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SURFACE DIFFERENCES BETWEEN ERYTHROCYTES FROM ARBITRARILY CHOSEN (PRESUMABLY HEMATOLOGICALLY NORMAL) INDIVIDUALS DETECTED BY CELL PARTITIONING

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Differences in membrane surface properties (both charge-associated and lipid-related) of erythrocytes from any two arbitrarily selected individuals can be detected by use of a purely physical method: cell partitioning in dextran-poly(ethylene glycol) aqueous phase systems. The procedure consists of isotopically labeling (with [^{51}Cr]-chromate) aliquots of red blood cell populations to be compared. Such labeled cells are mixed with an excess of unlabeled erythrocytes from the other individual and the mixtures subjected to countercurrent distribution in either a charge-sensitive or a non charge-sensitive aqueous phase system. As control we also prepare mixtures of labeled cells with unlabeled cells from the same individual to ascertain that the label *per se* has no influence on the cells' partitioning behavior. The distribution curves are analyzed for total cells (in terms of hemoglobin absorbance) and labeled cells (in terms of counts/min). Changes in the relative specific activities through the distribution curves are routinely obtained when cells from different individuals are used and are indicative of subtle differences in surface properties of such erythrocyte populations.

Partitioning of cells in dextran-poly(ethylene glycol) aqueous phase system is a highly sensitive and versatile method for the separation and subfractionation of cell populations (1,2). The sensitivity stems from the fact that surface characteristics reflected by partitioning, unlike most other separatory procedures, are related to the partition coefficient K (defined as the quantity of cells in the top phase as a percentage of total cells added) in an exponential manner. The versatility is due to the finding that manipulation of polymer concentration and ionic composition and concentration yields phase systems with appreciably different physical properties. Thus with appropriate phase system composition one can determine to a great

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

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extent whether the separation is due to surface charge-associated, lipid-related or receptor-dependent properties (3-5).

We have developed and recently reported a method that permits the detection of surface differences by partitioning between closely related cell populations the K's of which would fall within experimental error if one were to compare the countercurrent distribution (CCD, i.e., multiple extraction) curves of such populations run separately (6). In brief, the method entails isotopically labeling aliquots of the two cell populations (in the case of red cells with [^{51}Cr]-chromate), mixing each of these populations with an excess of unlabeled cells of the population to which it is to be compared and also, as control, mix labeled cells with an excess of unlabeled cells of the same population. These four preparations are then separately but simultaneously subjected to CCD. Determination of relative specific activities through the distribution curves indicates whether differences between the investigated populations exist. Even slight differences are indicated by a deviation of relative specific activities from 1.00. Mixing labeled cells with the unlabeled cells from the same population is essential as an indicator that the isotopic labeling procedure per se has no effect on the surface properties reflected by partitioning.

This method has been established (6) by examining the behavior of cell populations the relative partition coefficients of which were known. It was then applied to the detection of differences between human young and old red cells, differences which had previously (7,8) not been detectable; as well as to cells from the left and right ends of a human red cell CCD (8). Thus the presence of subpopulations in human red blood cells, differing in surface properties, and detectable by partitioning, was established (6).

Our interest now turned to an examination of the relative charge-associated and lipid-related surface properties of red blood cells from hematologically normal individuals and from those having selected disease states in which red cell membrane differences have either been reported or are suspected (e.g., sickle cell anemia, muscular dystrophy, etc.). In order to determine

to what extent differences detected by the described mixed cell experiments are in excess of those found between normal individuals, we set up to determine "baseline" values by examining the behavior of mixed red cell populations from randomly chosen hematologically normal individuals. We were surprised to find partitioning differences between red cells from any such arbitrarily chosen individuals in phase systems which reflect either charge-associated or lipid-related surface properties. These results are described here.

EXPERIMENTAL METHODS

The procedures employed to collect blood from presumably hematologically normal individuals, anticoagulant used, [^{51}Cr]-chromate labeling procedures, preparation of dextran-poly(ethylene glycol) aqueous phase systems and the method of countercurrent distribution have all previously been detailed (6). In the present experiments blood, each time from two arbitrarily chosen individuals, was obtained. To compare the surface properties of two such red cell populations ("A" and "B") 0.15 ml of [^{51}Cr]-labeled red cells (washed as previously indicated, 6) were pipetted into a centrifuge tube containing 5 ml of phosphate-buffered saline, pH 7.0. 0.6 ml of unlabeled, washed red blood cells with which the labeled cells were to be compared were pipetted into the same tube. Cells in the tubes were gently mixed and washed an additional three times with phosphate-buffered saline. The packed cells in the tubes, after the final wash, were used to make the load mix for countercurrent distribution (6). The following 4 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 the [^{51}Cr]-labeling procedures per se has no effect on the surface properties of the red cells as reflected by partitioning (see Figs. 1 and 2).

Countercurrent distribution was carried out separately but simultaneously (6) on the 4 cell mixtures described above using either a charge-sensitive phase system (Fig. 1) or a non charge-sensitive system (Fig. 2). The former reflects charge-associated surface properties of cells while the latter measures lipid-related membrane parameters (1,2).

Total cell distribution, in terms of hemoglobin absorbance, gives in each case predominantly the distribution of the unlabeled cell population since these are the cells present in great excess. The distribution of the labeled cell population is given in counts/min. The deviation of relative specific activities (6) from 1.00 (i.e., the relative specific activity of the original, unfractionated cell mixture) through the distribution curve reflects the degree of displacement and, hence, of differences between any two such red blood cell populations.

RESULTS AND DISCUSSION

Typical results are presented in Figs. 1 and 2. When labeled cells are mixed with unlabeled cells from the same person (A + A, B + B) there is perfect overlap of labeled and total cells. Labeled cells mixed with unlabeled

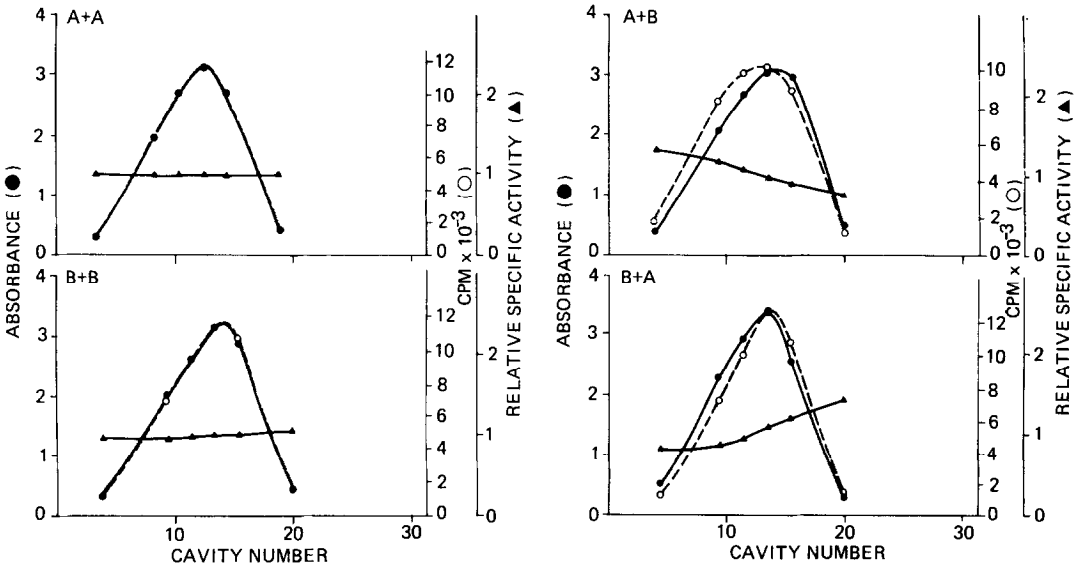

Fig. 1. Comparison of surface properties of red blood cell populations obtained from two randomly* selected, presumably hematologically normal individuals. Blood was drawn from two individuals (A and B). Aliquots of red cells from each were labeled with [^{51}Cr]-chromate. Labeled cells were washed and mixed with an excess of unlabeled red cells from the other individual (A + B, B + A). As control, aliquots of labeled cells were also mixed with an excess of unlabeled cells from the same person (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 membrane properties. Phase system composition: 5% (w/w) dextran T500, 4% (w/w) poly(ethylene glycol) 8000, 0.11 M Na-phosphate buffer, pH 6.8. Thirty transfers were completed at 4°C using a settling time of 6 min and a shaking time of 22 s. ● - ●, indicates total cell distribution in terms of hemoglobin absorbance at 540 nm; ○ - ○, shows the distribution of the labeled cells (in terms of counts/min); and ▲ - ▲, gives the relative specific activities (with 1.00 being the relative specific activity of the original, unfractionated cell mixture in each case). For additional details see text; for procedural details see ref. 6.

*The individuals A and B depicted in Figs. 1 and 2 were actually both A+.

red cells from another individual (A + B, B + A) give countercurrent distribution curves in which labeled cells and total cells are displaced.

Note that in the experiment depicted in Fig. 1 labeled red cells A have a lower partition coefficient (i.e., are to the left) than do red cells B (see part A + B). When labeled red cells B are mixed with unlabeled erythrocytes A (B + A), red cells A again have the lower partition coefficient. This result indicates that we are dealing not with an artefact but with a real difference between surface properties of erythrocytes from A and B and, in the case presented, A has a lower and B a higher partition coefficient. Since the

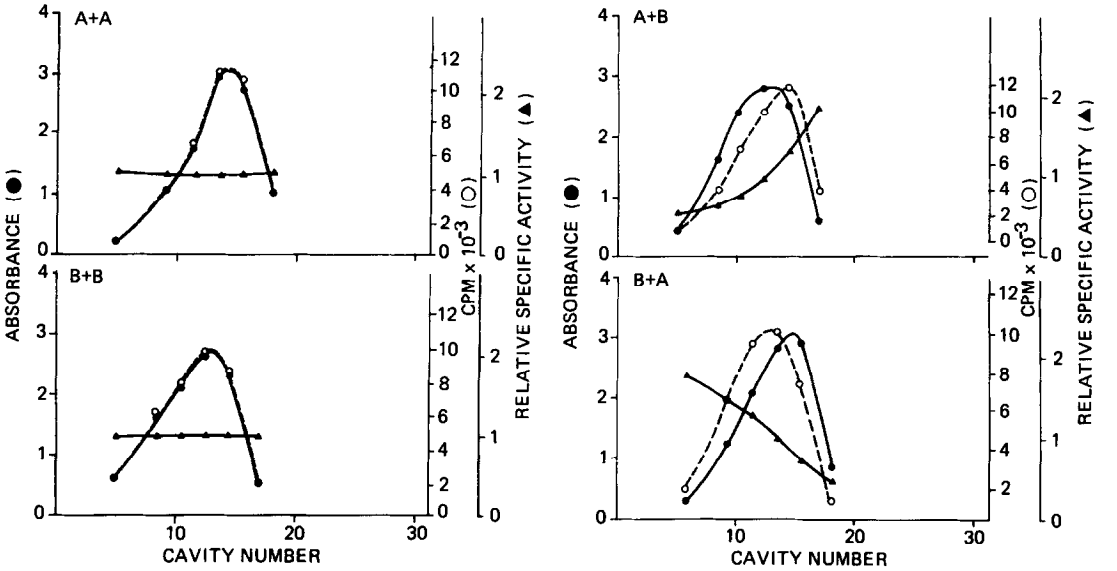

Fig. 2. Experiment as in Fig. 1 except that the phase system used measured lipid-related membrane parameters. Phase system composition: 4.85%(w/w) dextran, 3.3% (w/w) poly(ethylene glycol), 0.15 M NaCl and 0.01 M Na-phosphate buffer, pH 6.8. Settling time used was 8 min. All other conditions and symbols as in Fig. 1. Blood used for this experiment was from the same individuals as used for the experiment shown in Fig. 1.

phase system used for the experiment depicted in Fig. 1 is charge-sensitive, the differences found are presumably charge-associated (1,2).

An experiment (Fig. 2) with blood from the same individuals as in Fig. 1 was carried out in a non charge-sensitive phase system (with lower polymer concentrations, 3), which probably measures some lipid-related surface parameters (3). Note that in the non charge-sensitive phase system A (which had a lower partition coefficient than B in the charge-sensitive phases, Fig 1) has a higher partition coefficient. This reversal of relative partition coefficients in charge-sensitive and non charge-sensitive phase systems was seen in about half of the experiments comparing red cells from arbitrarily selected individuals.

Experiments on 2 sets of individuals were run twice (with either 1 or 3 months intervening) and, in each case, the differences established in the first experiment were repeated and the direction of difference (i.e., either A or B in a given experiment having the higher partition coefficient than B or A) was duplicated. In all 11 experiments in the charge-sensitive system

differences were found between two arbitrarily chosen people in each experiment. (In one case in which erythrocytes from a hematologically normal individual and a patient having hereditary spherocytosis were compared the cells showed no difference. This is probably indicative that a small percentage of sets of individuals have red cells which do not differ in surface properties in the charge-sensitive phase system.) In 1 experiment out of a total of 14 run in the non charge-sensitive system there was no difference between the individuals selected. In NO case were two arbitrarily selected individuals found who showed no difference in BOTH charge-sensitive and non charge-sensitive phase systems. Selection of individuals with the same ABO blood group (see Figs. 1 and 2) yields results comparable to those obtained with red cells from persons having different ABO groups.

Our results indicate surface differences detectable by cell partitioning between red blood cells from arbitrarily selected individuals.

We plan now to determine whether the observed surface differences between red cell populations from arbitrarily selected people have an acquired or a genetic basis by comparing red blood cells from identical siblings. A single experiment completed thus far with identical triplets revealed no difference between their erythrocytes' surface properties.

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REFERENCES

1. Walter, H. (1982) in: Cell Separation: Methods and Selected Applications (Pretlow, T.G. and Pretlow, T.P. eds) Vol. 1, Academic Press, New York, pp. 261-299.
2. Walter, H. (1977) in: Methods of Cell Separation (Catsimpoolas, N. ed) Vol. 1, Plenum Press, New York, pp. 307-354.
3. Brooks, D.E., Seaman, G.V.F. and Walter, H. (1971) Nature New Biol. (London) 234, 61-62.
4. Walter, H., Krob, E.J. and Brooks, D.E. (1976) Biochemistry 15, 2959-2964.
5. Flanagan, S.D., Barondes, S.H. and Taylor, P. (1976) J. Biol. Chem. 251, 858-865.
6. Walter, H. and Krob, E.J. (1983) Cell Biophys. 5, 205-219.
7. Walter, H., Krob, E.J., Tamblyn, C.H. and Seaman, G.V.F. (1980) Biochem. Biophys. Res. Commun. 97, 107-113.
8. Walter, H., Krob, E.J. and Ascher, G.S. (1981) Biochim. Biophys. Acta 541, 202-215.