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PARTITIONING BEHAVIOR OF ERYTHROCYTES IN AQUEOUS TWO-PHASE SYSTEMS CONTAINING HYDROXYPROPYL STARCH AND POLYETHYLENE GLYCOL

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SUMMARY

The partitioning behavior of erythrocytes in Reppal PES 200 (a hydroxypropyl starch produced by Reppe Glykos AB, Växjö, Sweden)–polyethylene glycol (PEG) and in dextran (Dx)–PEG aqueous phase systems made isotonic with phosphate is similar in a number of ways: (i) There is a correlation between the relative electrophoretic mobilities and partition ratios, P, of red blood cells from different species; (ii) The cell P is reduced when, at constant polymer concentrations, phosphate is systematically replaced by sodium chloride (with the total concentration isotonic); (iii) The cell P is increased with reduced polymer concentrations (decreased interfacial tensions); (iv) Treatment of erythrocytes with neuraminidase results in a reduced P value; (v) Rat red cells of different ages can be fractionated by counter-current distribution; and (vi) Differences between red blood cells from genetically distinct rats or between humans can be detected. Aquaphase (a hydroxypropyl starch marketed by Perstorp AB, Lund, Sweden) has been tested as in ii–iv above with analogous results.

The partitioning behavior of erythrocytes in PES–PEG and Dx–PEG aqueous phase systems containing sodium chloride differs in a number of ways: (vii) The correlation, apparent in Dx–PEG systems, between the P value of red blood cells from different species and the ratio of their membrane poly- to monounsaturated fatty acids is absent in PES–PEG systems. It is replaced by a correlation as in i; (viii) The increase in P value in Dx–PEG observed from red blood cells after treatment with neuraminidase is replaced by a decrease in P value in PES–PEG or Aquaphase–PEG systems.

We conclude that PES (and Aquaphase) can be substitutes for dextran in cell partitioning studies when charge-sensitive phases are used (*e.g.*, those containing phosphate) while separations based on properties reflected by Dx–PEG systems containing sodium chloride are not duplicated by PES–PEG (and probably not by Aquaphase–PEG). The hydroxypropyl starch–PEG systems containing sodium chlo-

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ride, unlike the analogous Dx–PEG systems, have a significant electrostatic potential difference between the phases.

INTRODUCTION

When aqueous solutions of structurally distinct polymers are mixed above certain concentrations immiscible two-phase systems are obtained with one of the phases rich in one of the polymers and the other phase rich in the second polymer[1,2]. With appropriate selection of polymers such phase systems, when buffered and rendered isotonic, are mild and not deleterious to biological particulates (cells, organelles, membranes) partitioned in them[3]. Dextran (Dx) and polyethylene glycol (PEG) is the most widely used polymer combination and the physicochemical properties of Dx–PEG aqueous phase systems as well as the partitioning behavior of biomaterials in such phases have been studied in considerable detail[1,2].

In the past few years the use of aqueous two-phase systems has expanded into many areas of biotechnology[4]. The high cost of dextran has thus prompted the search for suitable yet cheaper polymer substitutes. Starch was an obvious condidate[5]. Yet starch presented a major problem because of its significant gel-forming tendencies in solution. Introduction of hydroxyalkyl ether groups into starch reduces gel formation[6].

Recently two hydroxypropyl derivatives of starch (Reppal PES-200, Aquaphase PPT) have been marketed commercially. Aquaphase has been examined and compared with dextran in aqueous two-phase systems formed between each of these polymers and PEG so far as protein partitioning is concerned[5,7]. Aquaphase–PEG and Dx–PEG phases have been found to give comparable results in these studies[5,7].

The partitioning of cells in Dx–PEG phase systems depends on the interaction of the surface properties of the cells with the physical properties of the phase systems[8]. The latter can, to a large extent, be manipulated by appropriate selection of polymer concentration and ionic composition and concentration[9]. Thus, charge-sensitive or non charge-sensitive phase systems can be prepared while the incorporation of a polymer-ligand, which itself favors one of the phases, can give rise to cell separations which depend on the interaction of a surface property or receptor with the ligand[9].

In the present study we tested whether a variety of phase parameters known to influence the partition ratios of cells in Dx–PEG systems[8] has analogous effects in PES–PEG and, in some cases, Aquaphase–PEG systems. The versatility, observed in Dx–PEG systems, in separating cells on the basis of different surface properties was examined in the new phases. The sensitivity of the phases to differences in surface properties which, as one example, permits detection, in Dx–PEG systems, of differences between closely related cell populations (*e.g.*, red blood cells from different rat strains[10]), was also explored. Because of the large amount of information available on red cell partitioning behavior in Dx–PEG systems[8], erythrocytes from different species provide an excellent model for probing factors which operate in other phase systems.

EXPERIMENTAL

Rat injection and bleeding

Male Sprague–Dawley rats (Charles River Labs., Wilmington, MA, U.S.A.), weighing 275–400 g, were used. In one experiment (for a purpose described below) the animals were male, spontaneously hypertensive (SHR) or normotensive Wistar–Kyoto (WKY) rats. The SHR and WKY were supplied by Harlan Sprague–Dawley (Indianapolis, IN, U.S.A.). Rats were exsanguinated by heart puncture and the blood collected in anticoagulant acid–citrate–dextrose (ACD) using a ratio of 2.5–5 parts blood to 1 part ACD.

Injection of rats (with about 15 μCi) of [^{59}Fe]ferrous citrate (ICN, Irvine, CA, U.S.A.) via the saphenous vein as well as the ^{59}Fe counting procedures used have previously been detailed[11]. Such rats were bled as described above at different times after injection. Red cell populations are obtained thereby in which the cells that are labeled correspond in age to the time elapsed between isotope injection and bleeding.

Blood collection from other species

Quantities of 5–10 ml of blood from human, dog, pig and sheep were obtained by venipuncture and that from rabbit by nicking the ear marginal vein. ACD, in the ratios to blood indicated above, was used as anticoagulant in all cases.

Neuraminidase treatment of erythrocytes

Human or rat red blood cells from approximately 1.5 ml of blood were washed and incubated with or without neuraminidase (*Vibrio cholerae*; Calbiochem–Behring, La Jolla, CA, U.S.A.) and washed again as previously described[12]. The treated and untreated red cells were used in partitioning experiments described below.

Labeling of erythrocytes with [^{51}Cr]chromate in vitro and the comparison of surface properties of closely related cell populations

The labeling of red blood cells with [^{51}Cr]chromate, the counting procedures used and the preparation of mixtures of labeled and unlabeled red blood cells for analysis by counter-current distribution (see below) have all been described previously in detail[13–15]. In short, approximately 10 μCi of [^{51}Cr]chromate (ICN) was used per ml of an aliquot of anticoagulated blood suspension. Both labeled and unlabeled cells were then washed five times with isotonic aqueous salt solution. To compare the surface properties of erythrocytes from two rats, A and B (*e.g.*, WKY and SHR), 0.15 ml of [^{51}Cr]chromate labeled, washed red blood cells is pipetted into a centrifuge tube containing 5 ml of saline. Then 0.6 ml of unlabeled, washed red blood cells with which the labeled red blood cells are to be compared is pipetted into the same tube. Cells in the tubes are gently mixed and centrifuged. The supernatant solution is discarded, and the mixed cells are used to make the "load mix" (see below). An excess of unlabeled cells is mixed with labeled cells so that subsequent analysis of cell distribution curves by hemoglobin absorbance will reflect primarily the unlabeled cell population, while the distribution of the labeled cell population can be obtained by isotope counting. Four mixtures are examined in such an experiment: ^{51}Cr-labeled red blood cells A plus unlabeled red blood cells B (A + B); ^{51}Cr-labeled red blood cells B plus unlabeled red blood cells A (B + A); ^{51}Cr-labeled

red blood cells A plus unlabeled red blood cells A (A + A); and ^{51}Cr-labeled red blood cells B plus unlabeled red blood cells B (B + B). Mixing ^{51}Cr-labeled red blood cells with unlabeled red blood cells that are of the same population is an essential control to indicate that the ^{51}Cr-labeling procedures per se have no effect on the surface properties[13–15].

Preparation of phase systems

Aqueous two-phase systems were prepared from polymer and salt stock solutions as described previously[16]. Dextran T500 [number average molecular weight, M_n, (180–220) · 10^3, lot G1 21917; Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.], Reppal PES 200 (PES, M_n = 17 000, batch No. 023/14, Reppe Glykos, Växjö, Sweden) or Aquaphase PPT [Aquaphase, M_n (30–35) · 10^3; Perstorp AB, Lund, Sweden] were used in the current work as one of the polymers while PEG 8000 [M_n (7–9) · 10^3, "Carbowax 8000"; Union Carbide, New York, NY, U.S.A.] was the second polymer in each case.

To avoid having to repeat *in toto* the phase system compositions used throughout the text the following notation is employed. The first number gives the percentage of the first polymer (Dextran, PES or Aquaphase as indicated). The number after the hyphen gives the percentage of PEG. (Both percentages are w/w). The integer following the # sign indicates the salt composition as follows: #1 contains 0.11 *M* sodium phosphate buffer, pH 6.8 (composed of equimolar quantities of monobasic and dibasic sodium phosphates); #2 consists of 0.09 *M* sodium phosphate buffer and 0.03 *M* sodium chloride; #3 of 0.06 *M* sodium phosphate buffer and 0.075 *M* sodium chloride; #4 of 0.03 *M* sodium phosphate buffer and 0.12 *M* sodium chloride; and #5 of 0.01 *M* sodium phosphate buffer and 0.15 *M* sodium chloride.

The effect of salt composition and concentration as well as polymer concentration on the physical properties of Dx–PEG phase systems and the interaction of these with the surface properties of cells have been described[8,9]. These previous findings will be compared in the Results and discussion section with those obtained in the current work with aqueous phases containing PES–PEG and Aquaphase–PEG.

After preparation, phase systems for counter-current distribution (see below) were equilibrated in a separatory funnel at 4–5°C. Top and bottom phases were then separated. Phases for single tube partitioning experiments (see below) were equilibrated in 10 ml calibrated test tubes at 22–24°C. After equilibration top and bottom phase volumes were adjusted to be equal and the top phase volume was recorded.

Partitioning of erythrocytes

The partition procedure used with erythrocytes has previously been described[12] and will be outlined only in brief. A known aliquot of washed, packed cells (usually 0.1 ml) was added to a partition tube containing (about 10 g of) the phase system to be used with top and bottom phase volumes equalized (see above). Tubes were well mixed and the phases were permitted to settle by the clock (see tables for phase compositions and settling conditions) with the tubes in either vertical or horizontal position (to speed settling times of phases, see ref. 8). After settling, a known aliquot of the top phase was withdrawn and analyzed for the quantity of cells present (see below).

Counter-current distribution (CCD) of erythrocytes

Our thin-layer CCD plates have 120 concentric cavities (Workshop, University of Lund, Sweden). The bottom plate cavities have a capacity of 0.7 ml. Washed, packed red blood cells were suspended in ten times their volume of top phase ("load mix") of the system in which CCD was to be carried out. 0.5 ml of bottom phase was delivered into each of the 120 cavities. When four different populations were to be examined by CCD concurrently, 0.7 ml of one "load mix" was pipetted into cavities 0–2, a second "load mix" into cavities 30–32, a third into cavities 60–62, and the fourth into cavities 90–92. All other cavities received 0.7 ml top phase. When only two populations were to be compared cavities 0–2 and 60–62 received the two load mixes. With the phase system used, the partition ratios of the rat red blood cells were such that 39 transfers could be carried out even with four preparations simultaneously and without overlap. A settling time of 8 min and a shaking time of 36 s was used with phase system 11 PES–6 PEG #1. Runs were performed at 4–5°C.

Analysis of cells after CCD or single-tube partitioning

Cell concentration. After CCD the cells in each cavity were collected, by use of a fraction collector, directly into centrifuge tubes. Isotonic aqueous salt solution (0.7 ml) was added to each tube thereby reducing the polymer concentrations and giving rise to a single homogeneous suspending medium. Cells in each three adjacent tubes were pooled. They were centrifuged at 1200 *g* for 10 min, the supernatant solution discarded, and the cells lysed by addition of 3 ml of 20 milliosmolar sodium phosphate buffer, pH 7.2. The tubes were then centrifuged at high speed to remove the stromal residue and the hemoglobin absorbance of the supernatant solution was measured at 540 nm. In an analogous manner the concentration of cells was obtained (in terms of hemoglobin absorbance) in the top phase after a single tube partitioning (see above) as well as in an aliquot equal to total cells added to the partition tube.

Isotope determination. In experiments in which subpopulations of cells were isotopically labeled (see above), a known aliquot of each hemolysate prepared after CCD was counted in a Beckman scintillation well-counter set to either the ^{59}Fe or ^{51}Cr setting (depending on the isotope used in the experiment).

Presentation of data

The partition ratio, *P*, of cells in the various tables is given as the Quantity of cells in the top phase as a percentage of total cells added to the phase system.

In Fig. 1, the log distribution ratio of erythrocytes (ratio of number of cells in top phase per number of cells at interface plus bottom phase) from a number of species in phase systems of indicated compositions is plotted against either the electrophoretic mobility of red cells ($\mu m\ s^{-1}\ V^{-1}\ cm$) of the indicated species[17] or the species' red cell membrane ratio of poly- to monounsaturated fatty acids[18].

Total cell distribution of CCD curves is given in terms of hemoglobin absorbance (at 540 nm). Labeled (^{59}Fe or ^{51}Cr) cell populations are given in counts per min (cpm). A relative specific activity is also shown through the distribution curves and reflects the extent of displacement and, hence, of difference between any two such red cell populations. It is defined as: (cpm per unit hemoglobin absorbance in a given cavity)/(cpm per unit hemoglobin absorbance in the original cell population prior to CCD).

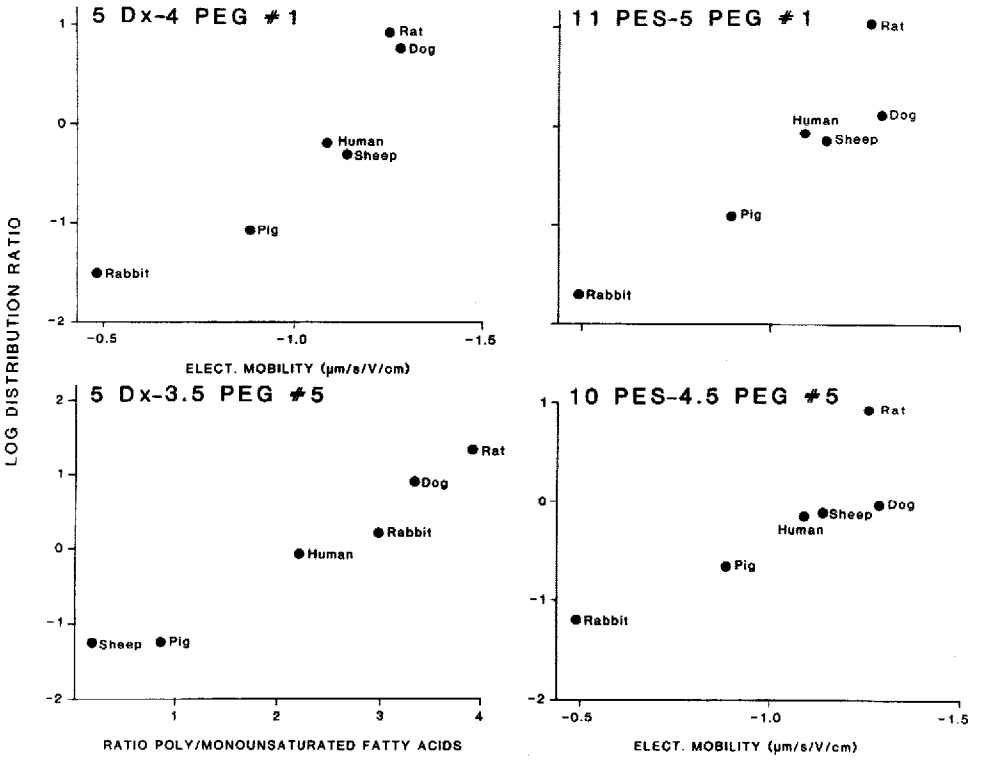

Fig. 1. (Top, left): log distribution ratio (ratio of number of cells in the top phase per number of cells at interface plus bottom phase) in a charge-sensitive dextran (Dx)–polyethylene glycol (PEG) phase system (*i.e.*, 5% Dx T500–4% PEG 8000 #1) plotted against the relative electrophoretic mobility of erythrocytes from a number of different species. (Bottom, left): log distribution ratio in a non charge-sensitive Dx–PEG phase system nearer the critical point (*i.e.*, 5% Dx–3.5% PEG #5) plotted against the ratio of membrane poly- to monounsaturated fatty acids of erythrocytes from a number of different species. (Top, right): experiment as in upper left panel but in a phase system 11% PES 200–5% PEG 8000 #1. (Bottom, right): experiment as above but in 10% PES 200–4.5% PEG 8000 #5. Settling times were as in Tables II and III for the respective phases; 5% Dx–3.5% PEG #5 was permitted to settle 7 min in the horizontal plus 1 min in the vertical position.

RESULTS AND DISCUSSION

Partitioning behavior of red blood cells from different species in PES–PEG vs. Dx–PEG systems containing phosphate.

Although dextran and PEG are non-ionic polymers some salts, notably phosphate, have different affinities for the two phases[9]. An electrostatic potential difference between the phases results, top phase positive, and cells, in such charge-sensitive phases, tend to have partition ratios in which surface charge plays a role. In the case of red blood cells from different species a correlation exists between their partition ratios and electrophoretic mobilities[19] (Fig. 1, upper left panel). Analogous results are found when red cells from different species are partitioned in a PES–PEG system containing phosphate (upper right panel, Fig. 1). It therefore appears that the PES–PEG phases containing phosphate also have an electrostatic potential difference between them.

TABLE I

EFFECT OF REDUCING THE POLYMER CONCENTRATION (AND INTERFACIAL TENSION) ON THE PARTITION RATIO OF ERYTHROCYTES

Partition ratio is the quantity of cells in the top phase as a percentage of total cells added.

*Phase system**	*Partition ratio*	
	Human RBC	*Rat RBC*
11% PES–5% PEG #1	50	85
10% PES–5% PEG #1	52	92
10% PES–4% PEG #1	95	87
11% PES–5% PEG #5	17	26
10% PES–5% PEG #5	29	43
10% PES–4% PEG #5	88	93

* Settling time: 10 min in the horizontal position plus 1 min in the vertical position.

Partioning behavior of red blood cells from different species in PES–PEG vs. Dx–PEG systems containing sodium chloride

If, instead of phosphate, sodium chloride is the predominant salt used in Dx–PEG systems there is virtually no potential difference between the phases[9] and, at some distance from the critical point (*i.e.*, at higher polymer concentrations), the cells, under such conditions, tend to be at the interface. As the polymer concentrations are reduced (giving rise to lower interfacial tensions, see below) the cells once again partition probably due to their surfaces' interaction with PEG[12]. The cell partition ratio in such non charge-sensitive phases correlates, at least in the case of red blood cells from different species, with the ratio of their membrane poly- to monounsaturated fatty acids[12] (Fig. 1, lower left panel), a lipid parameter.

If the partitioning of erythrocytes from different species is examined in PES–PEG phases close to the critical point and containing sodium chloride one finds that the partition ratios of cells correlate with the cells' electrophoretic mobilities (compare lower left and right panels in Fig. 1). Thus, those properties reflected by non charge-sensitive Dx–PEG phase systems (Fig. 1, lower left panel) are not duplicated by the PES–PEG systems. It appears that PES–PEG phase systems containing sodium chloride, unlike Dx–PEG systems, have an appreciable electrostatic potential difference between the phases.

Partitioning behavior with decreasing polymer concentrations

One can think of the normal position of cells in phase systems as being the interface[8]. That is where cells have their lowest energy. When, at constant ionic composition and concentration, the polymer concentration is reduced the partition ratio of cells in Dx–PEG systems increases[20]. This is a consequence of the concomitant reduction in interfacial tension which permits cells to be more easily pulled out of the interface by the factors acting on them.

When the polymer concentrations of PES–PEG phase systems (irrespective of whether they contain phosphate or sodium chloride), are reduced the partition ratios of (red) cells increase (Table I) just as in the case of Dx–PEG systems. Similar results

TABLE II

PARTITION RATIO OF RAT AND HUMAN ERYTHROCYTES IN A PES–PEG PHASE SYSTEM OF CONSTANT POLYMER CONCENTRATION BUT CONTAINING DIFFERENT QUANTITIES OF SODIUM PHOSPHATE BUFFER (pH 6.8) AND SODIUM CHLORIDE

*Phase system**	*Partition ratio*	
	Human RBC	*Rat RBC*
11% PES–5% PEG #1	40	83, 75
11% PES–5% PEG #2	24	47, 47
11% PES–5% PEG #3	14	39, 39
11% PES–5% PEG #4	7	36, 30
11% PES–5% PEG #5	5	23, 27

* Settling time: 10 min in the horizontal position + 1 min in the vertical position.

(not shown) were also obtained with Aquaphase–PEG systems. 12.5 Aquaphase–5 PEG and 14 Aquaphase–5 PEG were used for partitioning human and rat red cells.

Partitioning behavior of erythrocytes with changes in salt composition (ratio of phosphate to chloride)

If at constant Dx–PEG polymer concentrations phosphate is decreased and sodium chloride increased in a manner such that the overall ionic concentrations are essentially isotonic for red cells, the cells will have their highest partition ratio in the system containing phosphate and the lowest partition ratio in that containing sodium chloride[20]. That is because the electrostatic potential difference between the phases is being systematically reduced under such conditions[8].

Table II shows the partition ratios obtained with human and rat erythrocytes when they are partitioned in PES–PEG phase systems of constant polymer concentrations but changing ratio of phosphate to chloride (total salt concentration approximately isotonic in each case). A decrease in partition ratio is in evidence, as is the case in Dx–PEG systems[20], with a decrease in the indicated ionic ratio. Similar results (not shown) were also obtained with Aquaphase–PEG systems using 14 Aquaphase–5 PEG or 12.5 Aquaphase–5 PEG and salt compositions as in Table II. Human and rat red cells were partitioned.

Although (see Fig. 1, lower right panel) PES–PEG systems containing sodium chloride reflect surface properties that differ from those reflected by the analogous Dx–PEG systems (Fig. 1, lower left panel), the data in Table II imply that PES–PEG systems containing sodium chloride have lower electrostatic potential differences between the phases than do PES–PEG systems of the same polymer concentrations but containing phosphate.

Effect of neuraminidase treatment of erythrocytes on their partitioning behavior in phase systems containing phosphate or sodium chloride

When human or rat red blood cells are treated with neuraminidase their partition ratios diminish in charge-sensitive Dx–PEG systems (*e.g.*, those containing phosphate) but increase in non-charge-sensitive Dx–PEG phases (*e.g.*, those with sodium chloride)[12]. Neuraminidase releases sialic acid, the erythrocytes' main char-

TABLE III

EFFECT OF NEURAMINIDASE TREATMENT ON THE PARTITION RATIO OF HUMAN ERYTHROCYTES IN PES–PEG *vs.* DX–PEG SYSTEMS

Cell	*Partition ratio in phase**			
	A	*B***	*C*	*D*
Human RBC	22, 21	54	48, 41	91
Human RBC after neuraminidase treatment	82, 68	8	24, 22	31

* Phase system compositions and settling times: (A) 5% Dx–3.6% PEG #5, 7 min horizontal plus 1 min vertical; (B) 5% Dx–4% PEG #1, 20 min vertical; (C) 10% PES–4.5% PEG #5, 10 min horizontal plus 1 min vertical; (D) 10% PES–4.5% PEG #1, 10 min horizontal plus 1 min vertical.

** From ref. 12.

ge-bearing group, from the cell surface thereby reducing their surface charge. The increase in partition ratio in non-charge-sensitive systems may be due to an enhanced proximity between cell surface and PEG in the absence of sialic acid[12].

The effect of neuraminidase treatment on the partitioning behavior of human erythrocytes in PES–PEG and Dx–PEG systems is contrasted in Table III. The partition ratio of neuraminidase-treated cells decreases in a PES–PEG system containing phosphate (*e.g.*, 10 PES–4.5 PEG #1). However, unlike the results in a Dx–PEG system containing sodium chloride the human erythrocytes' partition ratio in 10 PES–4.5 PEG #5 is lower following the cells' treatment with neuraminidase. This result supports the conclusion (see also Fig. 1, lower right panel) that both of these PES–PEG systems are charge-sensitive.

Results similar to those found with neuraminidase-treated red cells in PES–PEG #1 and #5 systems (Table III) were also in evidence (data not shown) in Aquaphase–PEG systems (12 Aquaphase–4.5 PEG #1 and #5). We conclude, therefore, as we did above for PES–PEG systems, that both Aquaphase–PEG #1 and #5 are charge-sensitive.

Sensitivity of phase systems in detecting surface differences between closely related cell populations and subpopulations

By combining isotopic labeling and CCD techniques with Dx–PEG systems one can detect subtle differences between rat red blood cells of different ages[8], from different individuals (independent of major blood group)[21] or from different rat strains[10].

Rat red blood cells of different ages. Bleeding of rats at different times following their injection with ^{59}Fe yields erythrocytes in which those cells are labeled which correspond in age to the time elapsed between injection and bleeding[8]. Analysis of total and labeled cell distributions following CCD reveals that rat red cells of different ages have distinct partition ratios. Reticulocytes have the lowest partition ratios of any cells in the population[8]. Maturation of these cells to young erythrocytes is accompanied by a rapid increase in partition ratio so that the mature youngest erythrocytes have the highest partition rato of any red cells in the peripheral blood[8]. The partition ratio then diminishes as the erythrocytes age over their entire life-span[8].

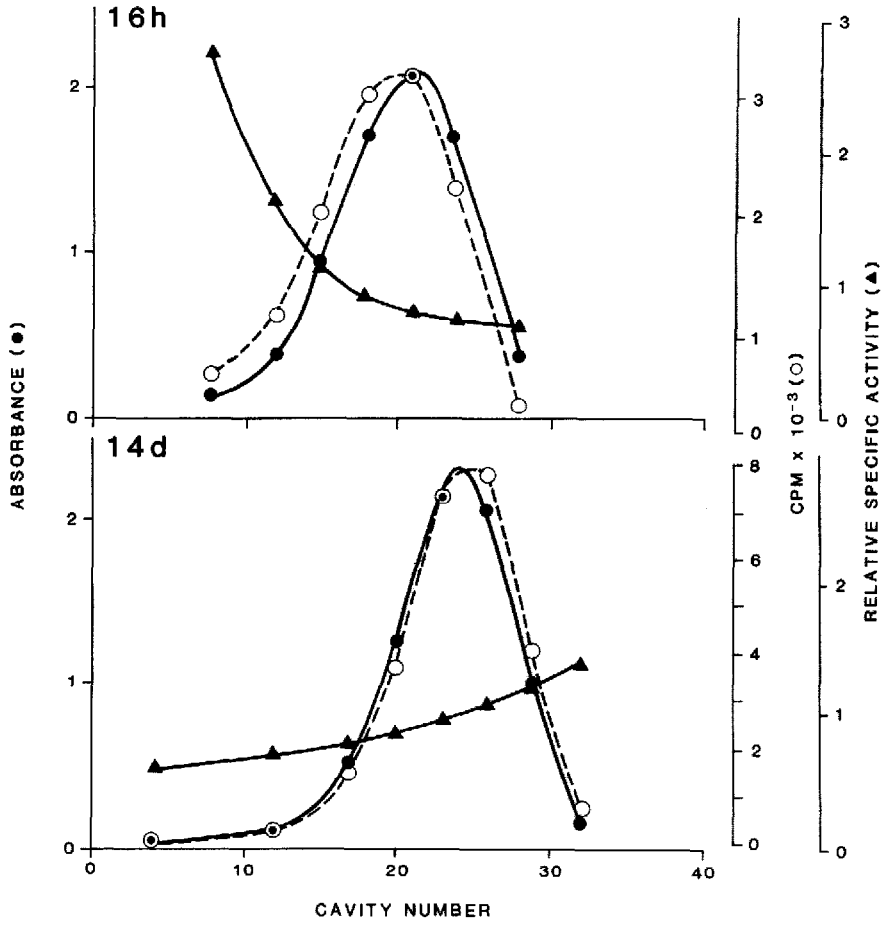

Fig. 2. Rats were injected with [^{59}Fe]ferrous citrate and bled either 16 h or 14 days after injection. This gave rise to rat red cell populations in which cells corresponding in age to the time elapsed between injection and bleeding were labeled. Such cell populations were subjected to countercurrent distribution in a phase system containing 11% PES 200–6% PEG 8000 #1 (39 transfers, 8 min settling, 36 s shaking). The runs were analyzed for total cell distribution (●, in terms of hemoglobin absorbance at 540 nm), and labeled cell distribution (○, in terms of cpm). A relative specific activity is also presented (▲) with 1.0 being the specific activity of the original, unfractionated cell population. The data indicate a distribution of 16 h old cells (top) to the left and 14 d old cells (bottom) to the right of the entire rat red cell population.

CCD experiments in a PES–PEG system with rat red cell populations in which reticulocytes (Fig. 2, top) or 14-day old erythrocytes (Fig. 2, bottom) were labeled give results similar to those obtained in Dx–PEG[8]. Reticulocytes have a low partition ratio (*i.e.*, are to the left of the bulk of red cells) while 14-day old erythrocytes have a higher partition ratio. Thus charge-sensitive PES–PEG systems reflect subtle differences in surface properties as do charge-sensitive Dx–PEG systems.

Erythrocytes from different rat strains. Surface differences between closely related cell populations can often be detected by labeling aliquots of cell populations

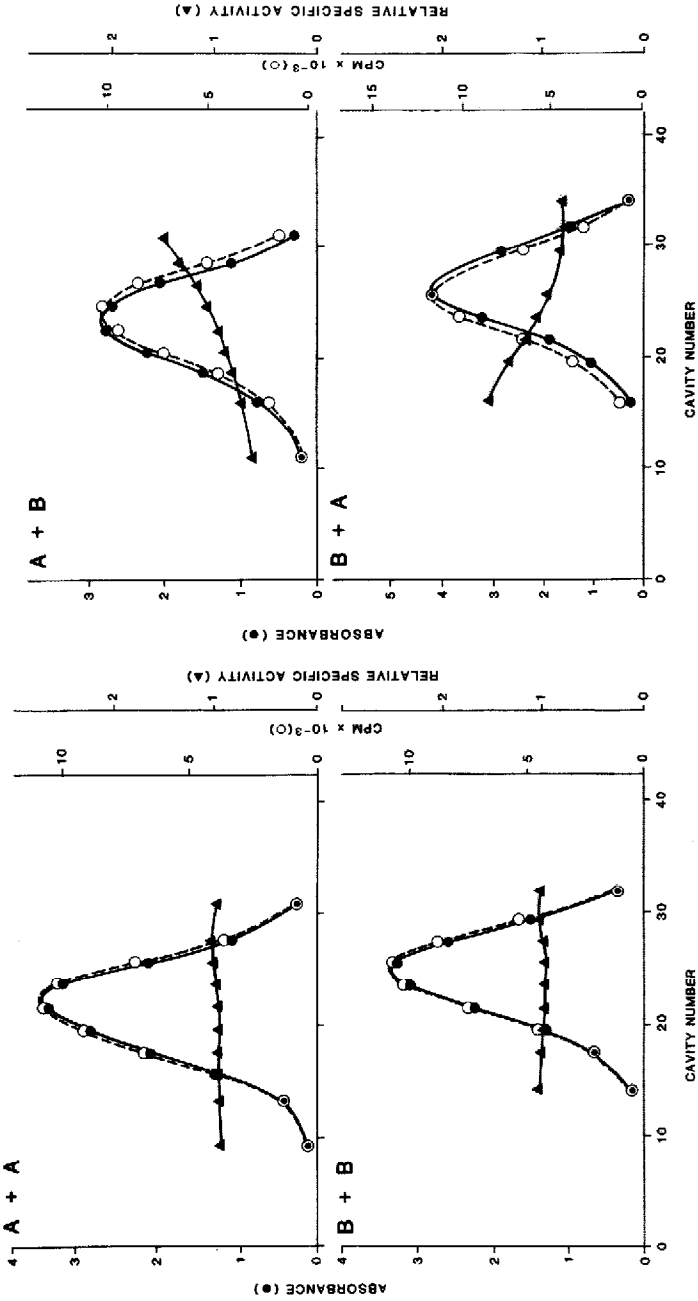

Fig. 3. Comparison of surface properties of red blood cell populations obtained from two genetically distinct strains of rats, WKY (A) and SHR (B). Blood was drawn from A and B. Aliquots of red cells from each were labeled with [^{51}Cr]chromate. Labeled cells were washed and mixed with an excess of unlabeled red cells from the other rat (A + B, B + A). As controls, aliquots of labeled cells were also mixed with an excess of unlabeled cells from the same rat (A + A, B + B). The four mixtures were subjected to CCD in a system composed of 11% PES 200–6% PEG 8000 #1 which is charge-sensitive (see Results and discussion). Thirty-nine transfers were completed at 4°C with a settling time of 8 min and a shaking time of 36 s. Analysis of distribution curves was analogous to that described in Fig. 2. Note that WKY red cells (A) have a higher partition ratio than do SHR erythrocytes (B).

with [^{51}Cr]chromate, mixing such cells with an excess of cells with which they are to be compared, subjecting the mixture to CCD in a Dx–PEG system and determining the extent of overlap or displacement of labeled and unlabeled cell populations[10,13–15,21].

In Fig. 3 we show an experiment in which such a CCD was carried out in a PES–PEG phase system. It is clear (upper right-hand part) that "A" (open circles) representing the red cells from a WKY rat has a higher partition ratio than do the erythrocytes from an SHR rat (solid circles). In the lower right-hand part of Fig. 3 the reverse mixture indicates once again that the red cells from WKY ("A", now solid circles) have the higher partition ratio. In the left parts of the figure we show the perfect overlap of labeled "A" with unlabeled "A" and labeled "B" with unlabeled "B", control experiments required to ascertain that the labeling procedure per se does not affect the cells' partition ratios. Erythrocytes from two rat strains, WKY and SHR, can thus be differentiated in charge-sensitive PES–PEG phases. Furthermore, the red cells from WKY have a higher partition ratio than those from SHR. These results are identical to the ones reported using a charge-sensitive Dx–PEG phase system[15]. Charge-sensitive PES–PEG systems are hence capable of revealing surface differences between cell populations as do charge-sensitive Dx–PEG phases.

CONCLUSION

We find the partitioning behavior of cells in the charge-sensitive Dx–PEG and PES–PEG (or Aquaphase–PEG) phase systems containing phosphate to be quite comparable. Separations based on properties reflected by the non-charge-sensitive Dx–PEG systems containing sodium chloride are, however, not duplicated by PES–PEG (or Aquaphase–PEG). The hydroxypropyl starch–PEG systems containing sodium chloride, unlike the analogous Dx–PEG systems, have a significant electrostatic potential difference between the phases.

When these data were presented at the recent Partitioning Conference[22] it was revealed[23] that the hydroxypropyl starches, unlike dextran, have, as a consequence of their preparation, a negative charge. The latter may be related to the potential difference between the phases of hydroxypropyl starch–PEG systems. It is possible that the selection of salts other than sodium chloride (*e.g.*, sodium bromide[24]) might reduce the potential difference between the phases. Perstorp is currently changing its manufacturing process to reduce the negative charge on Aquaphase[23].

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