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Detection of Surface Differences Between Two Closely Related Cell Populations by Partitioning Isotopically Labeled Mixed Cell Populations in Two-Polymer Aqueous Phases

II. A Correction

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This publication is to correct the inadvertent reproduction of one figure twice and the omission of a second figure in paper (I) of this series (Cell Biophysics ***5,*** *205–219, 1983). The original abstract is reproduced here together with all of the figures in correct sequence.*

Abstract

The partition behavior of cells in dextran–poly(ethylene glycol) aqueous phases (i.e., the cells' relative affinity for the top or bottom phase or their adsorption at the interface) is greatly dependent on the polymer concentrations and ionic composition and concentration. Appropriate selection of phase system composition permits detection of differences in either charge-associated or lipid-related surface properties. We have now developed a method that can reveal differences by partitioning that fall within experimental error if one were to compare countercurrent distribution (CCD) curves of two closely related cell populations run separately. One cell population is isotopically labeled in vitro (e.g., with ^{51}Cr-chromate) and is mixed with an excess of the unlabeled cell population with which it is to be compared. The mixture is subjected to CCD and the relative specific radio-activities are determined through the distribution. As control we also examine a mixture of labeled cells and unlabeled

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cells of the same population. The feasibility of this method was established by use of cell mixtures the relative partition coefficients of which were known. The procedure was then used to test for human erythrocyte subpopulations. ^{51}Cr-chromate-labeled human young or old red blood cells were mixed with unfractionated erythrocytes and subjected to CCD in a phase system reflecting charge-associated properties. It was found that older cells had a high, young cells (probably only reticulocytes) a low partition coefficient. Because of the small differences involved these results were not previously obtained. It was further determined, by repartitioning ^{51}Cr-labeled cells from the left or right ends of a CCD of human red blood cells admixed to unlabeled unfractionated erythrocytes, that a subpopulation with higher partition coefficient exists (probably constituting the old red cells). These experiments serve to illustrate (a) that human red blood cells, contrary to a previous report, can be subfractionated by partitioning and (b) the usefulness of this new method in detecting smaller surface differences between closely related cell populations than was heretofore possible by partitioning alone.

Index Entries: Surface differences, in human rbc subpopulations; cell surface differences, in human rbc subpopulations; partitioning, of isotopically labeled rbc populations; isotopically labeled rbc populations, partitioning of; human rbc subpopulations, partitioning of; red blood cell populations, partitioning of; blood cell populations, partitioning of.

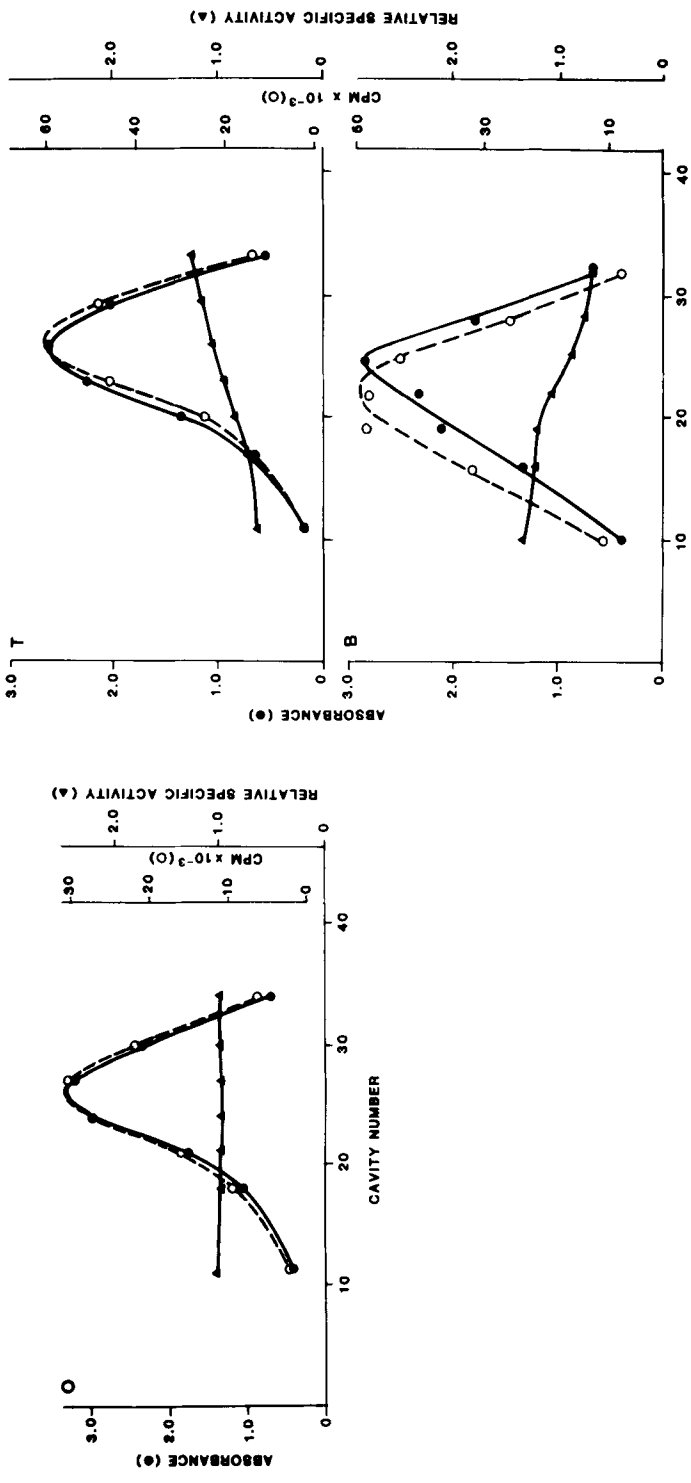

Fig. 1. Countercurrent distribution patterns of mixtures of ^{51}Cr-labeled unfractionated rat red blood cells + unlabeled, unfractionated red cells from the same rat (O); ^{51}Cr-labeled rat young red cells + unlabeled, unfractionated rat red cells (T); and ^{51}Cr-labeled rat old red cells + unlabeled, unfractionated rat red cells (B). Young and old cells were obtained by a centrifugal method as described in the text. Countercurrent distribution was carried out in phase system 2, which reflects surface charge-associated properties. Forty transfers were completed using 6 min settling and 22 s shaking. Temperature was 3–5°C. The distribution of the entire red cell mixture is given in terms of hemoglobin absorbance at 540 nm (●); the distribution of the isotopically labeled cell population in cpm (○). A relative specific activity (▲) is also shown and indicates the extent to which the labeled and unlabeled cell populations in a given mixture differ. For additional details see text.

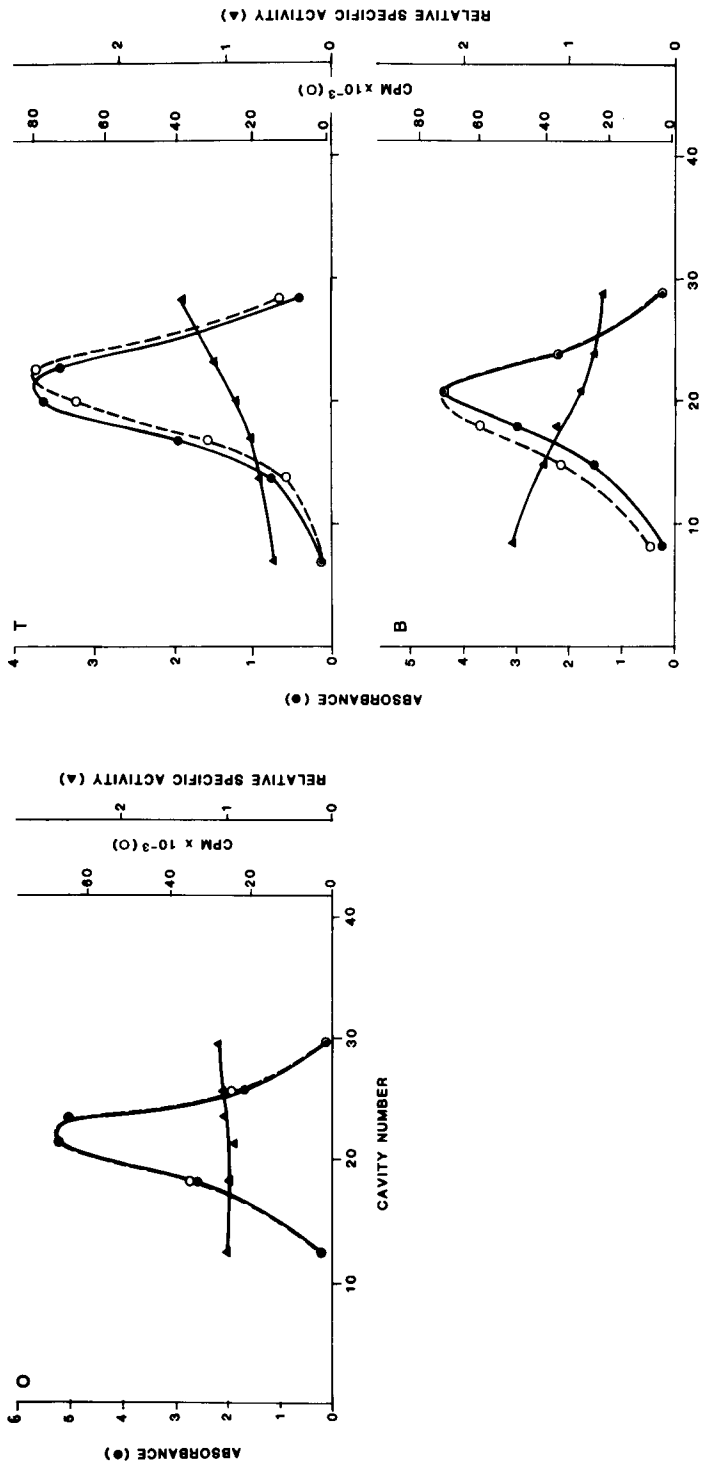

Fig. 2. Experiment similar to that shown in Fig. 1, except that phase system 3, which reflects surface lipid-related properties, was used. Settling time used was 7 min. All other conditions and symbols as in Fig. 1.

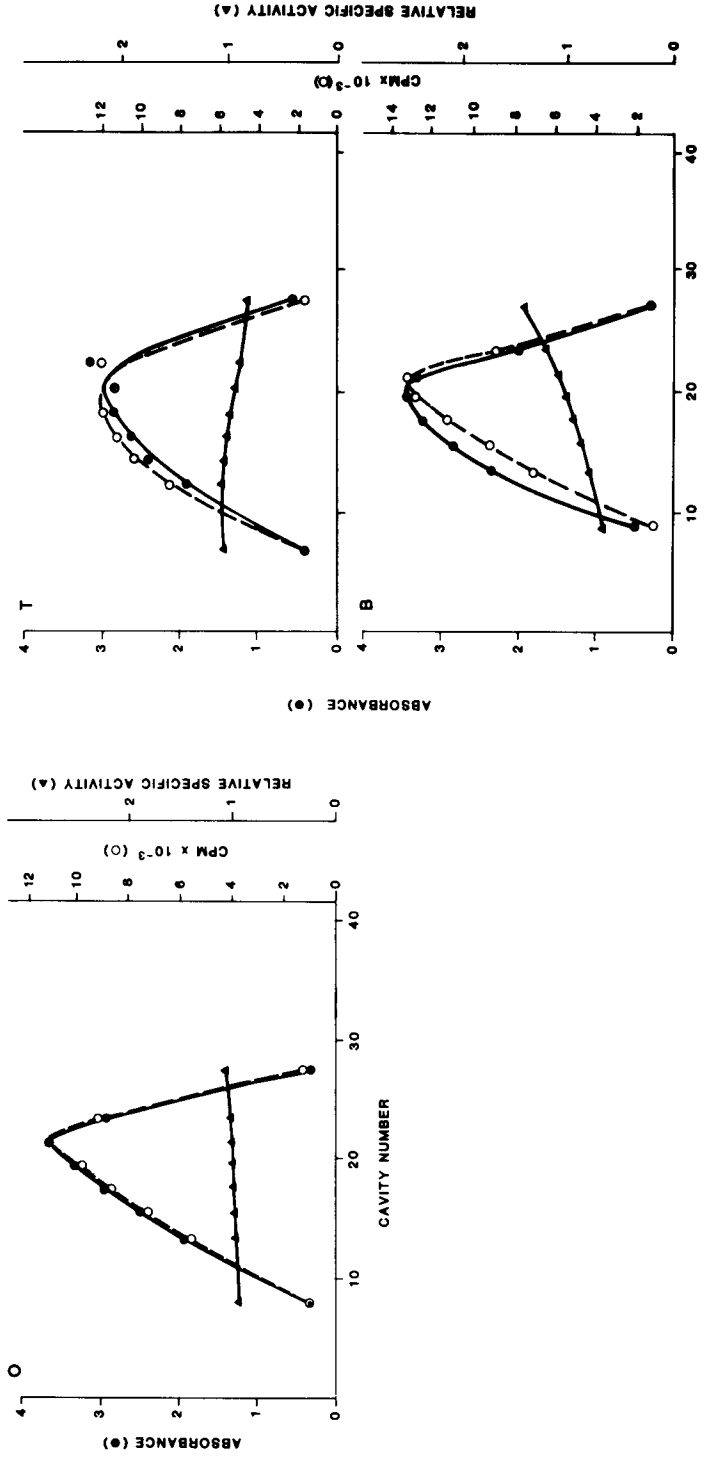

Fig. 3. Experiment similar to that shown in Fig. 1, except that human red blood cells (from which white cells had been removed) were studied. Phase system 1, which reflects charge-associated properties, was used. All other conditions and symbols as in Fig. 1.

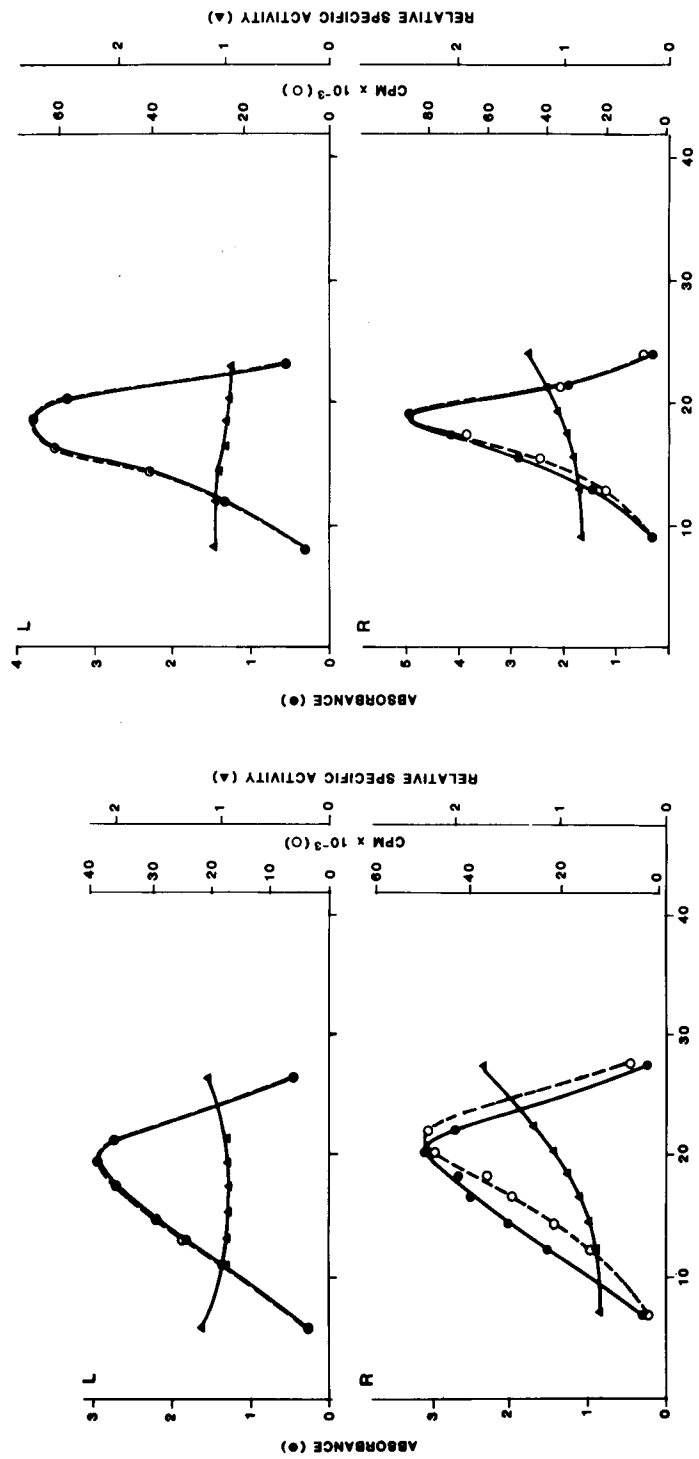

Fig. 4. Human red blood cells were subjected to countercurent distribution in either phase system 1 or 4. The former reflects charge-associated properties and the latter lipid-related ones. Sixty transfers were carried out, and 30–35% of the cells under the left and a similar percentage of cells under the right ends of the distributions were separately pooled. Cells were labeled with ^{51}Cr-chromate and mixed with an excess of unlabeled, unfractionated red cells from the same individual. These mixtures were subjected to CCD in a phase system having the same composition as used in the original respective CCD runs. The results of these re-CCD runs are depicted in this Figure. The left half of Fig. 4 gives results obtained in phase system 1; the right half in phase system 4. The upper parts of the figure show mixtures of the ^{51}Cr-labeled left end of the original CCD + unlabeled, unfractionated red cells (L); the bottom parts ^{51}Cr-labeled right end + unlabeled, unfractionated red cells (R). Settling time for phase system 4 was 8 min. Other conditions and symbols as in Fig. 1. See text for details and discussion.