# THE SEPARATION OF YOUNG AND OLD RED BLOOD CELLS BY COUNTER-CURRENT DISTRIBUTION

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We wish to report preliminary results on the separation of young and old erythrocytes by counter-current distribution. The recent development by Albertsson (1960) of a two-phase system, consisting of a mixture of two aqueous solutions of different polymers, for the separation of particles and cells, and the known change in specific gravity, size and surface charge of red blood cells on aging prompted us to attempt such a segregation of different aged cells.

## Experimental Methods

One of the buffered, dextran-polyethylene glycol (PEG), aqueous, two-phase systems described by Albertsson and Baird (1962) was used in this work. The materials were Dextran # 500 (Pharmacia, Uppsala, Sweden) and "Carbowax 6000" (PEG) (Carbide and Carbon Chemicals Company, New York, N.Y.). A 5% (w/w) dextran solution and a 4% (w/w) polyethylene glycol solution, each containing equimolar amounts of $NaH_2PO_4$ and $Na_2HPO_4$ (total conc. 0.09 M phosphate) and 0.03 M NaCl, were made up separately, then mixed and the phases separated.

An all-glass counter-current apparatus, manually operated, constructed according to the standard design, and with a bottom phase capacity of 10 ml was used. 10.7 ml top phase were fed into the apparatus at each transfer.

Nine ml of bottom phase (dextran solution) was placed into each of the forty-nine tubes of the extraction train with the exception of tube three. Tubes 1, 2 and 4 to 9 also received 11.7 ml of top phase (polyethylene glycol solution).

Sprague-Dawley rats weighing 200-250 grams were injected intravenously via the saphenous vein with about 10 μCs of $Fe^{59}$ in the form of ferrous citrate. Rats were exsanguinated by heart puncture two or forty-nine days after injection and the blood collected in heparin. The red blood cells were washed five times with isotonic saline. Two ml of the washed red blood cells were pipetted into a beaker containing 7 ml of bottom phase and 11.7 ml of top phase. After agitation, this mixture was transferred to tube three in the extraction train of the counter-current distribution apparatus.

The contents were mixed by inverting the tubes twenty times and phase separation was permitted to proceed for twenty minutes by the clock. A transfer was made, the interface permitted to remain with the stationary bottom phase (see Albertsson and Baird, 1962). 10.7 ml of top phase were added to the first tube and the procedure was repeated. A total of forty-seven such transfers were completed. The entire procedure was carried out at room temperature.

The red blood cells from each tube were centrifuged and the supernatant discarded. The cells were lysed in a small volume of water (five ml) and the concentration of hemoglobin determined by measuring the absorbancy of this solution at 540 mμ on a Beckman DU spectrophotometer. The radioactivity of each hemolysate was measured by counting an aliquot on a scintillation well-counter.

The data in the Figures are presented in terms of the hemoglobin absorbancy and Relative Specific Activity (RSA) determined for each tube. The RSA is defined as:

$$\frac{\text{counts per minute (cpm) per absorbancy unit in a tube}}{\text{cpm per absorbancy unit in a hemolyzed aliquot of the original whole cell pop.}}$$

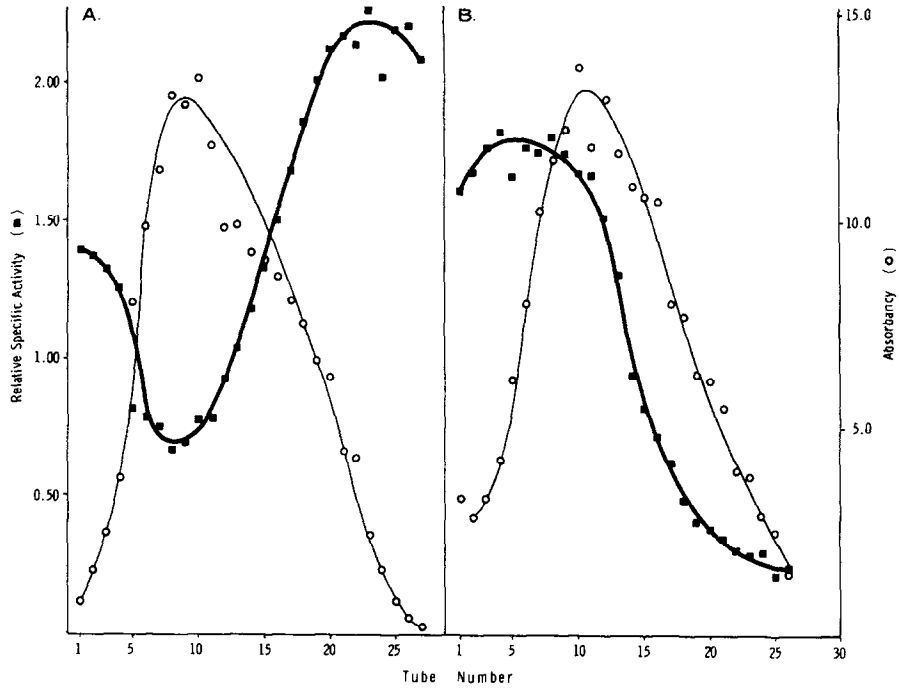


Fig. 1. Results of counter-current distribution of rat red blood cells labeled with $Fe^{59}$. ■ = relative specific activity; o = red blood cell concentration (in terms of hemoglobin absorbancy).
A - Young cells labeled (relative specific activity above 1.00 indicates cells younger than average). B - Old cells labeled (relative specific activity above 1.00 indicates cells older than average). For details see text.

## Results and Discussion

The life-span of the red blood cell in the rat is about 55 days (Levy et al., 1959). It is therefore clear that the labeled cell population 49 days after injection of a rat with radioactive iron represents old cells. Conversely, the labeled erythrocyte population obtained only two days after isotope administration represents the young cells.

Figure 1 depicts the results obtained on 47 transfers in a counter-current distribution of rat red blood cells in which the young cells are radioactively labeled. Since a relative specific activity above 1.00 in this Figure indicates cells younger than average, it is apparent that two populations of young cells are differentiated on counter-current distribu-

tion. One of these is to be found in those tubes corresponding to the right end of the distribution curve of the total cell population. The other component of young cells is associated with the very first one or two tubes of the extraction train. A similar experiment in which it is the old erythrocytes that are radioactively labeled is shown in Figure 2. Here the peak activity is in tubes 6 to 8 (depending on the individual run), and is followed by a marked decrease in relative specific activity in the subsequent tubes.

We have therefore found that counter-current distribution discriminates between two populations of young red blood cells, associated with opposite ends of the distribution curve of the total red blood cells. Further, that the old cells, which can be found near the left end of the absorbancy curve, are distinct from both young cell populations.

The two young cell populations reported here are perhaps analogous to those described by Simon and Topper (1957), who differentiated young cells on the basis of their resistance to hypotonic lysis. They found one group highly fragile and the other most resistant when compared to the average red blood cells. We have suggested that one of these may well represent reticulocytes while the other may be comprised of young mature erythrocytes (Sass et al., 1963).

While centrifugation, making use of the different specific gravity of young and old red blood cells, has previously been used for the separation of these cells (Prankerd, 1958; Rigas and Koler, 1961), this method is often unreproducible and is coupled with difficulties in sampling the layered cells. Most important, there is little indication that one can separate anything but the youngest from the average-aged cells in the population. Further, there is no method at present available for the isolation of truly old red blood cells. The counter-current distribution method holds out the hope that one can obtain red blood cells of different ages differing by only a few days in age. We are investigating this possibility.

In the present isolation procedure, it must be pointed out, some of the cells have a tendency to aggregate. This may present a stumbling block to those whose primary interest is to obtain cells of different ages in their original state. To us it is not of great importance as our purpose is the study of the contents (lysates) of cells of different ages (Walter, 1963a, b). Present indications are that such hemolysates are not adversely affected by the tendency of the cells to cohere somewhat. Still, we are trying to diminish the aggregation of cells by investigating their behavior in aqueous solutions of some other polymers.

A more detailed report on the separation of young and old red blood cells by counter-current distribution will be published elsewhere.

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