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SURFACE ALTERATIONS OF ERYTHROCYTES WITH CELL AGE: RAT RED CELL IS NOT A MODEL FOR HUMAN RED CELL

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SUMMARY

The charge-related surface properties of human and rat young and old erythrocytes were examined by partitioning in an aqueous dextran-poly(ethylene glycol) phase system having an electrostatic potential difference between the phases and by electrophoresis. It was found (a) that while rat red cells undergo changes in surface charge-related properties as a function of cell age which are detectable by partitioning, human erythrocytes do not; (b) that analytical particle electrophoresis after countercurrent distribution confirms both the alteration in rat and its absence in human erythrocytes; and (c) that particle electrophoresis cannot detect the age-related charge-associated alteration in rat red cells without prior cell partitioning.

INTRODUCTION

Mixtures of aqueous solutions of dextran and of poly(ethylene glycol) give rise to two-phase systems suitable for the separation and subfractionation of cell populations by partitioning (1,2). Certain salts (e.g., phosphates) have different affinities for the two phases (3) and an electrostatic potential difference between the phases results, with the top phase positive (4). Cells, when added to such systems at appropriate polymer concentrations, will partition according to surface charge-associated properties (5,6). Using isotopic labeling techniques we have previously found that rat erythrocytes of different ages have characteristic partition coefficients, K [i.e., quantities of cells in the top phase as a percentage of total cells added]. They can thus be

Abbreviations: K, partition coefficient; CCD, countercurrent distribution.

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subfractionated by countercurrent distribution (CCD) in dextran-poly(ethylene glycol) aqueous phase systems which contain phosphate (7). Since the K of mature rat erythrocytes decreases with cell age and there is a concomitant decrease in cell electrophoretic mobility with decreasing K through the CCD extraction train (6), it appears that rat red cells of increasing in vivo age have decreasing surface charge as reflected both by partitioning and electrophoresis. In contrast, human young and old red blood cells prepared by the density fractionation method of Murphy (8) have the same electrophoretic mobilities (9,10). Since the charge which determines partitioning in charged dextran-poly(ethylene glycol) aqueous phase systems is not necessarily the same as that measured by electrophoresis (11) we decided to examine, under similar conditions, the partitioning and electrophoretic behavior of human versus rat red blood cells of different in vivo ages obtained by use of the Murphy centrifugation technique (8).

EXPERIMENTAL METHODS

Blood collection

Ten ml of blood was obtained by venipuncture from human subjects and by heart puncture from adult, male Sprague-Dawley rats. Blood (10 ml) was defibrinated by gentle manual swirling in a 50 ml Erlenmeyer flask containing five 5 mm diameter glass beads.

Preparation of young and old red blood cell populations

Young and old red cells were separated by the method of Murphy (8) but employing centrifuge tubes of 0.5 cm bore and 8 cm length. It was verified that the top 5% (young) and the bottom 5% (old) of the centrifuged cell column as used in the experiments were enriched with respect to young or old cells, respectively, by measuring the levels of aspartate aminotransferase (a marker for young red cells, 12,13). The specific enzyme activity ratio of cells from top/bottom was between 2 and 3.

Preparation of phase systems

The phase systems used were selected as described by Walter (2) and had the following compositions: 5% (w/w) dextran T500, lot no. 5556 (Pharmacia Fine Chemicals), 4% (w/w) poly(ethylene glycol) 6000 (recently renamed "8000" by Union Carbide) and either 0.09 M Na-phosphate buffer, pH 6.8, 0.023 M NaCl, and 5% (w/w) heat-inactivated fetal calf serum (Grand Island Biological Co.) or 0.105 M Na-phosphate buffer, pH 6.8, and 5% (w/w) heat-inactivated fetal calf serum. Phases were equilibrated at 4°C in separatory funnels and then top and bottom phases were separated.

Countercurrent distribution of red cells

Countercurrent distribution was carried out on young and old red cells (from human and rat) as previously described (14) except that the total quantity of cells loaded was between 1 and 2 x 10^8. Since our countercurrent plates have 120 cavities (15), 39 or 59 transfers were carried out on the

young and old red cells in a given experiment, simultaneously and without overlap by loading the two preparations on opposite sides of the plate. Conditions for the countercurrent distribution experiments are given in the captions to Figures 1 and 2.

Electrophoretic mobilities of red cells

Mobilities of cells were measured in a cylindrical chamber apparatus at 25°C using Ag/AgCl electrodes (10). Cell samples were washed three times in standard saline and examined in this medium.

Electronic cell counting

Cell counts for the load mix and in different cavities along the extraction train were obtained by use of an Electrozone Celloscope (Particle Data, Chicago, IL), fitted with a 76 μm orifice tube.

RESULTS AND DISCUSSION

These studies were undertaken to clarify some of the physicochemical changes which occur in red cell membranes during aging in vivo. Our electrophoretic observations confirm earlier reports (9,10) that human young red blood cells have the same electrophoretic mobility as old erythrocytes implying that the surface charge density remains constant during aging. An interpretive problem with this observation is that sialic acid, which is the main charge-bearing moiety on the surface of red cells (16,17), decreases on a per cell basis during human erythrocyte aging (10,18). The dilemma can be resolved as there appears to be less membrane associated with human old red cells (18-20) and it would therefore be possible to maintain the density of charge while having less sialic acid per cell. In Table I it is seen that human young, old and unfractionated red blood cells have the same mobility. Surprisingly, in view of our earlier CCD results (see above and refs. 6,7), rat young, old and unfractionated erythrocytes also display no difference in mobility.

The mobilities of the cells after overnight exposure to phase followed by washing are unaltered (see Table I). The fact that the mobilities of rat red cells is the same after exposure to phase and washing of cells (Table I) implies that the increase in mobilities observed with increasing K through the CCD extraction train (6) is not a consequence of irreversible and differential binding of polymer to the surface of cells of different ages. Such an effect has been reported to be the basis for the altered mobility of stored human erythrocytes which have been exposed to polymer (6).

Table I

Electrophoretic mobilities (μm/sec/V/cm) in standard saline of human and rat red blood cell populations and of subpopulations enriched with respect to young and old erythrocytes by Murphy's method (8). Mobilities are also given for these populations after exposure overnight to top phase of the system used for CCD and washing cells three times with saline.

	Human	Rat
Whole red cell population (a)	-1.08 ± 0.05	-1.23 ± 0.05
Young red cells (b)	-1.09 ± 0.04	-1.28 ± 0.09
Old red cells (c)	-1.08 ± 0.06	-1.24 ± 0.06
(a) After exposure to phase	-1.09 ± 0.05	-1.24 ± 0.05
(b) After exposure to phase	-1.08 ± 0.06	-1.25 ± 0.06
(c) After exposure to phase	-1.09 ± 0.05	-1.22 ± 0.06

CCD was carried out on rat young and old red blood cells in a charged (phosphate-containing) phase system. Fig. 1A depicts the distribution of rat young red cells, 1B of rat old red cells. The young erythrocytes have a higher mean K (i.e., are displaced to the right) than the old cells. Measurement of particle electrophoretic mobilities along the extraction train (Figs. 1A, B) of both rat young and old red cells not only reveals the increase in electrophoretic mobilities with increasing K reported earlier (6) for distribution curves obtained with whole rat red cell populations (i.e., populations not previously subfractionated by Murphy's method) but also that the mean mobility of the young red cell population is higher than the mean mobility of the old red cell population (each indicated by an arrow in Fig. 1). The difference found is, however, small enough so as to fall within experimental error when mobilities are measured on the young and old cell populations prior to CCD (Table I).

Similar studies were carried out with human young and old red cells (Figs. 2A, B). Note that the CCD curves obtained for young and old cells overlap. The electrophoretic mobilities of cells found along the extraction train are constant and also the same as those for the original unfractionated red blood cell population (Table I). It appears that neither electrophoresis nor parti-

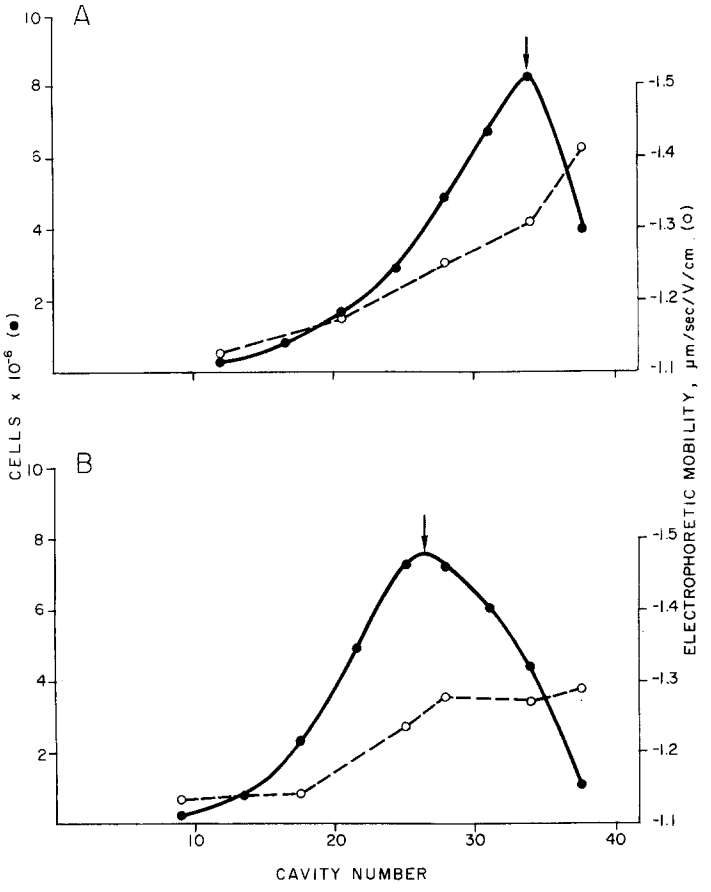

Fig. 1. Countercurrent distribution patterns for rat young (A) and old (B) red blood cells. Young and old red cell subpopulations were obtained by centrifugation of blood. Countercurrent distribution was carried out at 4°C in a charged phase system composed of 5% (w/w) dextran T500, 4% (w/w) poly(ethylene glycol) 6000 (renamed "8000"), 0.09 M Na-phosphate buffer, pH 6.8, 0.023 M NaCl and 5% (w/w) heat-inactivated fetal calf serum. 39 transfers were completed using a settling time of 6 min and a shaking time of 25 sec. ●, gives the distribution in terms of number of cells found in the different cavities along the extraction train. Cells from selected cavities were washed three times with saline and their electrophoretic mobilities were then determined. ○, shows the mobilities of cells in different cavities in µm/sec/V/cm.

tioning alone or in combination reveal any change in charge or charge-associated surface properties in human red cells as a function of their *in vivo* age.

Thus no difference in electrophoretic mobility between young and old blood cells from either species prepared by the method of Murphy (8) was found. CCD shows that rat old erythrocytes have a lower mean K than rat young red cells, while the CCD curves of human young and old erythrocytes overlap. Elec-

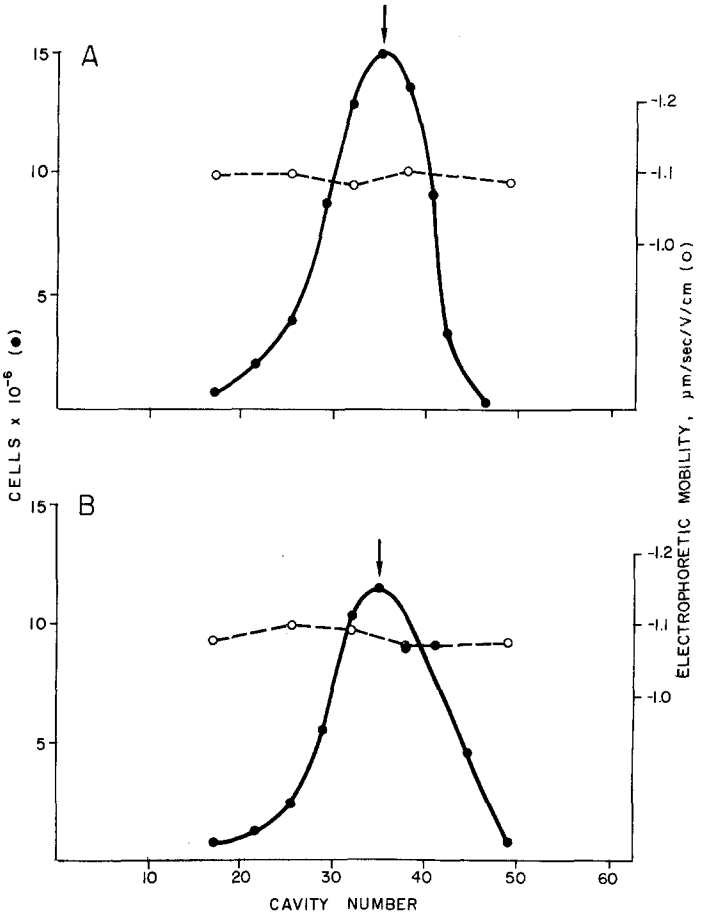

Fig. 2. Countercurrent distribution patterns of human young (A) and old (B) red blood cells, procedure as given for Fig. 1. Phase system was composed of 5% (w/w) dextran T500, 4% (w/w) poly(ethylene glycol) 6000 (renamed "8000"), 0.105 M Na-phosphate buffer, pH 6.8, and 5% (w/w) heat-inactivated fetal calf serum. 59 transfers were completed in this case to increase the detectability of possible differences between young and old red cells. Symbols as in Fig. 1.

trophoretic mobilities of human erythrocytes taken from different cavities along the CCD extraction train are the same while rat red cells display increasing mobilities which parallel the increasing K values, with the mean mobility of rat old red cells being lower than the mean mobility of rat young red cells.

The basis for the observed differences in aging of the mature erythrocyte membrane in rat and in human is currently under investigation.

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