

Letter to the Editor

Residual Permeability in Red Cell Ghost Preparations

Received 24 August 1970

I agree in principle with the findings of P. G. LeFevre and S. J. Masiak as it has been apparent for sometime now from my own observations that the so-called "binding" by erythrocyte membranes of ghost preparations was a selective transport into vesicular remnants.

The selective property of the membranes was demonstrated in our laboratory as follows. Aliquots of "pink" ghosts prepared by the Levine-Stein method were dispensed into individual tubes to which a mixture of $^3\text{H-D-glucose}$ and $^{14}\text{C-L-glucose}$ was added. At time intervals ranging from 15 min to several hours, portions were centrifuged, and samples of both supernatant and pellet were treated with trichloroacetic acid and assayed for radioactivity. The results indicated that D-glucose is completely equilibrated by the time the first observation is made. The subsequent slow equilibration of L-glucose can be followed by the gradual change in ratio of $^3\text{H-D-glucose}/^{14}\text{C-L-glucose}$ in both supernatant and pellet; the ratio in the pellet decreases with time whereas that in the supernatant increases until the equilibrium value is reached.

Another method we have used was a comparison of the uptake of $^{14}\text{C-L-sorbose}$ with that of $^{14}\text{C-L-sorbitol}$ into ghosts and intact red cells. The conventional mercuric chloride stopping medium was employed to halt the translocation after a given time. A marked difference in uptake of sorbose compared with that of sorbitol indicated the presence of a selective system in "pink" ghosts. However the results were variable compared with intact red cells, ranging from 1% to 20% of the original red cell capacity.

When "white" ghosts prepared by the method of Dodge *et al.* (J. T. Dodge, C. Mitchell & D. J. Hanahan, 1963. *Arch. Biochem.* **100**:119) were similarly assayed, there was also evidence for a functional transport system, but in general this was much reduced compared with most batches of "pink" ghosts. Under phase contrast, however, "white" ghosts appeared as intact spheres.

When "white" ghosts were dispersed in Triton and dialyzed exhaustively against Tris buffer, a precipitate appeared which no longer distinguished between sorbose and sorbitol. However, white ghosts dispersed in either 1 M NaCl or NaI and similarly re-aggregated were found to retain some of their selective transport property.

It would appear, therefore, that in our hands the selective transport system is retained to a variable extent in preparation of both "pink" and "white" ghosts.

M. LEVINE
Dept. of Biological Chemistry
University of Manchester
Manchester, England