A Simple Technique of Measuring Membrane Permeability of Human Erythrocytes to Water and Other Highly Permeable Solutes

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A new way of measuring high diffusional permeabilities P_d of erythrocyte membranes is presented using THO and ^{14}C-glycol as test solutes. The method is essentially based on the work of Redwood et al. (1). The underlying principle is to measure tracer diffusion through a column of tightly packed erythrocytes in phosphate-buffered isotonic saline (hematocrit 98%). A bulk diffusion coefficient, D_{ef}, is thus obtained. Based on an appropriate physical model, diffusional permeabilities of the membrane may be calulated from D_{ef}, if three other quantities are known:

- The diffusion coefficient D_1 for the transport of the solute through the intercellular fluid;
- the diffusion coefficient D_2 for the transport through the intracellular fluid, measured in concentrated hemoglobin solutions regarded as reconstituted intracellular solution;and
- the fractional intercellular volume V_f.

The precise determination of D_{ef}, D_1 and D_2 posed severe problems when carried out according to the procedure described in (1), due to the very different viscosities of the fluids under investigation and to the fact that D_1 is larger than D_{ef} by about two orders of magnitude. Consequently, another principle, first introduced by Wang (2) was applied for measuring D_1, D_2 as well as D_{ef}. It involves tracer diffusion from a homogeneously loaded capillary into an infinite sink of solvent. Exact, reproducible data could be obtained by this technique, well suited for routine measurements. The results were evaluated by the set of equations given in (1).

In order to calculate absolute values of diffusional permeabilities P_d from D_{ef}, D_1, D_2 and V_f the procedure requires calibration, since the equations contain two additional parameters reflecting geometrical properties of the system, which cannot be determined experimentally.

Consistent data for the permeabilities of THO and ^{14}C-glycol could be obtained as yet. The new technique may also be of potential value for permeability measurements in other single cell or vesicle systems.

(1) Redwood, W.R., Rall, E., and Perl, W. (1974), J. Gen. Physiol. 64, 706 - 729

(2) Wang, J.H. (1951). J. Am. Chem. Soc. 73, 510 - 517

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