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AGING OF ERYTHROCYTES RESULTS IN ALTERED RED CELL SURFACE PROPERTIES IN THE RAT, BUT NOT IN THE HUMAN

STUDIES BY PARTITIONING IN TWO-POLYMER AQUEOUS PHASE SYSTEMS

HARRY WALTER, EUGENE J. KROB and GALLI S. ASCHER

Laboratory of Chemical Biology and Laboratory Service, Veterans Administration Medical Center, Long Beach, CA 90822, and Department of Physiology and Biophysics, College of Medicine, University of California, Irvine, CA 92717 (U.S.A.)

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Summary

Aqueous solutions of dextran and of poly(ethylene glycol) when mixed give rise to two-phase systems useful in separating cells, on the basis of their surface properties, by partitioning. Depending on whether salts with unequal or equal affinity for the two phases are chosen, phases with or without an electrostatic potential difference between the phases are obtained. At appropriate polymer concentrations the former yield cell partition coefficients (i.e., the quantity of cells in the top phase as a percentage of total cells added) based on charge-associated surface properties while the latter reflect membrane lipid-related parameters. With increasing cell age, rat erythrocytes have diminishing partition coefficients in both charged and uncharged phases. Using the elevated aspartate aminotransferase levels of younger red cells as a marker, we have now found that young mature erythrocytes of human do not have the highest partition coefficient in the red cell population as they do in rat. Experiments with isotopically labeled dog red cells yield results similar to those found with human erythrocytes. Furthermore, density-separated young and old red cells from human give overlapping countercurrent distribution curves. Finally, countercurrent distribution of human red blood cells followed by pooling of cells from the left and right ends of the distribution and subjection of these cells to a redistribution gives curves that overlap with each other and with the original countercurrent distribution. This indicates that not only are human red cells not subfractionated based on possible age-related surface alterations, but also that they are not subfractionated by partitioning based on any surface parameter.

These results are consistent with our previous findings that membrane sialic

acid/hemoglobin absorbance is essentially constant through the extraction train after countercurrent distribution of human erythrocytes in a charged phase system; and with the recent reports of others that there is no difference in electrophoretic mobility between human young and old red cells.

Introduction

Aqueous solutions of dextran and of poly(ethylene glycol) when mixed above certain concentrations give rise to immiscible, liquid two-phase systems which, when buffered and rendered isotonic, are suitable for the separation of cells by partitioning [1]. Depending on whether salts with unequal or equal affinity for the two phases are chosen, phases with or without an electrostatic potential difference are obtained [2,3]. In the former phases (at appropriate polymer concentrations), the partition coefficient, K, of cells (i.e., the cells' relative affinity for the top or bottom phase or their adsorption to the interface) depends predominantly on charge-associated surface properties, while in the latter phase systems, K is lipid-related [4].

Partitioning of cells in such aqueous phase systems is an extremely sensitive method not only for the separation of cells (based on differences in their K values), but also for tracing subtle alterations in the cells' surface properties as a function of normal in vivo processes (e.g., differentiation, maturation, age) [4]. Over the past few years we have studied, by combining radioisotopic labeling techniques with partitioning, the changes in charge-associated and lipid-related surface properties accompanying red blood cell aging in the rat from first appearance in the bone marrow [5] to final disappearance from the peripheral circulation [6—9].

Until recently, both we and others assumed that the surface alterations discerned in the rat erythrocyte with age were also representative of other species' red blood cells. This was especially true, since the original interpretation of the altered K values of rat peripheral red blood cells of different ages appeared to correlate with data in the literature showing a diminution of human erythrocyte electrophoretic mobilities with cell age [10,11]. Recently, however, these electrophoretic mobility measurements have come into dispute [12,13] with a number of investigators reporting their inability to repeat them. We have thus undertaken a study of the partitioning behavior of human (and dog) red blood cells as a function of age. We have found, much to our surprise, that not only can human mature red blood cells not be subfractionated by partitioning on the basis of age-related surface alterations (at least not under conditions analogous to those successfully employed with rat mature erythrocytes), but also that subfractionation by partitioning of these cells is not in evidence on any basis.

Materials and Methods

General

The following experimental methods have previously been presented in detail (Refs. 8 and 14; and Seaman, G.V.F., Tamblyn, C.H., Colvin, N., Krob,

E.J., Ascher, G.S. and Walter, H., unpublished data): (a) preparation of phase systems; (b) procedure and apparatus used in countercurrent distribution; (c) collection of cells after countercurrent distribution and analysis of cell concentration by lysing and measuring hemoglobin absorbance or by electronic cell count, (e) assay of aspartate aminotransferase in red blood cell lysates; and (f) microscopic determination of reticulocyte counts after countercurrent distribution.

Specific

Blood collection. Blood from human and dog was collected by venipuncture; from rat (Sprague-Dawley) by heart puncture. Acid/citrate/dextrose was used as anticoagulant except in experiments in which the method of Murphy [15] for young and old red cell separation and/or the method of Beutler et al. [16] for white cell removal from blood were used. Defibrinated blood was used in the latter instances.

Two-polymer aqueous phases. A number of different two-polymer aqueous phase systems, having different physical properties and selected as described by Walter [4], were used. Their compositions were as follows: system 1 contained 5% (w/w) dextran T500, lot Nos. 3936 or 5556 (Pharmacia Fine Chemicals, Piscataway, NJ), 4% (w/w) poly(ethylene glycol), ('Carbowax 6000', recently renamed '8000', Union Carbide, NY), and 0.11 M sodium phosphate buffer, pH 6.8 (composed of equimolar quantities of mono- and dibasic phosphates); system 2 contained the same polymer concentrations but 0.09 M sodium phosphate buffer, pH 6.8, and 0.03 M NaCl; system 3 also had the same polymer concentrations but 0.09 M sodium phosphate buffer, pH 6.8, 0.023 M NaCl and 5% (w/w) heat-inactivated fetal calf serum; and, finally, system 4 consisted of 4.85% (w/w) dextran T500, 3.3% (w/w) poly(ethylene glycol) 6000 and 0.15 M NaCl and 0.01 M sodium phosphate buffer (pH 6.8). System 1 has a high, systems 2 and 3 low and system 4 no electrostatic potential difference between the phases. Phase systems were equilibrated at 4—5°C in a separatory funnel. Top and bottom phases were then separated and used in countercurrent distribution experiments as described below.

Centrifugal preparation of human red blood cell subpopulations enriched with respect to young or old cells. The method of Murphy [15], which consists of centrifuging blood cells at 30°C followed by collection of the upper and lower 5% of the centrifuged blood cell layer, was used to obtain red blood cell populations enriched with respect to young (top layer) or old (bottom layer) cell subpopulations. 100 ml of defibrinated blood were used and the cells were centrifuged in 16 × 100 mm plastic centrifuge tubes each containing 7.5 ml of red cells. The relative enrichment of young and old red blood cells was monitored, after removal of the bulk of white blood cells using the method of Beutler et al. [16] (see section following), by assay of aspartate aminotransferase which is an enzyme marker for young red blood cells [14]. The ratio of specific activities of aspartate aminotransferase (i.e., enzyme units/hemoglobin absorbance, Ref. 14) of top/bottom fractions obtained by centrifugation was usually between 2 and 3.

Removal of white blood cells from red blood cells in experiments utilizing enzyme marker assays for young red cells. Preparations of young and old red

blood cells prepared by the method of Murphy [15] as well as other red cell populations on which aspertate aminotransferase assays were to be carried out were first put through small columns containing microcrystalline cellulose (Sigmacell Type 50) and α-cellulose (Sigma Chemical Co., MO). The procedure which removes white blood cells was carried out as described by Beutler et al. [16]. The extent of white cell depletion was examined by hemocytometer count on cell samples before and after passage through the columns and was found to be between 97—99%.

Injection of dog with [^{59}Fe]ferrous citrate. A medium-sized dog (13—15 kg) was injected intravenously via the cephalic vein with 250 μCi of [^{59}Fe]ferrous citrate (Mallinckrodt). The dog was bled periodically (as indicated in the caption to Fig. 7) and the washed red blood cells subjected to countercurrent distribution in system 2, as described below.

Countercurrent distribution of red blood cells. Our thin-layer countercurrent distribution plates have 120 concentric cavities [17]. The bottom plate cavities have a capacity of 0.7 ml. In the experiments depicted in Figs. 1 and 2, 0.9 ml of washed, packed red blood cells were suspended in 8 ml of top phase ('load mix') of system 2. Cavities 0—7 received 0.5 ml of bottom phase and 0.9 ml load mix. All other cavities received 0.6 ml bottom phase and 0.8 ml top phase. Plates were loaded in this manner to give a stationary interface (see Ref. 1 for full discussion). 119 transfers were completed using a settling time of 6 min and a shaking time of 25 s. Countercurrent distribution was carried out at 4—5° C. At the end of the run cavities were emptied and red cell counts or hemoglobin absorbance determined as previously described [8]. The experiment shown in Fig. 3 was carried out in an analogous manner except that only cavities 0—5 received the load mix and the phase system used was system 1.

The distributions in Fig. 4 were run on opposite sides of the countercurrent distribution plates. By loading 'young' cells in cavities 0—4 and 'old' cells in cavities 60—64 and completing 59 transfers, this comparative experiment could be undertaken at the same time, in the same phase system and without overlap. The phase system used was system 4 (an uncharged system) which, being closer to the critical point, needs a longer settling time. 8-min settling and 25-s shaking times were used. The run was performed at 4—5° C.

In Figs. 5A and 6A, five cavities were loaded with cells and 59 transfers carried out using 6.5-min settling and 25-s shaking times in phase system 3. The run was performed at 4—5° C. Afteer countercurrent distribution, hemoglobin absorbance was determined of cells in every fifth cavity (to obtain the distribution curve). Based on this analysis about 40% of the remaining cells in the original distribution to the left of the striped area (Figs. 5A, 6A) were pooled as were about 40% of the remaining cells to its right. Cells from the right and left ends of this original countercurrent distribution were centrifuged, the supernatant solution discarded, and cells resuspended in the top phase. They were subjected to a redistribution using a phase system of identical composition and the same shaking and settling times as above. Three cavities were loaded and 29 transfers completed. These countercurrent distributions were then analyzed by determining the hemoglobin absorbance of lysates of cells obtained from different cavities along the extraction train (Figs. 5B and 6B).

We have previously found (Walter, H., Tung, R. and Krob, E.J., unpublished

data) that smaller quantities of cells can have distribution curves of altered apparent K. Incorporation of fetal calf serum (Grand Island Biological Co., Grand Island, NY) or other protein results in distribution curves of identical K for the same cell population over all cell concentrations tested. Since the distributions of different quantities of cells are compared in Fig. 5A and B as well as in Fig. 6A and B, fetal calf serum was incorporated into the phase systems.

In Fig. 7 we show the distributions of total red cells and also that of radioactively labeled red cells obtained at different times after injection of a dog with [^{59}Fe]ferrous citrate. Five cavities were loaded with cells and a settling time of 7 min and shaking time of 25 s were used. 60 transfers were carried out in system 2. The run was performed at 4—5° C.

Presentation of data. Distribution curves of red blood cells are given either in terms of electronic cell counts (Seaman, G.V.F., Tamblyn, C.H., Colvin, N., Krob, E.J., Ascher, G.S. and Walter, H., unpublished data) obtained in the different cavities along the extraction train or in terms of the hemoglobin absorbance (at 540 nm) released on lysis of cells in the different cavities [8]. Aspartate aminotransferase activity is defined in arbitrary units: 1 unit = Δ 0.001 absorbance unit/min per ml of enzyme solution at 340 nm [14]. Specific enzyme activity is expressed either in terms of enzyme units per unit hemoglobin absorbance or per cell [14]. Isotope distributions are given in cpm. A relative specific activity is also presented and is defined [8] as:

$$\frac{\text{cpm/unit hemoglobin absorbance in a given cavity}}{\text{cpm/unit hemoglobin absorbance in the original unfractionated cell population}}$$

Results and Discussion

Background

Dextran/poly(ethylene glycol) aqueous phases containing phosphate have an electrostatic potential difference between the phases with the top phase positive with respect to the bottom [3]. Cells, which are negatively charged, when added to such a system will interact with the positively charged top phase and the partition coefficients (K) obtained will be charge-associated [4]. Phases containing NaCl instead of phosphate have no electrostatic potential difference between them ('uncharged phases'). At polymer concentrations close to the critical point (i.e., at concentrations close to those below which no phase separation occurs), the K values obtained in such systems have been shown, in the case of erythrocytes from different species, to correlate well with the ratio of their membrane poly/monounsaturated fatty acids and other membrane lipid components [18].

It has previously been found that when rat peripheral red blood cells containing radioactively labeled cells of different and distinct ages are subjected to countercurrent distribution (a multi-extraction procedure) in a charged phase system, the youngest reticulocytes have the lowest K. Within a few hours K increases dramatically so that the oldest reticulocytes in the population have the highest K of any red cell in the circulation [8]. The oldest reticulocytes then give rise to mature erythrocytes and these, with increasing age, have correspondingly diminishing K values. The oldest erythrocytes are found to have

K values very close to those of the youngest reticulocytes [6]. Thus, charge-associated membrane properties of rat red cells appear to increase during cell maturation and subsequently decrease during cell aging. Analogous experiments with rat red cells in uncharged phases indicate that lipid-related membrane parameters also change during the life-span of the red cell although the rapid changes of K values observed during the maturation of reticulocytes in charged phases are absent [9].

Countercurrent distribution of rat and of human red blood cells followed by determination of an enzyme marker for young cells along the extraction train

Aspartate aminotransferase has been shown to have elevated activities in young red cells of both human and rat [14,19]. Since one cannot use radioactive [^{59}Fe]ferrous citrate in quantities required to study the aging of red blood cells in human (as we have done earlier in rat), we undertook to analyze aspartate aminotransferase activities along the extraction train following countercurrent distribution of human (and for comparison of rat) red blood cells. Defibrinated human or rat blood was passed through a column containing cellulose, prior to countercurrent distribution, to remove virtually all of the white blood cells. This is important so that the higher levels of aspartate aminotransferase in white cells will not interfere with the use of this enzyme as a marker for young red cells.

Fig. 1 shows the countercurrent distribution pattern of rat red cells obtained in a charged phase system (system 2). Increased aspartate aminotransferase levels are associated with the left and right limbs of the curve; and the specific enzyme activity (expressed as enzyme units/cell) rises at the ends of the dis-

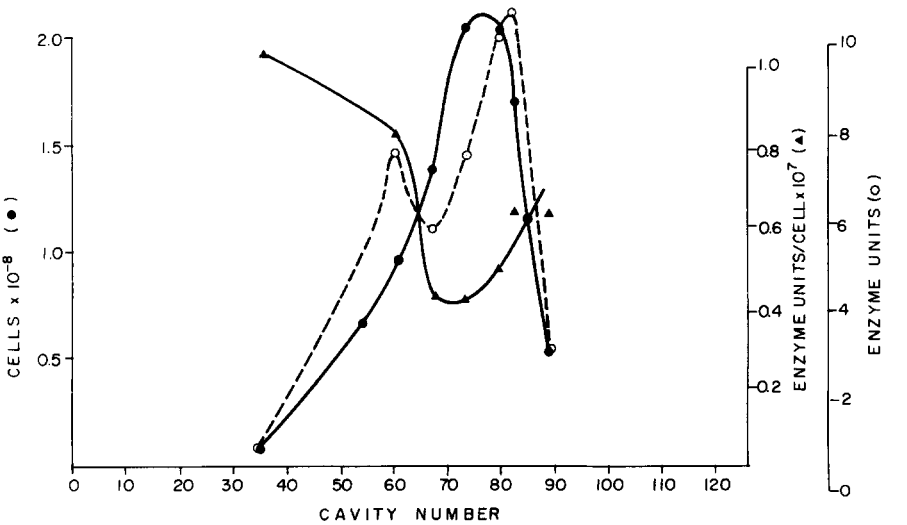

Fig. 1. Countercurrent distribution pattern of rat red blood cells and the aspartate aminotransferase activity associated with cells along the extraction train. Aspartate aminotransferase is a marker enzyme for young red blood cells and the elevated levels at the left and right ends of the cell distribution indicate, respectively, the presence of reticulocytes and young mature erythrocytes. ●, cell distribution curve (electronic cell count); ○, enzyme activities (arbitrary units); ▲, specific enzyme activity (enzyme units/cell). 119 transfers were carried out in phase system 2 using a settling time of 6 min and a shaking time of 25 s. The run was performed at 4—5° C. For other details see text.

tribution and dips in the middle. These findings are in line with our earlier detailed analysis of the distribution of rat red cells of different ages [8] in which the youngest reticulocytes have the lowest K values (i.e., are to the left) while the youngest mature erythrocytes have the highest K values (i.e., are to the right).

Fig. 2 shows an analogous experiment with human red blood cells. Note that the aspartate aminotransferase distribution is displaced slightly to the left of the red cell distribution and that the increased enzyme activities associated with the right end of the distribution of rat red cells (Fig. 1) are absent. There is a small decrease in the specific aspartate aminotransferase activity with increasing K of cells. Our preliminary conclusion by analogy with the data from rat, to be further examined below, is that there is a difference between the charge-associated membrane surface properties of human reticulocytes and erythrocytes, but that no difference between young and old mature red cells is in evidence.

Countercurrent distribution of human red blood cells followed by microscopic count of reticulocytes in different cavities along the extraction train

Slides were prepared of cells obtained from different cavities along the extraction train after countercurrent distribution of human red cells. These were stained with new methylene blue and counted for percentage of reticulocytes present (Fig. 3). Note that the highest percentage of reticulocytes is to the left of the distribution curve and that it decreases with increasing cell K. (Similar results were obtained when human red cells were subjected to countercurrent distribution in an uncharged phase system). These data are thus analogous to those obtained with rat red cells [8] and indicate that the increased aspartate aminotransferase levels found associated with cells of low K (Fig. 2) appear indeed to be due to the reticulocytes.

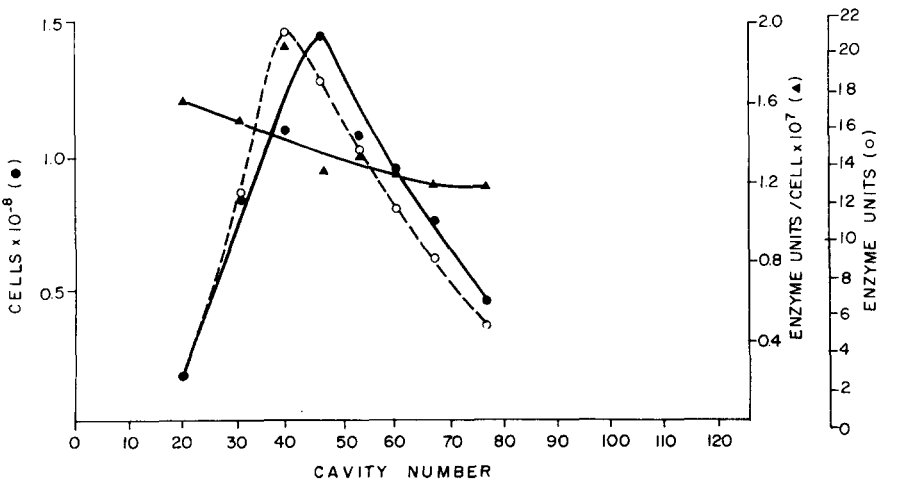

Fig. 2. Countercurrent distribution pattern of human red blood cells and the aspartate aminotransferase activity associated with cells along the extraction train. Symbols and conditions are as in Fig. 1. Note the displacement of the enzyme distribution to the left of the cell distribution but the absence of elevated enzyme activities at the right end. For discussion see text.

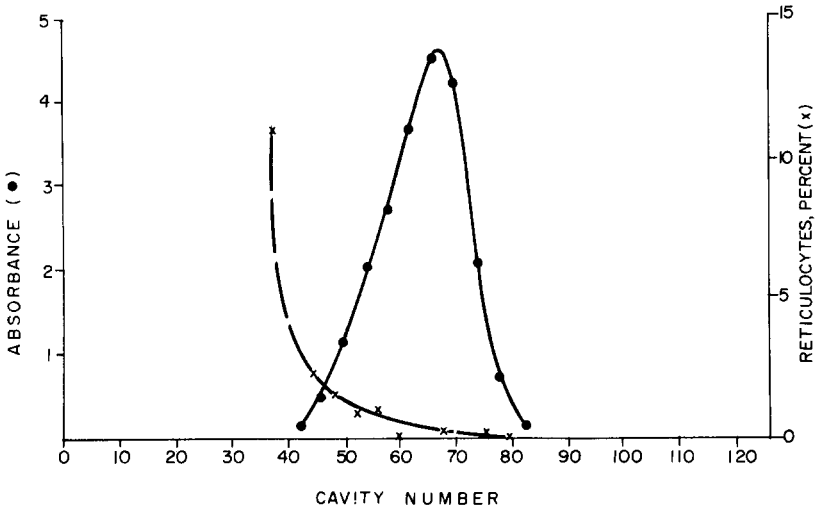

Fig. 3. Countercurrent distribution pattern of human red blood cells followed by microscopic determination of the percentage of reticulocytes in the different cavities along the extraction train. •, cell distribution (in terms of hemoglobin absorbance); X, percentage of reticulocytes. Experimental conditions were as in Fig. 1 except that phase system 1 was used. See text for discussion.

Separation of human young and old red blood cells by centrifugation and comparison of countercurrent distribution patterns of cells so obtained

Human young and old red blood cells were obtained by centriguging defibrinated blood according to the method of Murphy [15] and removing the top (young) and bottom (old) 5% of the centrifuged cell layer. Young and old red cells were then put through cellulose columns [16] to remove contaminating white cells. Countercurrent distribution was subsequently carried out on the young and old red cells in an uncharged phase system (system 4). Fig 4 presents results of such an experiment. Fig. 4A shows the cell distribution, aspartate aminotransferase distribution and specific aspartate aminotransferase activity of human young red cells; Fig. 4B of old red blood cells. That we are truly working with younger and older red cells is indicated by the approx. 2-fold higher mean level of aspartate aminotransferase activity in the top as compared to bottom graph. The most striking aspect of the distribution curves is that, within experimental error, they appear to overlap indicating no discernible difference by partitioning between the bulk of young and old red cells. The slight displacement of the aspartate aminotransferase distribution to the left of the cell distributions again indicates the difference of surface properties between human reticulocytes and mature erythrocytes. As there are more reticulocytes present in the top than in the bottom fractions, the difference observed is larger in Fig. 4A than in Fig. 4B. Since reticulocytes constitute only a small percentage of the total red cell population, their presence has no apparent effect on the distribution curve of the latter.

The results presented in Fig. 4 are similar to those we have observed using a charged phase system [20]. We conclude that human young and old red cells, at least as prepared by a generally used centrifugation method, do not differ in

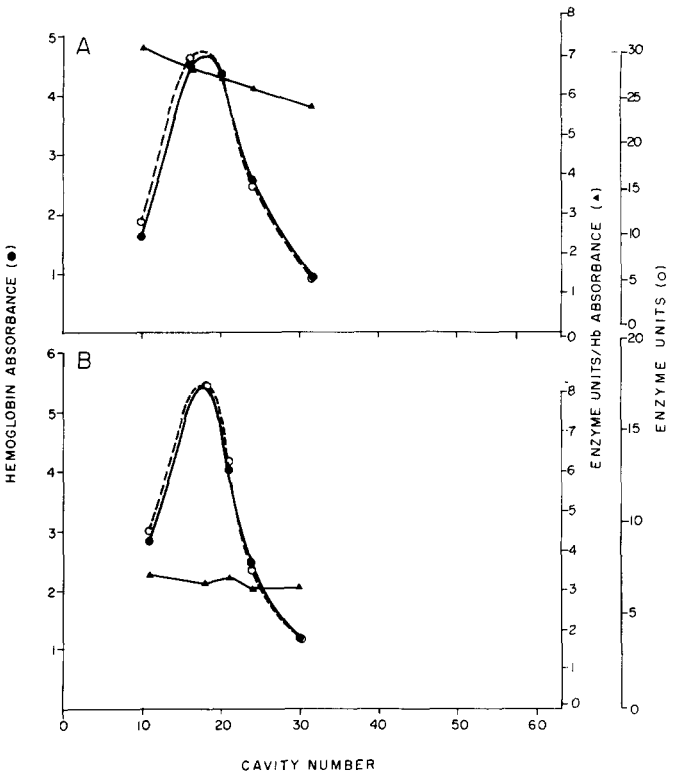

Fig. 4. Countercurrent distribution patterns of human young (A) and old (B) red blood cell subpopulations and of the aspartate aminotransferase activities associated with cells along each extraction train. Young and old red cell subpopulations were obtained by the centrifugation method of Murphy [15] and subjected to countercurrent distribution in phase system 4. 59 transfers were carried out using a settling time of 8 min and a shaking time of 25 s. The run was performed at 3—5°C. Symbols as in Fig. 1 (except that the cell distribution is given in terms of hemoglobin absorbance). Note that, within experimental error, the two distribution curves overlap. See text for discussion.

either their lipid-related or charge-associated surface properties as reflected by partitioning.

Countercurrent distribution of rat and of human red blood cells followed by redistribution of cells under the left and right ends of the extraction train

The curves obtained on countercurrent can be due to a random distribution of cells in which every cell has an equal chance of being under any part of the curve (i.e., all cells have the same K) or it can be due to true differences in surface properties in which cells found to the left of the distribution have a greater chance of being found there and those to the right a greater chance of being on the right (i.e., cells have increasing K values from left to right through the distribution).

A simple and standard manner in which to test for differences in separated cells is to subject cells from the left and right ends of a countercurrent distribution to a second distribution in a phase system of the same composition. If cells have increasing K values through the distribution, then cells obtained from the

left part of the original countercurrent distribution should give, on redistribution, a curve displaced to the left of a curve obtained with cells from the right part.

Rat red blood cells were subjected to countercurrent distribution in a charged phase system (system 3). The distribution curve obtained after 60 transfers is depicted in Fig. 5A. Cells to the left of the striped area were then pooled, as were cells to the right of the striped area. These two cell subpopulations were separately but simultaneously subjected to a redistribution in the same phase system. Fig. 5B shows the curves obtained (after 30 transfers). They are presented superimposed for better visualization. Cells obtained from the left of the original countercurrent distribution (Fig. 5A) are to the left; cells obtained from the right are to the right. The results clearly indicate that rat red blood cells obtained from different parts of the distribution curve differ in surface properties. This result is expected based on our previous findings [6,8] in which cells of different ages were shown to be under different parts of the countercurrent distribution. Furthermore, cell electrophoresis of rat erythrocytes from different parts of the extraction train revealed a correlation

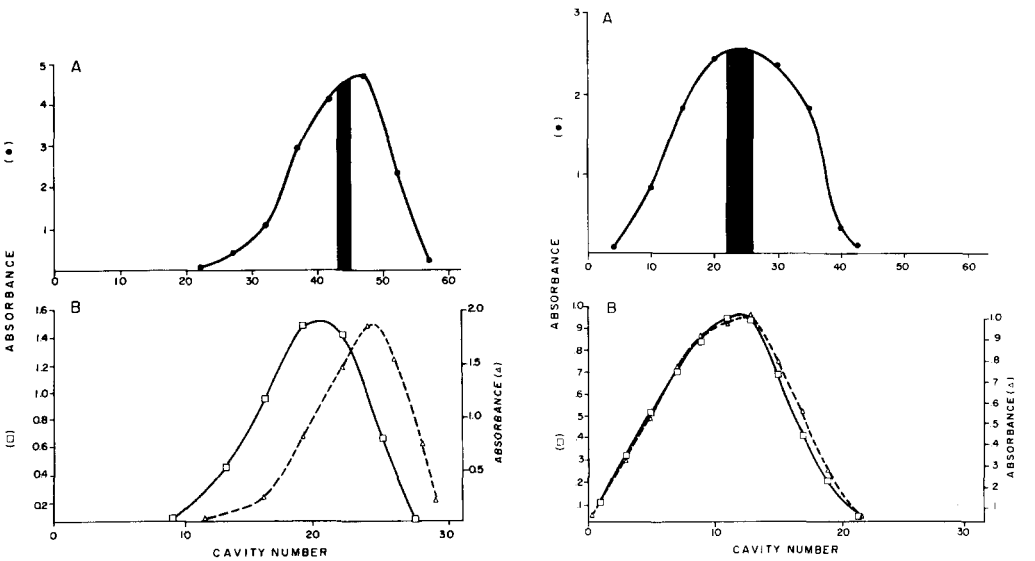

Fig. 5. Countercurrent distribution of rat red blood cells (A) was followed by pooling cells from the left and right ends of the distribution (i.e., cells to the left and right, respectively, of the striped area). These cells were subjected separately, but simultaneously, to a second countercurrent distribution (B). 59 transfers were carried out in A and 29 transfers in B using phase system 3 in each case. Settling time was 6.5 min and shaking time 25 s. Runs were performed at 3—5° C. ●, original cell distribution (in terms of hemoglobin abosrbance); □, distribution obtained with cells from left end of distribution in A; △, distribution obtained with cells from right end of distribution in A. Note that cells from left end of A are displaced to left of cells originating from right end of A thus indicating membrane surface differences between these cells. See text for additional details.

Fig. 6. Countercurrent experiment as in Fig. 5 but with human red blood cells. Other conditions as in Fig. 5. Note the overlap on redistribution of human red blood cells (B) from the left and right ends of the original distribution (A). No membrane surface differences in human red cells are thus discernible by partitioning. See text for discussion.

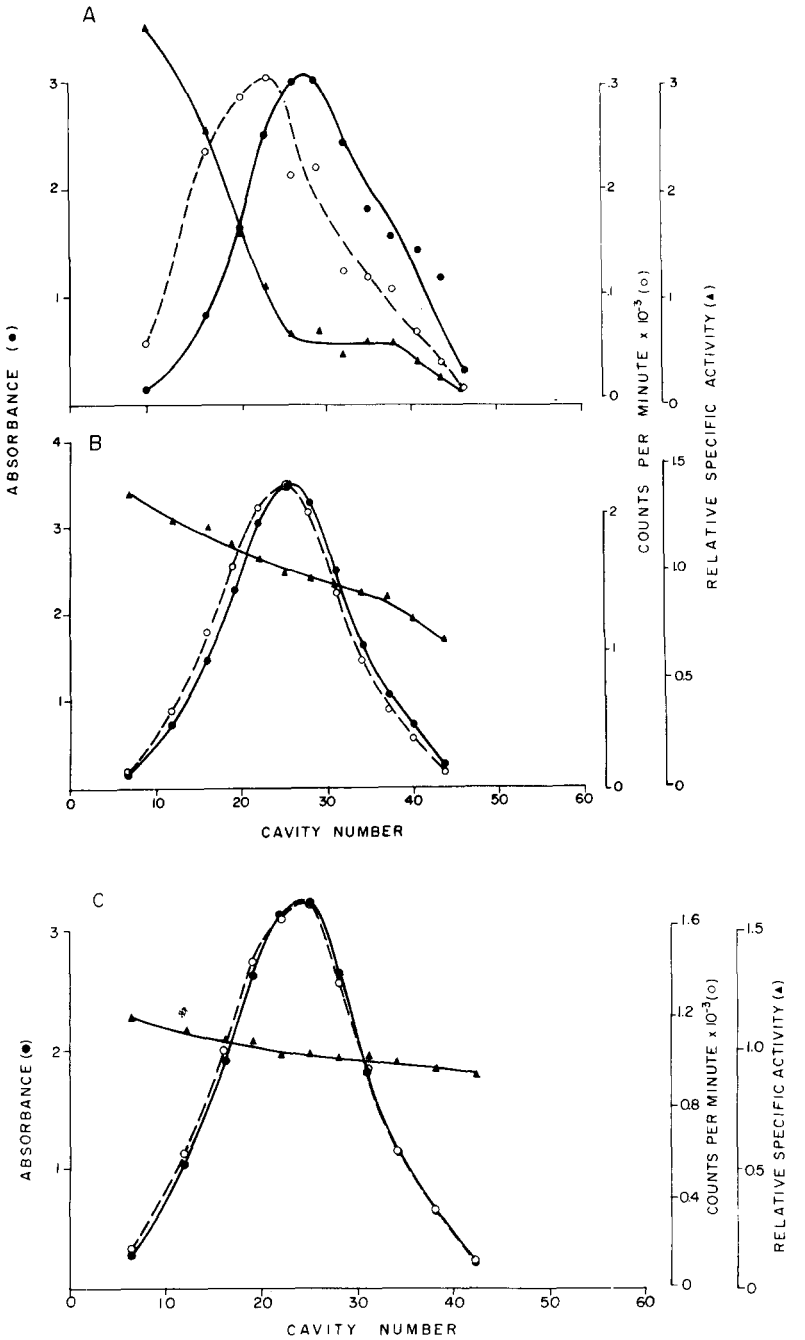

Fig. 7. Countercurrent distribution patterns of dog red blood cells labeled with [^{59}Fe]ferrous citrate. A dog was bled at different times after injection giving rise to labeled red cell populations of distinct ages. Relative specific activities above 1.00 indicate higher concentrations of red cells of ages corresponding to the time elapsed between injection and bleeding. 60 transfers were carried out in system 2 using a settling time of 7 min and a shaking time of 25 s. Runs were performed at 3—5°C. ●, cell distribution (in terms of hemoglobin absorbance); ○, isotope distribution (in counts/min); ▲, relative specific activity. Dog red blood cells were obtained at (A) 46 h, (B) 13 days and (C) 27 days after isotope injection. For discussion and additional details see text.

between increasing K and increasing mobility of cells through the distribution [21].

Fig. 6 shows an analogous experiment with human red blood cells. It is clear that cells obtained from the left and right ends of the original countercurrent distribution (Fig. 6A) overlap on redistribution (Fig. 6B). It thus appears that, at least under conditions in which rat red blood cells are fractionated into cells of distinct ages, human red blood cells are not only not so fractionated but also are not fractionated by partitioning on any basis.

Countercurrent distribution patterns of ^{59}Fe-labeled dog red blood cells of different ages

Since it now appears that under analogous conditions rat red cells can and human red cells cannot be subfractionated by partitioning based on their charge-associated or lipid-related surface properties, it became of interest to study another species. Dogs were injected with [^{59}Fe]ferrous citrate and bled at different times after injection giving us cell populations in which cells of different but distinct ages were labeled. These cells were then subjected to countercurrent distribution (60 transfers) in a charged phase system (system 2) and analyzed for (a) cell distribution, (b) radioisotope distribution (cpm), and (c) relative specific activity. 46 h after injection (Fig. 7A) the labeled cells (reticulocytes) are distinctly displaced to the left of the total cell distribution. This fact is also clearly reflected by the high relative specific activity to the left and its decrease to the right through the distribution curve. The data are thus similar to those obtained with rat red cell populations at short times after isotope injection [8]; and also with the distribution of aspartate aminotransferase activity in human red cells (Fig. 2). 13 days after injection (Fig. 7B), the isotope distribution curve is still slightly to the left of the cell distribution; and the relative specific activity shows a much smaller difference than in Fig. 7A. These results are quite distinct from those obtained with labeled rat red cells at any time from about 48 h to nearly 30 days after isotope injection [6]. In the rat there is a dramatic shift of the labeled cells from the left end to the right end of the distribution within 2—3 days (reticulocyte maturation) followed by a slow decrease in partition coefficient over the entire life-span of the mature red cell [8,6]. The difference is even further emphasized by an experiment carried out 27 days after isotope injection (Fig. 7C). The labeled dog cells are still very slightly displaced to the left, with the relative specific activity almost flat. The slight displacement of labeled cells (Fig. 7B and C) probably reflects small quantities of isotope continuing to be incorporated into reticulocytes, while the bulk of labeled mature erythrocytes appear not to have charge differences detectable by partitioning. We conclude that dog mature red blood cells, like human mature erythrocytes, are not subfractionated by partitioning. Dog reticulocytes and erythrocytes (again as in human) do appear to have differences in surface properties reflected by partitioning.

Conclusion

Previous studies have shown that the partition coefficient, K, of the rat red blood cell changes continuously during the cell's life-span in both charged (e.g.,

phosphate-containing) and uncharged (e.g., NaCl-containing) dextran/poly-(ethylene glycol) aqueous phases [6,8,9]. Since correlations exist between increasing K values in charged phases and increasing electrophoretic mobilities of erythrocytes from different species [7], and between increasing K values in uncharged phases and increasing ratios of the red cells' membrane poly/mono-unsaturated fatty acids [18], we concluded that both charge-associated and lipid-related membrane surface properties change during rat red cell maturation and aging [9].

When our first results in charged phase systems were obtained [6], the decrease in K with increasing rat mature erythrocyte age appeared (by analogy) to fit in with the reported diminution of electrophoretic mobility of human mature erythrocytes with age [10,11]. Subsequently, we also found that, on countercurrent distribution, increasing K values of rat red cells through the extraction train were concomitant with increasing electrophoretic mobilities [21]. Recent reports, however, indicate that the original mobility results with human erythrocytes of different ages cannot be reproduced [12,13]. It has also become clear that while sialic acid (the main charge-bearing surface group of red cells [22]) and various membrane lipid and protein components diminish [23,24] during human red cell aging when expressed on a per cell basis, membrane composition appears constant when one membrane component is expressed in terms relative to other membrane components (e.g., sialic acid/membrane protein). It has therefore been suggested (e.g., Refs. 13, 24, 25) that, on aging, segments of membrane are 'shed' resulting in lower membrane components per cell but maintaining constant ratios between them.

Because of the great sensitivity with which partitioning reflects membrane surface properties [4] and the fact that the charge-associated properties which determine K (in charged systems) are not necessarily the same as the charge measured by cell electrophoresis [26] we decided to study, by partitioning, the surface of human red blood cells of different ages. Our current results indicate that, using the partitioning technique under conditions analogous to those previously employed with rat red cells, we cannot detect a change in either charge-associated or lipid-related surface properties in human mature erythrocytes as a function of age. Dog red blood cells which were studied in a charged phase system give results similar to those obtained with human erythrocytes. The fact, confusing until now, that there is no difference in sialic acid/hemoglobin absorbance when human erythrocytes in different cavities along the extraction train are analyzed after countercurrent distribution is thus also clarified.

By pooling separately human red cells under the left and right ends of a countercurrent distribution and subjecting these to a second distribution we find the distributions of such populations to be strictly superimposable on each other as well as on the original cell distribution. Hence, not only is there no subfractionation based on possible age-related alterations in surface properties of human mature erythrocytes by partitioning in a charged phase system (again under conditions in which rat red cells are subfractionated,) but also no subfractionation by partitioning based on any surface property is so reflected.

The basis for the observed difference in mature erythrocyte aging patterns in rat and in human is under current investigation.

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