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# OBSERVATIONS ON LEVITT'S "NEW THEORY OF TRANSPORT"

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## Summary

Levitt (1974) (Biochim. Biophys. Acta 373, 115—131) has recently developed a "New Theory of Transport for Cell Membrane Pores" based on the supposition that equivalent pores in the red cell membrane are so small that water and small solute molecules such as urea can not pass each other. Levitt's concept is based on the implicit assumption that urea and water are spherical molecules. We have shown, using a scale model, that Levitt's supposition is not in agreement with the actual molecular shapes. Levitt has further asserted that there is a serious methodological error in measurements reported fifteen years ago by Goldstein and Solomon (1960) (J. Gen. Physiol 44, 1—17). We have shown that the supposed "methodological error" lies in the fact that Levitt made his mathematical analysis of the appropriate equations under conditions significantly different from those employed by Goldstein and Solomon. A computer solution of the equations under the actual conditions used shows that Levitt's assertion is not justified.

In a recent article in this journal, Levitt [1] has made two statements about the passage of solutes and solvents through small aqueous pores. His first statement concerns the diffusion of urea through a 3.5 Å pore, about which he asserts, "The sum of the diameter of the urea molecule (> 4 Å) plus the water molecule (≈ 3 Å) is greater than the pore diameter so that the urea molecule effectively blocks the pore and the continuum idea that the water flows around the urea molecule is completely wrong". In fact, Levitt has neglected to consider that neither urea nor water is a spherical molecule and his conclusion can readily be shown to be unsupportable by an examination of CPK molecular models.

Levitt goes on to examine the published results of experiments carried out by Goldstein and the senior author about fifteen years ago [2] and concludes that "there is a serious methodological error in the measurements

made by Goldstein and Solomon of the red cell reflection coefficients..." In this instance also, Levitt's statement can not be justified, as can be shown by a computer solution of the phenomenological equations governing the passage of solute and solvent across red cell membranes under the exact conditions specified by Goldstein and Solomon. Levitt's solution to the phenomenological equations applies to experimental conditions appreciably different from those actually employed.

We have used CPK models and an equivalent pore made from lucite tubing to illustrate the true nature of the geometric constraints on solute transport through narrow cylinders. The equivalent pore radius in human red cells has been given as 4.4 Å by Solomon [3] based on a comparison of all the available methods of estimation. We have taken a cylinder of lucite, 4.2 inches high × 4.21 inches in diameter, which is equivalent on a CPK scale to a height of 8.5 Å and a radius of 4.3 Å, the nearest commercially available size. Fig. 1a shows this cylinder with a urea CPK model suspended lengthwise in the center, since Soll [4] has provided evidence that solutes permeate equivalent pores in the lengthwise configuration. Fig. 1b shows the same cylinder, still containing the urea together with 18 molecules of $H_2O$. The water surrounds the urea molecule so completely that the urea can no longer be seen. Thus solute and solvent can readily pass one another. Since water molecules can even get past urea lying transversely in a 3.8 Å pore, this conclusion is independent of the orientation of the urea molecule. If the equivalent pore radius were 3.5 Å, an equivalent pore 8.5 Å high would still contain about 12 molecules of $H_2O$ in addition to the urea and the water could still easily pass as long as the urea molecule were randomly oriented in its passage through the pore. In a 0.5 M urea solution there are about 100 water molecules for every urea, so the proportion of water to urea in an equivalent pore ≈ 45 Å long would be much greater than the 18:1 ratio shown in the 8.5 Å length illustrated in Fig. 1.

Goldstein and Solomon [2] gave explicit information about the experimental conditions they used. Red cells in isosmolar buffer were rapidly

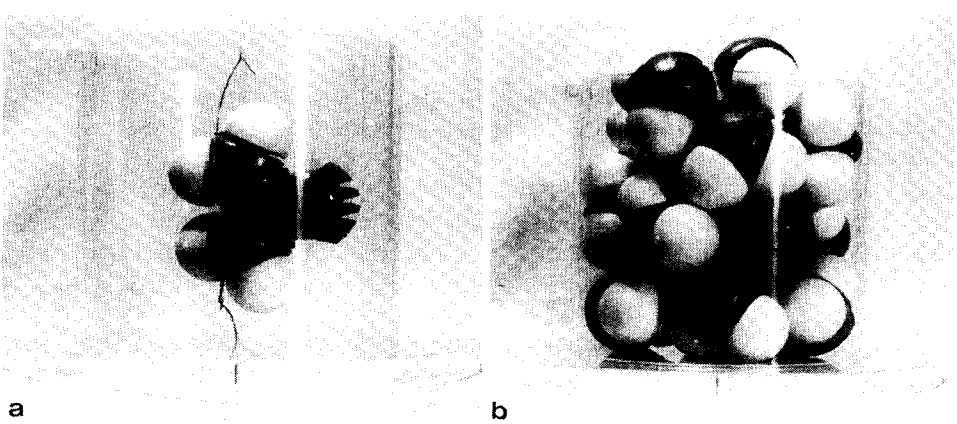


Fig. 1. (a) Lucite cylinder measuring 8.6 scale Å in diameter by 8.5 scale Å in height containing a CPK scale model of urea. (b) The same cylinder containing 18 CPK models of water in addition to the urea model.

mixed with nonelectrolyte solutions of 0.3, 0.4 and 0.5 M, in a proportion of 1:2.5. The mixture of cells and solute then passed down a tube in which cell volume could be measured photometrically at 40, 90, 140 and 190 ms after mixing. In the case of urea, these conditions led to an initial external salt concentration of 83 mosM and initial external urea concentrations of 214, 286 and 357 mosM. Milgram* has developed a computer program which integrates the equations of Kedem and Katchalsky used to describe the system both by Goldstein and Solomon and Levitt. Taking the value of $L_p$ given by Rich et al. [5] as $0.9 \cdot 10^{-11}$ cm$^3$ · dyne$^{-1}$ · s$^{-1}$ (for 370 mosM) and the value of $\omega$ given by Sha'afi et al. [6] as $14.1 \cdot 10^{-15}$ mol · dyne$^{-1}$ · s$^{-1}$, the curves given in the left hand section of Fig. 2 have been obtained, calculated on the basis of $\sigma = 0.62$ as given by Goldstein and Solomon**. In order to determine $\sigma$ by the method of Goldstein and Solomon it is necessary to measure the slope at $t = 0$. Levitt asserted that these authors' extrapolations to obtain the zero time slope could not have been accurate, because of the minimum in cell volume that occurs shortly after mixing when red cells are placed in very concentrated solutions of permeating solutes. However at the concentrations actually used, there was no minimum, as the left hand section of Fig. 2 clearly shows.

Examination of the lowest curve in the left hand side of Fig. 2 suggests that the increased rate of curvature as the time approaches the origin would not have been apparent from the four points that Goldstein and Solomon could measure. This would have led to their result being an underestimate

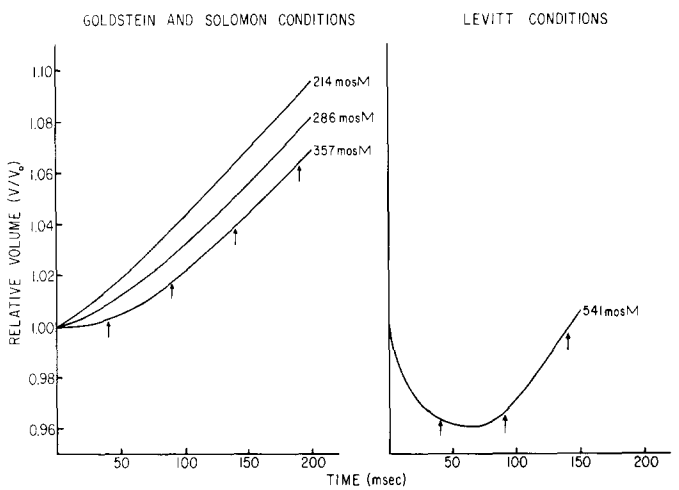


**Fig. 2. Left section. Computer simulation of solution of Kedem and Katchalsky equations under the conditions used by Goldstein and Solomon. The osmolalities of the permeant solute, urea, are shown at the left hand side of the curves. The ordinate is cell volume relative to the initial volume, and the arrows denote the four times at which Goldstein and Solomon were able to make observations. Other conditions are described in the text. Right section. Levitt's solution to the Kedem and Katchalsky equations redrawn from Fig. 3 of ref. 1.**

* Milgram, J., personal communication.

** These computations are based on a fractional cell water of 0.717 of isosmolal red cell volume, and a value of 0.80 for the fraction of "apparent osmotic volume" [7]. Levitt does not give his values for these parameters.

by 10—20%, but there is no evidence to support the gross errors that Levitt has imputed.

For comparative purposes, one curve for urea permeation, given in Levitt's paper (his Fig. 3) has been redrawn in the right hand section of Fig. 2. This curve shows a clearly defined minimum between 50 and 100 ms, and if these data were relevant to the Goldstein and Solomon experiment, their extrapolations would indeed have been misleading. Levitt's initial conditions are: external salt concentration = 67 mosM, urea initial concentration = 541 mosM, $L_p = 1.2 \cdot 10^{-11}$ cm$^3 \cdot$ dyne$^{-1} \cdot$ s$^{-1}$, $\omega = 15 \cdot 10^{-15}$ mol $\cdot$ dyne$^{-1} \cdot$ s$^{-1}$ and $\sigma = 0.8$. The salt and urea concentrations as well as Levitt's value of $\sigma$ are markedly different from the conditions in the Goldstein and Solomon experiment. On the basis of Milgram's computer simulation, the Kedem and Katchalsky equations follow a time course essentially equivalent to that computed by Levitt*. The thrust of our argument is not that Levitt's integration of the equations is wrong, but rather that Levitt did not confine his criticism of the Goldstein and Solomon method to the conditions they actually employed.

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* The major effect of the differences between his and our value of $L_p$ is primarily to shift the minimum volume with little effect on the time when the minimum is reached