Biochimica et Biophysica Acta 855 (1986) 8–15
Elsevier

BBA 72859

Detection of surface differences between two closely related cell populations by partitioning. Erythrocytes from inbred and out-bred rats and rat strains

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(Received July 31st, 1985)

Key words: Cell surface; Phase partitioning; Chromate labeling; Cell separation; (Rat erythrocyte)

We have recently developed a new and powerful method capable of detecting, by purely physical means, surface differences between closely related red (or other) cell populations. The procedure consists of isotopically labeling (with [^{51}Cr]chromate) aliquots of red blood cell populations. Such labeled cells are mixed with an excess of unlabeled red cells to which they are to be compared. The mixtures are subjected to countercurrent distribution in either a charge-sensitive or a non-charge-sensitive dextran-poly(ethylene glycol) aqueous phase system. The distribution curves are analyzed for total cells (in terms of hemoglobin absorbance) and labeled cells (in terms of cpm). Changes in the relative specific activities through the distribution curves are indicative of subtle differences in surface properties between such cell populations. Using this method we have found that erythrocytes from arbitrarily chosen (presumably hematologically normal) individuals differ. In the current work we have examined the surface properties of erythrocytes from Sprague-Dawley and from Lewis rats. This was done with a view to determining whether (a) differences of the type found between different humans can also be detected in other species and (b), if such differences do exist, to examine, by study of the highly inbred Lewis rat strain, whether the differences appear to have a genetic or an acquired basis. It was found that the surface properties of erythrocytes from Lewis and Sprague-Dawley rats differ as do erythrocytes among rats of the Sprague-Dawley strain. No difference was found between red blood cells from different rats of the inbred Lewis strain. These results indicate that the surface differences between red blood cells from different rats detected by partitioning have a genetic rather than acquired origin.

Introduction

Partitioning of cells in dextran-poly(ethylene glycol) aqueous phase systems is a sensitive and versatile method for the separation and subfractionation of cell populations [1,2]. By appropriate selection of polymer concentrations and ionic composition and concentration one can so manipulate the physical properties of the phase system that the cell segregations obtained will be due primarily to charge-associated or non-charge-related surface properties.

We have recently described a method that permits the detection, by partitioning, of surface differences between closely related cell populations, the partition ratios of which would fall within experimental error if one were to compare the countercurrent distribution (i.e., multiple extraction) curves of such populations run separately. The method, in brief, consists of isotopically label-

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ing aliquots of each of two cell populations to be compared, mixing labeled cells with an excess of unlabeled cells of the second population and subjecting such mixtures to countercurrent distribution in charge-sensitive or non-charge-sensitive phases. As control we also prepare mixtures of labeled cells and unlabeled cells from the same source to be certain that the label per se has no influence on the cells' partitioning behavior. Changes in the relative specific activities through a distribution curve are indicative of subtle differences in surface properties between the two cell populations [3,4].

By use of this method we have found that the surface properties of red blood cells from arbitrarily selected (presumably hematologically normal) individuals differ [5]. The detected difference is independent of ABO [5] or MN blood group (Walter, H., Krob, E.J. and Brooks, D.E., unpublished data).

In order to determine whether the differences between red blood cells from different individuals have a genetic or acquired basis, studies on identical siblings have been started. However, access to and acquisition of data from identical siblings is a slow process. We have therefore turned, as a model, to a highly inbred strain of rats and have compared the surface properties of erythrocytes from these animals to those of rats from the same strain, to rats of another strain, and of out-bred rats to each other. It was established that differences such as those found among humans are not species-specific since they are also present between Lewis rats and Sprague-Dawley rats and among Sprague-Dawley rats. No difference was in evidence between erythrocytes from the highly inbred Lewis rats. This indicates that there is a genetic basis for the surface differences, detected by partitioning, between erythrocytes from different animals of the same species.

Methods

Animals. Male rats weighing between 300 and 500 g were used in these experiments. Lewis rats were obtained from Charles River Labs. (Wilmington, MA). Sprague-Dawley rats were from Hilltop Lab. Animals (Scottdale, PA). Some experiments were also undertaken with Sprague-Dawley rats from Charles River and from Simonsen Labs. (Gilroy, CA).

Blood collection. Rats were bled by heart puncture and 10 ml of blood was collected in 4 ml acid/citrate/dextrose (ACD) anticoagulant. Red cells were used in experiments within 1 week of collection.

^{51}Cr-labeling of erythrocytes. Isotopic labeling of red cells with ^{51}Cr-chromate has been described in detail, as have been the counting procedures used [3,4]. In the present experiments, approx. 10 μCi of ^{51}Cr was used per ml of an aliquot of the anticoagulated blood suspension described above. Labeled and an aliquot of unlabeled red cells were then washed five times with phosphate-buffered saline (pH 7.0).

Preparation of mixtures of labeled and unlabeled erythrocytes for analysis by countercurrent distribution. To compare the surface properties of erythrocytes from two rats ('A' and 'B', i.e., two Lewis rats, two Sprague-Dawley rats or one Lewis and one Sprague-Dawley rat) 0.15 ml of ^{51}Cr-labeled red cells (washed as indicated above) were pipetted into a centrifuge tube containing 5 ml of phosphate-buffered saline. 0.6 ml of unlabeled, washed red cells with which the labeled red cells were to be compared were pipetted into the same tube. Cells in the tubes were gently mixed and centrifuged. The supernatant solution was discarded and the packed cells were used to make the 'load mix' (see below) for countercurrent distribution. Four mixtures were examined in each experiment: ^{51}Cr-labeled red cells 'A' + unlabeled red cells 'B' (A + B); ^{51}Cr-labeled red cells 'B' + unlabeled red cells 'A' (B + A); ^{51}Cr-labeled red cells 'A' + unlabeled red cells 'A' (A + A); ^{51}Cr-labeled red cells 'B' + unlabeled red cells 'B' (B + B). Mixing ^{51}Cr-labeled red cells with unlabeled red cells of the same population is an essential control to indicate that ^{51}Cr-labeling procedures per se have no effect on the surface properties reflected by partitioning (see Figs. 1–5).

Preparation and composition of phase systems. The preparation of two-polymer aqueous phase systems has previously been described [1–4]. In the present work, two phase systems were used. Phase system 1 contained 5% (w/w) dextran T500, lot No. 11648 (Pharmacia Fine Chemicals, Piscataway, NJ), 4% (w/w) poly(ethylene glycol) 8000

('Carbowax 8000', formerly called '6000', Union Carbide, NY), 0.09 M sodium phosphate buffer (pH 6.8) and 0.03 M NaCl. Phase system 2 contained 5% (w/w) dextran, 3.5% (w/w) poly(ethylene glycol), 0.15 M NaCl and 0.01 M sodium phosphate buffer (pH 6.8). Phase system 1 has an electrostatic potential difference between the phases and is charge-sensitive; phase system 2 does not have a potential difference and is non-charge-sensitive (see Refs. 1 and 2 for detailed discussion).

Countercurrent distribution of cell mixtures. 0.25 ml of the labeled + unlabeled red blood cell mixtures (see above) were suspended in 2.75 ml of top phase of the system to be used in countercurrent distribution ('load mix'). Three adjacent cavities (at 0–2, 30–32, 60–62, or 90–92), on our 120-cavity circular countercurrent plates [3,4], were loaded with one of the load mixes. Countercurrent distribution was then carried out, separately but simultaneously, as previously described [3,4] on the four cell mixtures in either phase system 1 or 2. 30 transfers were completed at 4–5°C.

Presentation of data. Total cell distribution, in terms of hemoglobin absorbance (at 540 nm), indicates primarily the unlabeled cell population in each case since these are the cells present in great excess. The labeled cell distribution is given in counts per minute (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/unit hemoglobin absorbance in a given cavity)/

(cpm/unit hemoglobin absorbance in the original mixed cell population prior to countercurrent distribution)

Results

Background

Aqueous solutions of dextran and of poly(ethylene glycol) when mixed above certain concentrations form immiscible, liquid two-phase systems with a dextran-rich bottom and a poly(ethylene glycol)-rich top. Such phases can be buffered and rendered isotonic and have proved highly useful in the separation and subfractionation of cell populations by partitioning [6–9]. Depending on the polymer concentrations and on the ionic composition and concentration chosen, the phases have greatly different physical properties [2]. Although both dextran and poly(ethylene glycol) are themselves non-ionic, some salts (e.g., phosphate) have different affinities for the two phases. An electrostatic potential difference between the phases results [10,11] and, at appropriately high polymer concentrations, the partitioning of cells in such phases is charge-associated [12]. Other salts (e.g., NaCl) have virtually equal affinities for the two phases and such systems have essentially no potential difference between them. In systems containing NaCl and adequately low polymer concentrations, cell partitioning depends on non-charge-related surface properties which, at least in the case of red blood cells from different species, has been shown to correlate extremely well with the cells' membrane ratio poly/monounsaturated fatty acids [13]. In the present studies, phase system 1 most likely reflects charge-associated red cell surface properties while partitioning in system 2 depends more on lipid-related parameters.

Surface properties of erythrocytes from rats belonging to the highly inbred Lewis strain

We compared the surface properties of red blood cells from different Lewis rats using the mixed, labeled cell procedure outlined under methods. Typical results, obtained in 5 of 5 experiments, are presented in Figs. 1 and 2. When labeled cells are mixed with unlabeled cells from the same rat (A + A, B + B) there is perfect overlap of labeled and total cells. This shows that the labeling of red cells with ^{51}Cr does not, per se, affect the cells' partitioning behavior. When labeled cells from one Lewis rat are mixed with unlabeled cells from a second Lewis rat (A + B, B + A) note that labeled and unlabeled cells again overlap. These results indicate that, in the highly inbred Lewis rat strain, no difference in erythrocyte surface properties is discernible by partitioning.

Surface properties of erythrocytes from rats belonging to the Sprague-Dawley strain

Experiments analogous to those described above were carried out with red blood cells from Sprague-Dawley rats. Fig. 3 depicts an experi-

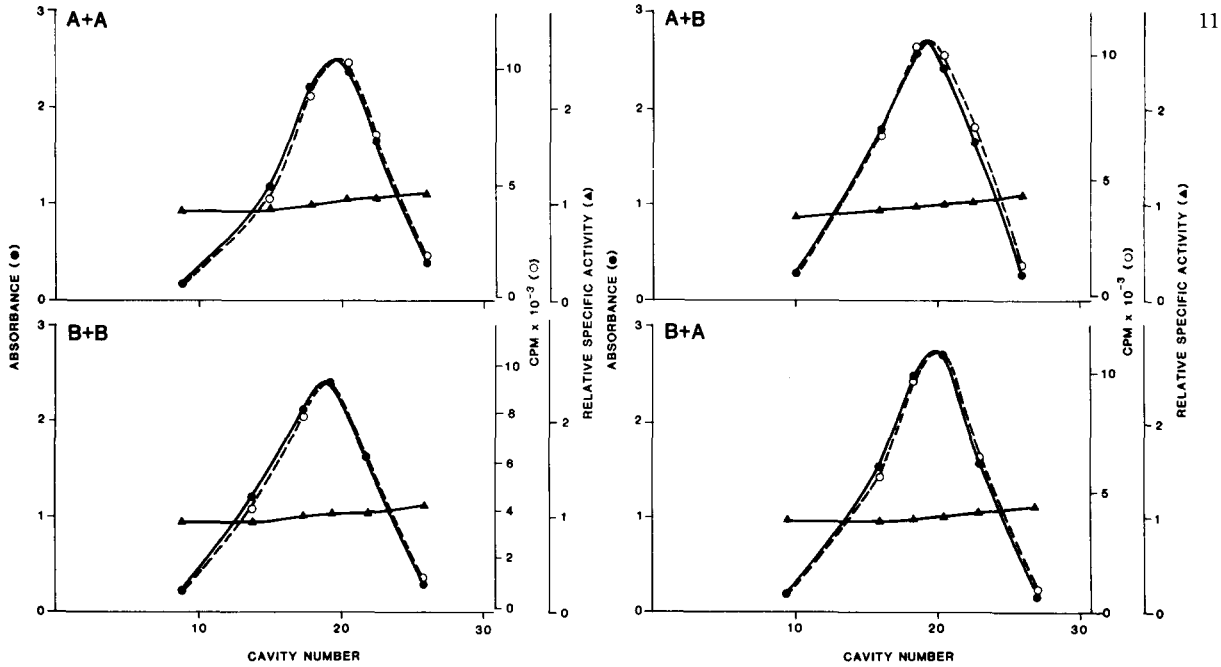

Fig. 1. Comparison of surface properties of red blood cell populations obtained from two rats of the highly inbred Lewis strain. Blood was drawn from two rats (A and B). Aliquots of erythrocytes from each rat were labeled with [^{51}Cr]chromate. Labeled cells were washed and mixed with an excess of unlabeled, washed red cells from the other rat (A + B, B + A). As control, 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 countercurrent distribution in a dextran-poly(ethylene glycol) aqueous phase system which reflects charge-associated surface properties. Phase system composition: 5% (w/w) dextran T500, 4% (w/w) poly(ethylene glycol) 8000, 0.09 M sodium phosphate buffer (pH 6.8) and 0.03 M NaCl. 30 transfers were completed at 4–5°C using a settling time of 6 min and a shaking time of 22 s. ●———●, Total cell distribution in terms of hemoglobin absorbance at 540 nm; ○———○, the distribution of labeled cells (in cpm); ▲———▲, the relative specific activities (with 1.0 being the specific activity of the original, unfractionated cell mixture in each case). For additional details, see text and Refs. 3–5.

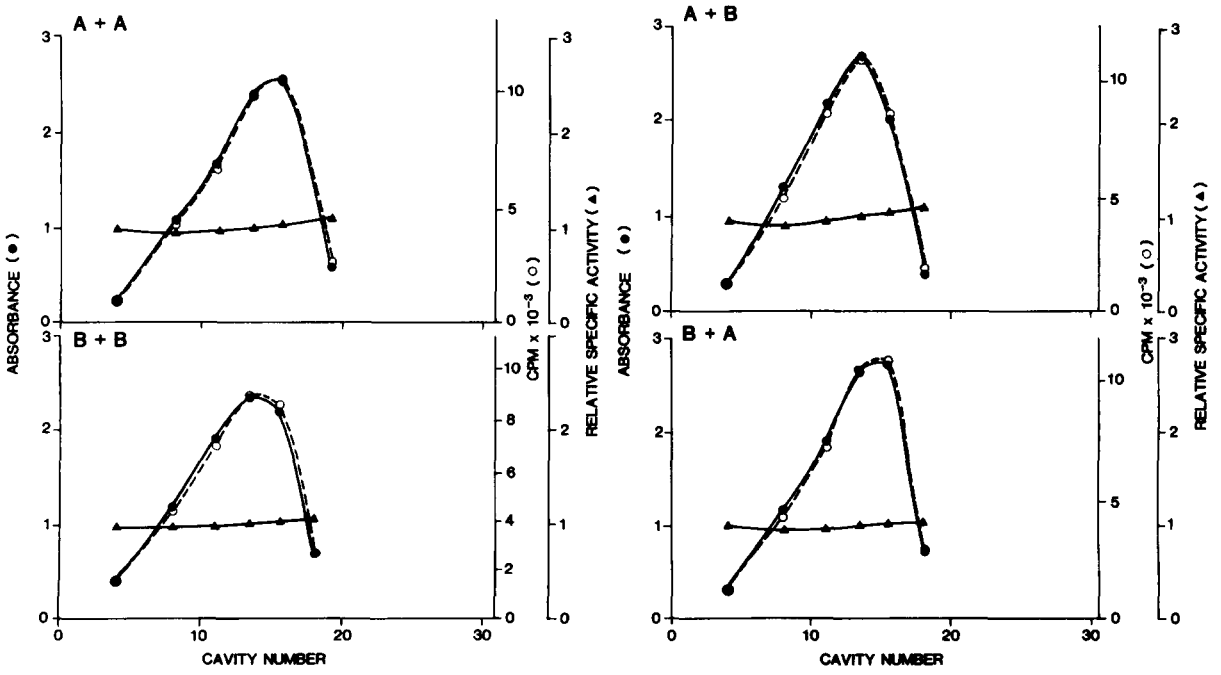

Fig. 2. Experiment as in Fig. 1 except that the phase system used measured lipid-related membrane parameters. Phase system composition: 5% (w/w) dextran T500, 3.5% (w/w) poly(ethylene glycol) 8000, 0.15 M NaCl and 0.01 M sodium phosphate buffer (pH 6.8). Settling time used was 7 min. All other conditions and symbols as in Fig. 1.

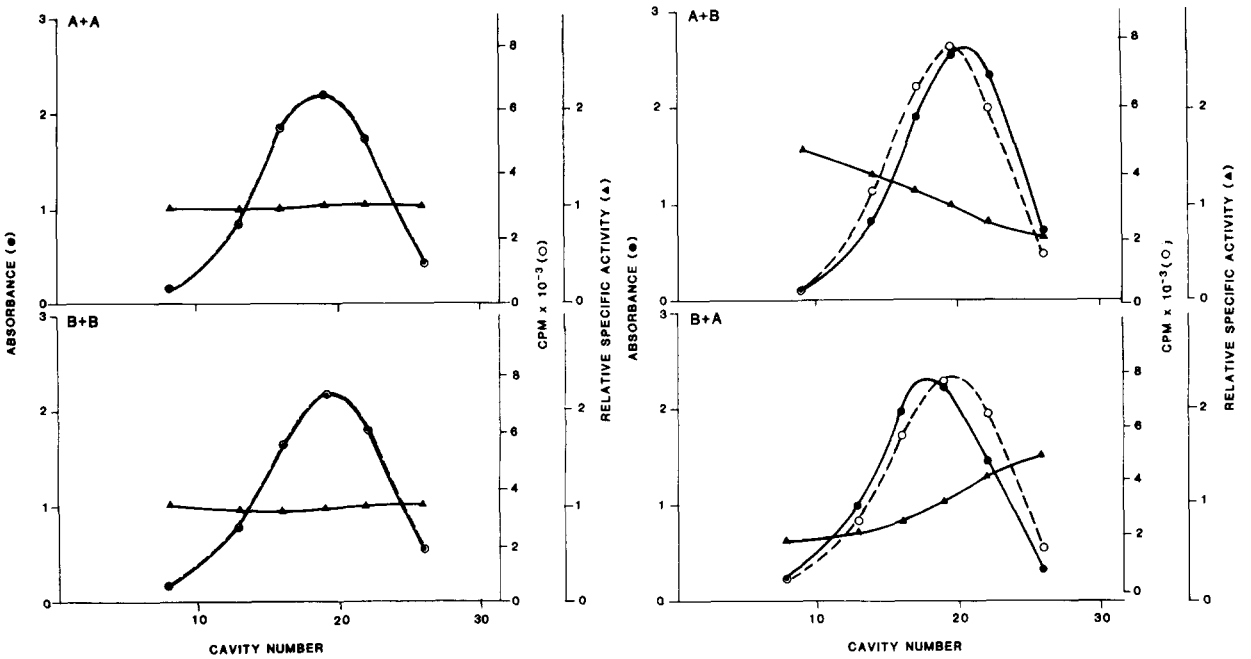

Fig. 3. Experiment as in Fig. 1 except that erythrocytes from two rats of the out-bred Sprague-Dawley strain were used.

ment, in phase system 1, in which the surface properties of erythrocytes from two Sprague-Dawley rats are shown to differ. This is revealed by the displacement of total cell distribution curves from isotope curves in (A + B) and in (B + A). In Fig. 3 note that in (A + B) labeled red cells A have a lower partition ratio (i.e., are to the left) than do red cells B. When labeled red cells B are mixed with unlabeled red cells A (B + A), red cells A again have the lower partition ratio. This, as has been previously discussed [5], indicates that we are not dealing with an artefact but with a real difference between surface properties of erythrocytes from A and B and that, in the present case, A has a lower and B a higher partition ratio.

The results obtained with Sprague-Dawley rats were more variable as compared to those with Lewis rats. Data depicted in Fig. 3 are representative of 10 out of 13 experiments. In the remaining three experiments no difference was found between erythrocytes from the particular rats examined.

Experiments (not shown) with erythrocytes from Sprague-Dawley rats using phase system 2 revealed differences between the surface properties of erythrocytes in 8 of 12 experiments.

Comparison of surface properties of erythrocytes from rats belonging to the Lewis strain with those belonging to the Sprague-Dawley strain.

Figs. 4 and 5 show results obtained in charge-sensitive and non-charge-sensitive phase systems, respectively, when comparing Sprague-Dawley ('A') and Lewis ('B') rat erythrocyte surfaces by partitioning.

In phase system 1 (Fig. 4), Sprague-Dawley rat red cells always have a lower partition ratio (i.e., are to the left) than Lewis rat erythrocytes (12 out of 12 experiments). In phase system 2 (Fig. 5), Sprague-Dawley rat erythrocytes usually have a lower partition ratio than Lewis red cells (6 of 8 experiments with two experiments showing no difference between the particular rats' red cells examined). The control experiments depicted in Figs. 4 and 5 (A + A, B + B) again serve to indicate that ^{51}Cr-labeling per se has no effect on the red cells' partitioning behavior (see above).

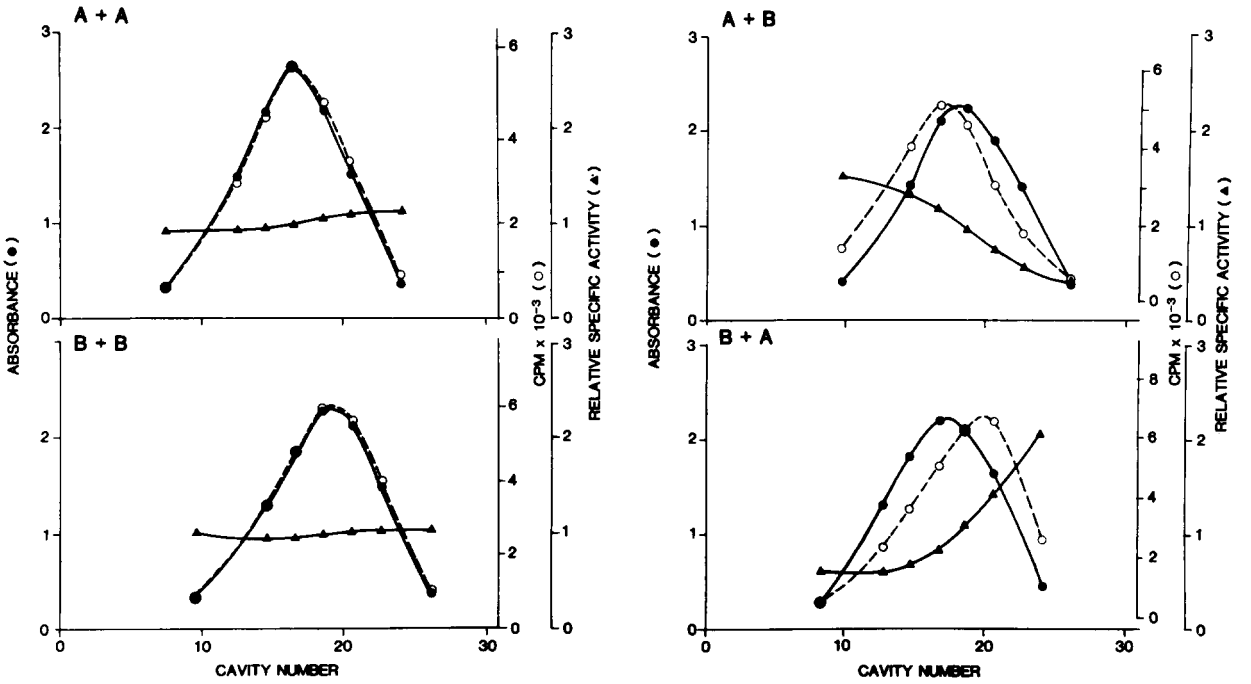

Fig. 4. Experiment as in Fig. 1 except that erythrocytes from one Sprague-Dawley rat ('A') and one Lewis rat ('B') were compared.

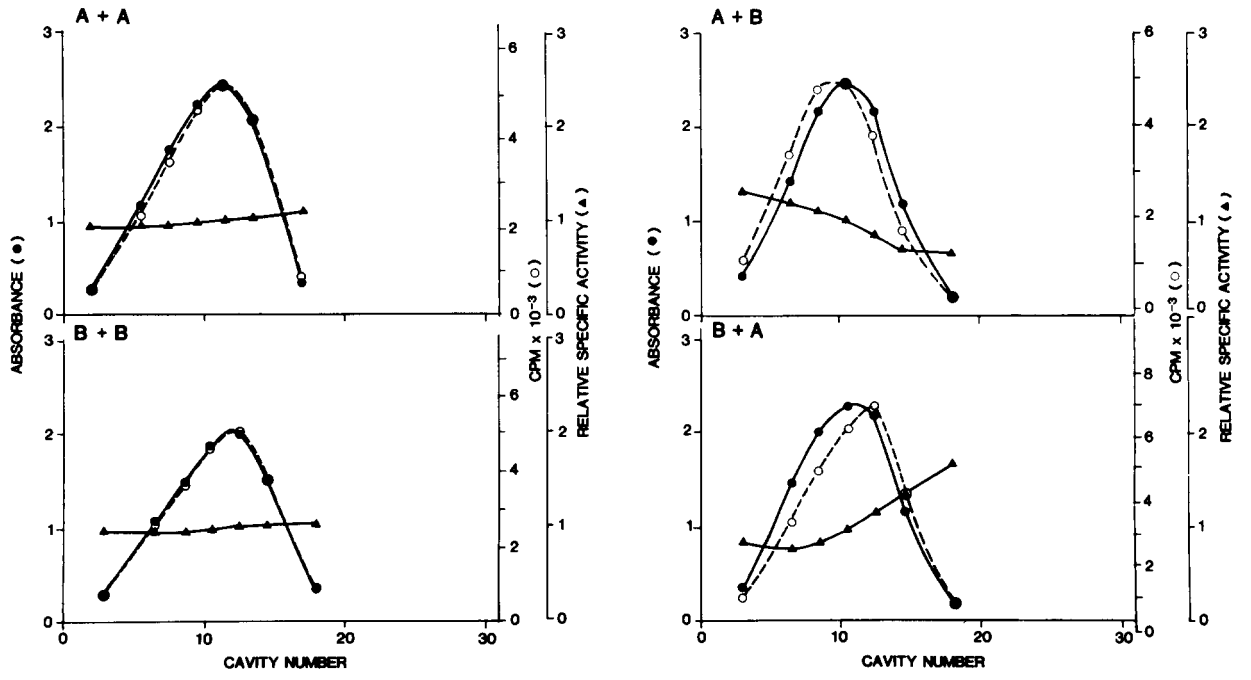

Fig. 5. Experiment as in Fig. 2 except that erythrocytes from one Sprague-Dawley rat ('A') and one Lewis rat ('B') were compared.

Discussion

The parameters which determine the partition ratio of cells (i.e., the quantity of cells in the top phase as a percentage of total cells added) are related exponentially to it [14]. Thus, by use of charge-sensitive systems (i.e., those with an electrostatic potential difference between the phases) or non-charge-sensitive systems (i.e., those with no potential difference between the phases and lower polymer concentrations), information on extremely subtle charge-associated or non-charge-related surface properties of cells can be obtained [1,2]. Furthermore, differences between the surface properties of two closely related cell populations can be detected by subjecting mixtures of isotopically labeled cells of one population and an excess of unlabeled cells from the second population to countercurrent distribution in dextran-poly(ethylene glycol) aqueous phases and noting whether the total cell distribution and the isotopically labeled cell distribution overlap or are displaced from one another [3–5]. Using this methodology we have found a difference in surface properties of erythrocytes (unrelated to ABO or MN blood group) from arbitrarily chosen (presumably hematologically normal) individuals [5]. To determine whether the observed differences have an acquired or a genetic basis we have begun to compare erythrocytes from identical siblings. In the only experiment completed thus far on one set of identical triplets no difference was observed [5]. In the experiments depicted in Figs. 1 and 2 we have found that neither charge-associated nor lipid-related differences can be detected by partitioning erythrocyte populations from inbred (i.e., syngeneic) Lewis rats. Analogous experiments with out-bred Sprague-Dawley rats (Fig. 3) revealed charge-associated surface differences between 10 of 13 pairs of rats examined. Lipid-related differences could also be observed in 8 of 12 experiments. It thus appears that the differences between red blood cells from different rats reflected by partitioning are genetic rather than acquired.

Highly reproducible differences (12 of 12 experiments in charge-sensitive and 6 of 8 in non-charge-sensitive phases) are in evidence (Figs. 4 and 5) between erythrocytes from the Lewis and Sprague-Dawley rat strains. In both cases, Sprague-Dawley erythrocytes have the lower partition ratio, probably indicating a lower surface charge, that is, less sialic per unit surface area [12], and possibly a lower ratio of membrane poly/monounsaturated fatty acids [13].

While we conclude that partitioning detects surface charge-associated or non-charge-related differences that are genetically determined, certain acquired surface alterations may be detectable as well. Thus, we are currently studying the alteration of red cell partitioning caused by viral infection of inbred rats. We are also examining the partition behavior of human red cells in certain selected disease states (e.g. paroxysmal nocturnal hemoglobinuria [15], sickle cell anemia, etc.). In the latter cases, differences, to be judged significant, between hematologically normal individuals and those having the disease state, must exceed the differences observed among hematologically normal individuals [5].

Acknowledgement

This work was supported by the Medical Research Services of the Veterans Administration.

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