presented ± S.D. with the number of experiments in parentheses. Note that the 5:3.5 #1 system reflects both charge-associated and non-charge-related surface properties of these cells, while 5:4 #1 reflects charge-associated properties of all species' erythrocytes except those from rat (where both charge-associated and non-charge-related properties are still reflected). At higher polymer concentrations charge-related properties determine the *P* value of all species' erythrocytes including those from rat. For a detailed discussion, see ref. 9.

[b] Settling time: 7 min horizontal + 1 min vertical.

[c] Settling time: 20 min vertical.

[d] Settling time: 10 min vertical.

tems, small decreases in *P* value, usually less than 10% and never more than 20%, were observed. These data indicate that at the usual sampling times the kinetics of partitioning have become sufficiently

TABLE III

PERCENTAGE OF RABBIT AND DOG ERYTHROCYTES BOUND TO BOTTOM PHASE DROPLETS IN THE TOP PHASE AND THE CELLS' PARTITION RATIO, *P*, IN A NON-CHARGE-SENSITIVE (5:3.5 #5) AND A CHARGE-SENSITIVE (5:3.5 #1) PHASE SYSTEM

The latter system, also being close to the critical point (*i.e.*, having the same lower polymer concentrations as the non-charge-sensitive system), reflects in addition to charge also non-charge surface parameters. Phases were permitted to settle for 7 min horizontal + 1 min vertical. Data are presented ± S.D. with the number of experiments in parentheses. See text for details.

Species	Phase system			
	5:3.5 #5		5:3.5 #1	
	P	% bound	*P*	% bound
Rabbit	76 ± 15(3)	80 ± 7(9)	65 ± 11(3)	97 ± 3(9)
Dog	100 ± 0(2)	90 ± 8(6)	102 ± 2(2)	1 ± 1(6)

TABLE IV

PARTITION RATIO, *P*, AND THE PERCENTAGE OF CHICKEN ERYTHROCYTES BOUND TO BOTTOM PHASE DROPLETS IN THE TOP PHASE OF A NON-CHARGE-SENSITIVE PHASE SYSTEM CONTAINING DIFFERENT AMOUNTS OF PEG–PALMITATE

See text for discussion.

PEG–palmitate (μg)	Phase system[a]			
	5:4.5 #5		5:5 #5	
	P	% bound	*P*	% bound
0	0	100	0	100
2	32	78		
4	71	30		
6	95	30		
8	97	5		
20			59	89
40			92	11
60			83	3
80			91	3

[a] Phase systems were composed of the indicated concentrations of dextran T500, poly(ethylene glycol) 6000, 0.15 *M* NaCl + 0.01 *M* sodium phosphate buffer (pH 6.8), and the indicated amounts of PEG 6000–palmitate. Partitions shown were carried out in 2-g phase systems (1 g of each phase) to which 0.01 ml of packed cells had been added. The *P* value was determined after 15 min of phase settling in the vertical position.

TABLE V

EFFECT OF DOUBLING THE "USUAL" SAMPLING TIME ON THE PERCENTAGE OF HUMAN ERYTHROCYTES BOUND TO BOTTOM PHASE DROPLETS IN THE TOP PHASE AND ON THE CELLS' PARTITION RATIO, *P*

Data are presented ± S.D. with the number of experiments in parentheses.

Settling time[a]	Phase system			
	5:3.5 #5		5:4 #1	
	P	% bound	*P*	% bound
7 min h + 1 min v	55 ± 5(3)	96 ± 1(3)	–	–
15 min h + 1 min v	58 ± 10(3)	95 ± 4(3)	–	–
20 min v	–	–	52 ± 7(6)	53 ± 13(6)
40 min v	–	–	49 ± 8(18)	61 ± 14(15)

[a] h = Horizontal; v = vertical.

slow that the choice of this time is not overly critical.

DISCUSSION

Microscopic analysis of erythrocytes partitioned in (dextran T500–PEG) non-charge-sensitive phase systems has previously revealed that cells tend to adhere to the surfaces of the phase droplets and streams (microphases) that are formed as phase separation proceeds and are delivered to the developing bulk interface [5,7]. These early events are complex and depend on the strength of attachment determined by the surface properties of the cell being partitioned and on the phase system composition [12,13]. They generate the *P* value since their kinetics determine the proportion of cells delivered to the bulk interface, by the time that the partition ratio is measured, and the proportion present in the top phase (either as attached to phase droplets or as free cells). The partition ratio, *P*, can be seen actually to reflect the sum of free[a] + droplet-bound cells in a bulk phase as a percentage of total cells added.

However, in most practical applications, such as the times employed in sampling a single partition tube to measure the *P* value or in the settling steps in countercurrent distribution, it is relatively late stages in the cell partitioning process, when the phases have separated to form distinct bulk phases, that prevail. We have therefore, unlike in previous reports, concentrated on an examination of these later stages of the partitioning behavior of erythrocytes.

The partitioning of erythrocytes in dextran T500–PEG 8000 (or 6000) phase systems takes place between the top phase and the interface in both charge-sensitive and non-charge-sensitive systems. The cells adhere to the PEG side of the dextran droplets found in the top phase and also to the PEG side of the relatively few PEG droplets found in the bottom phase at the time of sampling. The bulk phase into which the cells partition is thus reflected by the side of the phase droplet to which they adhere.

Partitioning of cells in non-charge-sensitive phase systems is possible only when the polymer concentrations are adequately low (low interfacial tension) to permit cells to be found in one of the bulk phases (either free or on droplets) at the usual time of phase sampling. At higher polymer concentrations all cells are at the bulk phase interface and no partitioning takes place. Hence it is particularly intriguing that erythrocytes from different species, virtually totally bound to the surface of phase droplets (Table I), the physical properties of which are presumably identical with those of the bulk interface, can still have different *P* values and be separated ([9], Table I). In fact the non-charge-sensitive phase system appears to be the only kind of phase system

[a] "Free" cells are those not bound to droplets of visible dimensions.

in which cells totally bound to droplets are separable at normal partition times (compare Tables I and II; also see ref. 14). Possible bases for these findings are suggested below.

The sequence of attachment/detachment of different species' red blood cells from phase droplets (Fig. 2) is a reflection of the cells' relative avidity for the interface. It indicates that whereas, in a given phase system (*e.g.*, 5:3.5 #5, Table I), cells from different species may, essentially, have the same percentage of cells bound to droplets, these are not equally tightly held on the droplet surface. The sequence of detachment parallels the cells' relative *P* values (rat > dog > rabbit > human) in such a non-charge-sensitive phase system (see Table I).

The effect of the difference in relative avidity of erythrocytes from different species for phase droplets on the early stages of phase separation has been summarized [11,15] and presents the most likely sequence of occurrences which results in the separation of cells all of which are found to be phase droplet-bound at the usual time of sampling.

Whereas the cell- and phase-specific kinetic differences in cell binding to droplets and of cell-bound droplet delivery to the bulk interface are dramatic in the early stages of phase separation, the change in *P* value observed with a doubling of the usual sampling time is not large (Table V; see also ref. 16) and, hence, the time of sampling is not so critical in optimizing cell separations when made beyong the time that a sharp interface has appeared.

To determine appropriate polymer concentrations for partitioning cells in charge-sensitive phases, the lowest polymer concentrations at which cells are totally at the interface in a non-charge-sensitive phase system (*e.g.*, one containing predominantly NaCl) are first established. Using the determined polymer concentrations one replaces the salt with one that produces an electrostatic potential difference between the phases (*e.g.*, phosphate). Partitions carried out in charge-sensitive phases at or above such polymer concentrations are deemed to be charge-sensitive; below such polymer concentrations (*i.e.*, in phases having lower interfacial tensions) partitioning can also reflect non-charge-associated surface properties [9,15,17].

Table II indicates that an inverse relationship exists between erythrocyte *P* values in charge-sensitive phases and the percentage of cells bound to dextran phase droplets in the top phase. Cells totally bound to droplets in charge-sensitive phases would be exclusively at the bulk interface. This differs from the behavior of cells in non-charge-sensitive phases (Table I) in which, as already indicated, cells virtually completely bound to phase droplets can still have different *P* values. In charge-sensitive phases the partitioning of cells appears to take place because cells are "pulled" off the phase droplets in relation to their charge-associated properties (giving rise to the cell partition sequence shown in Table II). Just as with non-charge-sensitive phase systems, the effect of doubling the usual sampling time on the *P* value observed in charge-sensitive phases is not large (see Table V and Results) and hence some leeway exists in its choice.

The partition ratio of erythrocytes from different species in dextran T500–PEG phase systems increases with increasing amounts of PEG–palmitate ([9], Table IV). The higher cell *P* values result from a decrease in the number of phase droplet-bound cells. Cells in phases containing PEG–palmitate behave as if their interaction with phase droplets is weakened. Evidence for this has been provided by the wetting behavior of erythrocytes in dextran–PEG systems [11]. By binding to the cell surface (hydrophobically), PEG–palmitate increases the tendency for the PEG phase to wet the surface (contact angle measurements [11,15]) and, as it were, "pull" the cell free and into the PEG phase.

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