

LETTER TO THE EDITOR

On Uridine Transport
in Human Red Blood Cells

Dear Sir:

In our work (1) we have made use of Stein and Lieb's (2) approach to obtain values of ratios of molecular rate constants related to transport steps. In order to rationalize our results we assumed at the time that in equilibrium-exchange conditions the concentration of the carrier-substrate complex was higher at the external surface of the cell and that the turnover rate of influx was proportionally smaller than that of efflux. This implied a different resistance of the membrane to the inward and outward movements, respectively, of the loaded carrier.

There is, however, another possibility which we have not previously considered, namely, that the loaded carriers are equally distributed across the membrane but that the unloaded carriers are not. That this is in fact the case for the uridine transport system of human red blood cells we shall attempt to demonstrate here.

Briefly, from Stein and Lieb's work (2) it can be shown that at steady state the concentration of the carrier at the inner side of the membrane is given by

$$E_1 = \frac{T(b_1 k_2 + b_2 k_2 + b_1 f_2 S_2)}{(b_1 + b_2)(k_1 + k_2) + (b_2 + k_2)f_1 S_1 + (b_1 + k_1)f_2 S_2 + f_1 f_2 S_1 S_2}, \quad (1)$$

while that on the outer side of the membrane is given by

$$E_2 = \frac{T(b_2 k_1 + b_1 k_1 + b_2 f_1 S_1)}{(b_1 + b_2)(k_1 + k_2) + (b_2 + k_2)f_1 S_1 + (b_1 + k_1)f_2 S_2 + f_1 f_2 S_1 S_2}, \quad (2)$$

where T is the total carrier concentration, f is the association rate constant, b is the dissociation rate constant, k is the rate of translocation of free carrier across the membrane, and S is the concentration of the substrate. Subscripts 1 and 2 refer to the inside and outside of the membrane, respectively.

From the principle of microscopic reversibility which states that

$$b_1 k_1 f_2 = b_2 k_2 f_1, \quad (3)$$

and from the fact that under equilibrium conditions

$$S_1 = S_2 = S, \quad (4)$$

we arrive at the following equality for the ratio of carrier concentration:

$$\frac{E_1}{E_2} = \frac{b_1 k_2 + b_2 k_2 + b_1 f_2 S}{b_2 k_1 + b_1 k_1 + b_2 f_1 S} = \frac{k_2}{k_1}. \quad (5)$$

In addition f/b is the association constant of the carrier-substrate complex on either side of the membrane, hence

$$f_1/b_1 = ES_1/E \cdot S_1 \quad f_2/b_2 = ES_2/E \cdot S_2. \quad (6)$$

Combining Eq. (4), (5), and (6) we learn that

$$\frac{b_1 k_1 f_2}{b_2 k_2 f_1} = \frac{ES_2}{ES_1} \quad (7)$$

and finally, using Eq. (3) and (7), we arrive at $ES_1 = ES_2$. This indicates that under equilibrium-exchange conditions, the concentration of carrier-substrate complex (i.e., loaded carrier) is equal at both sides of the membrane. Thus, any observed asymmetry should stem from uneven distributions of free carrier.

In fact from the kinetic parameters of uridine transport measured in equilibrium-exchange conditions (1) we obtain a k_2/k_1 value of 4.3. Thus, using this value and Eq. (5) we conclude that free carrier concentration on the inside of the membrane is 4.3 times higher than that on the outside, contrary to our previous assumption. It should be stressed, however, that the present treatment does not allow distinction between distribution of carriers as compared to conformational states of carriers.

On the basis of the figures of 10^4 carriers per cell (3) 1.43×10^{13} cells (packed) per liter cell water, and V_{\max} of uridine of 7.5 mM/min (at 25°C) from our previous work, we can now estimate a new overall turnover rate of 3.15×10^4 molecules of uridine per minute per carrier.

H. GINSBURG
Z. I. CABANTCHIK
Biophysics Group
Hebrew University of Jerusalem
Jerusalem, Israel

Dr. Ginsburg's present address is the Biochemistry Department, University of Virginia School of Medicine, Charlottesville, Virginia 22901, and Dr. Cabantchik's present address is the Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20014.

Received for publication 14 March 1977.

REFERENCES

1. CABANTCHIK, Z. I., and H. GINSBURG. 1977. Transport of uridine in human red blood cells. Demonstration of a simple carrier-mediated process. *J. Gen. Physiol.* **69**:75-95.
2. STEIN, W. D., and W. R. LIEB. 1973. A necessary simplification of the kinetics of carrier transport. *Israel J. Chem.* **11**:325-339.
3. CASS, C. E., and A. R. P. PATERSON. 1976. Nitrobenzylthioinosine binding sites in the erythrocyte membrane. *Biochim. Biophys. Acta.* **419**:285-294.