THE NATURE OF THE CELL MEMBRANE CHARGE MEASURED BY PARTITION IN AQUEOUS TWO-POLYMER PHASE SYSTEMS: DIFFERENTIATION OF CLASSES OF BEEF ERYTHROCYTES

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Summary: Beef erythrocytes are comprised of three major classes: those having low, intermediate or high partition coefficients in aqueous dextran-polyethylene glycol phases. All beef erythrocytes have the same electrophoretic mobility but cells having a high partition release far more sialic acid when treated with neuraminidase or trypsin than do cells belonging to the other two classes. The partition of cells in two-polymer aqueous phase systems is thus shown to measure charge-associated-properties much deeper into the membrane than does cell electrophoresis.

Aqueous solutions of different polymers (e.g. dextran, polyethylene glycol) when mixed above certain concentrations give rise to liquid two-phase systems (1). Such systems, when buffered and rendered isotonic, have proved useful in the partition (and separation by countercurrent distribution) of cells, particles, membranes and macromolecules (1-6). Subtle changes in the surface properties of cells as a function of *in vivo* or *in vitro* processes are often detectable by changes in the partition of the cells (7,8).

One of the major determinants in the partition of cells in dextran-polyethylene glycol phases is surface charge (9,10). This was first suggested on the basis of a correlation between the relative partitions and electrophoretic mobilities of erythrocytes from a number of species (9). The diminution of red cell partition with age (3) similarly found its counterpart in a concomitant reduction of electrophoretic mobility with increasing cell age (11). Surface charge is a major determinant in the partition of cells probably because of the unequal partition of some inorganic salts between the phases (12). This gives rise to an electrical potential between them (1). A zeta potential has been demonstrated between the two phases by electrophoresis of droplets of one phase in the other. The magnitude and sign of the potential was found to

depend markedly on the salt composition of the phase system (13).

In those cases in which partition and electrophoretic mobility do not correlate, surface properties other than electrokinetic charge must be measured by the phases. This communication presents evidence that with beef erythrocytes such is indeed the case and, furthermore, establishes the probable nature of the surface properties determined by partition.

Fresh beef blood was collected in acid-citrate-dextrose (ACD) at a local slaughterhouse and stored at 4 - 6°. The erythrocytes were used for the experiments described within a period of 4 days following blood collection. They were routinely washed three times with saline prior to examination.

Buffered, dextran-polyethylene glycol aqueous two-phase systems described by Albertsson and Baird (2) were used in this work. Phase system I contained 5% (w/w) dextran T500, batch 5996 (Pharmacia Fine Chemicals, N.J.), 4% (w/w) polyethylene glycol ("Carbowax 6000", Union Carbide, N.Y.), and 0.11 M Na-phosphate buffer, pH 6.8. Phase system II had the same polymer composition but 0.09 M Na-phosphate buffer, pH 6.8, and 0.03 M NaCl. The procedure for the partition of erythrocytes has been described in detail (14). Beef erythrocytes were partitioned in the above-indicated phase systems at room temperature with a settling time of 20 min (see Table I). Partition is expressed as the quantity of cells found in the top phase (percent of total cells added).

Countercurrent distribution of beef erythrocytes was also carried out as previously reported (7) using phase system II in the cold-room (4 - 6°) and 60 transfers. Cells in the different cavities of the extraction train were collected after countercurrent distribution; lysed and the hemoglobin absorbance (540 nm) measured. The results are presented in Figure 1.

Standard washed and centrifugally packed beef red blood cells were treated with Vibrio cholerae neuraminidase (Behringwerke, Marburg/Lahn) as described by Uhlenbruck, Seaman & Coombs (15) or with trypsin (Worthington Biochemical Corp.) as reported by Seaman and Heard (16). The supernatant fluids from the neuraminidase or trypsin-treated cells were analyzed for sialic acid by the

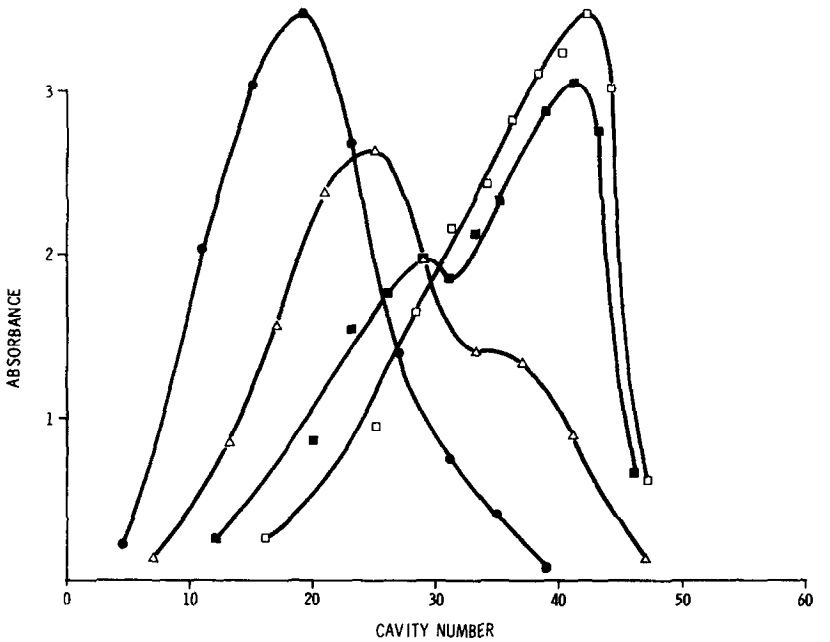

Fig. 1. Superimposed countercurrent distribution curves obtained with beef erythrocytes of different classes. Phase system II was used and 60 transfers were completed at 4 - 6°. (● - ●), beef erythrocytes of class 1; (Δ - Δ), class 2; (■ - ■) and (□ - □), patterns of two different animals belonging to class 3.

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method of Svennerholm (17). Microelectrophoresis was carried out on the beef red blood cells as previously described (18).

Beef red blood cells were reported to fit the correlation between partition and relative electrophoretic mobility found for a number of species (9,19). Two communications (20) have indicated that the partition of beef red cells, though reproducible for individual animals, varied greatly from animal to animal. The partition of beef red blood cells was therefore re-examined with larger numbers of animals than originally tested. It was found that beef red cell partitions fall into three classes: low, intermediate and high. Electrophoretic examination of beef erythrocytes gave a single mobility value and did not discriminate between the three classes.

It has been shown, however, that beef red cells can be subdivided into several groups on the basis of their agglutinability with various antisera (15).

TABLE I

PARTITION[a] AND MEMBRANE SIALIC ACID[b] OF BEEF ERYTHROCYTES

Class of Cell[c]	Partition in Phase System		Sialic Acid Released by	
	I	II	Neuraminidase	Trypsin
1	37 ± 13 (10)	13 ± 2 (8)	88 ± 8 (8)	47 ± 9 (8)
2	62 ± 9 (11)	17 ± 3 (8)	91 ± 8 (5)	49 ± 9 (5)
3	90 ± 11 (14)	27 ± 3 (11)	137 ± 11 (9)	105 ± 11 (5)

Results are presented as the mean ± S.D. with the number of experiments indicated in parentheses.

[a]Partition is expressed as quantity of cells found in top phase (percent of total cells added). Phase system I contained 5% (w/w) dextran, 4% (w/w) polyethylene glycol and 0.11 M Na-phosphate buffer, pH 6.8. Phase system II had the same polymer composition but 0.09 M Na-phosphate buffer, pH 6.8, and 0.03 M sodium chloride.

[b]Membrane sialic acid released by treatment with neuraminidase or trypsin (γsialic acid/ 10^{10} cells).

[c]Classes 1 + 2 and 3 correspond, on the basis of the quantity of sialic acid released by neuraminidase, to "agglutinable" + "moderately agglutinable" and "inagglutinable" erythrocytes, respectively, as described by Uhlenbruck, Seaman and Coombs (15). Countercurrent distribution of beef erythrocytes reveals the presence of a still greater variety and heterogeneity than can suitably be described by three classes. See Figure 1 and discussion in text.

Although these red cells have the same electrophoretic mobilities different quantities of sialic acid are released by neuraminidase or pronase (15). Those erythrocytes from which the largest quantity of sialic acid is released by enzyme action make up the inagglutinable class while those from which the smallest quantity of sialic acid is released are agglutinable. An erythrocyte class intermediate between these was also described.

We have now determined the partition of beef erythrocytes in dextran-polyethylene glycol phases and the quantity of sialic acid released by both neuraminidase and trypsin treatment (Table I). It was found that those beef erythrocytes having a low or intermediate partition yield a smaller quantity of sialic acid on enzyme treatment while those having the highest partition yield a much larger quantity of sialic acid. The electrophoretic mobilities were

TABLE II

ELECTROPHORETIC MOBILITIES OF NORMAL, NEURAMINIDASE-TREATED OR TRYPSIN-TREATED BEEF RED BLOOD CELLS

Electrophoretic Mobilities[a] of cells treated with		
Nothing (Normal)	Neuraminidase[b]	Trypsin[c]
0.92 ± 0.03 (14)	0.20 ± 0.03 (10)	0.86 ± 0.03 (12)

[a]Cell electrophoretic mobility measured in saline (18) and expressed as μ/sec/V/cm.

[b]See reference (15).

[c]See reference (16).

found to be the same for all samples of beef red blood cells (Table II). Furthermore, removal of surface membrane-bound sialic acid by neuraminidase yielded beef erythrocytes with greatly reduced but identical electrophoretic mobilities irrespective of partition class to which the cells belonged. Similarly, trypsin treatment resulted in the same (slightly reduced) electrophoretic mobilities for all classes of beef red blood cells (Table II).

One must therefore conclude that partition in two-polymer phases measures cell charge-associated-properties much deeper into the membrane than does electrophoresis under normal conditions.

Beef erythrocytes with a low or intermediate partition release about the same quantity of sialic acid on proteolytic enzyme treatment. This is most probably a reflection of different quantities of non-sialic acid charged groups in the peripheral zone of these red cells [e.g. protein carboxyl groups of low pKa's (21)].

Beef erythrocytes representing the (at least) three classes of cells were also subjected to countercurrent distribution to test for additional cell population heterogeneities (Figure 1). It can be seen that differences found in single tube partitions are borne out by the distributions obtained. Furthermore, erythrocytes of intermediate partition (class 2), and some of high partition

(class 3), show the presence of two cell populations (in the form of a double-peak) in the distribution curve (Figure 1). Thus, not only do beef red cells of different animals fall into classes with respect to differing sialic acid (or other charge) composition (but apparently the same sialic acid content at the plane of shear), but some individual animals actually have multiple red cell populations in their blood.

Samples of beef red blood cells from different cavities along the countercurrent extraction train showed no difference in electrophoretic mobility when compared with a sample of control cells which had not been subjected to countercurrent distribution. It should be noted that this is in contrast to the results obtained with human and rat red blood cells (10). When beef red blood cells from the left and right halves of a countercurrent distribution were pooled separately and subjected to countercurrent distribution once again, the distribution curves obtained for the left and right halves were identical and, furthermore, were both shifted to the left. This, too, is in contrast to results obtained with mammalian red blood cells of other species and may indicate a time-dependent aggregation of beef erythrocytes in the phases (22). The basis for these phenomena is under study.

The small number of beef red cell samples originally examined by partition led to statistical sampling of cells of low partition which precluded our finding the different beef red cell classes. Our report of a correlation between beef erythrocyte partition and electrophoretic mobility (9,19) for this class (class 1) of beef red cells remains intact.

In conclusion, it has been found that the partition of cells in two-polymer aqueous phase systems can measure membrane-associated charge much deeper into the membrane than can cell electrophoresis which measures only the charge effective at the plane of shear. Furthermore, the differentiation by partition of two classes of beef erythrocytes (those having a low or intermediate partition) which release identical quantities of sialic acid when treated with neuraminidase or trypsin and having identical electrophoretic mobilities apparently reveals

the presence of different quantities of charged groups other than sialic acid on their membranes. Such physico-chemical differences in the structure of the peripheral zone of red blood cells may well be responsible for the lack of correlation between the relative electrophoretic mobilities and partitions of erythrocytes from the horse, rat and mouse (9).

The foregoing results suggest that partition of cells in aqueous two-polymer phases depends on cell surface properties which cannot readily be measured by other means.

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