A New Quantitative Relation Between Internal and External Steady-State Concentrations in Active Transport

Probably the most common model for active transport into tissues or cells is the combination of an uptake process which obeys Michaelis-Menten kinetics, and an exit process which obeys the laws of simple diffusion, although it may involve a carrier[1]. The rate law for this model is

$$\frac{dC_i}{dt} = \frac{V_{max}\, C_0}{C_0 + K_m} + k_D\,(C_0 - C_i) \qquad (1)$$

where C_i is the internal concentration, C_0 is the external concentration, V_{max} and K_m are the maximum velocity and the Michaelis constant for the uptake processes, and k_D is the rate constant for the exit process. At steady state, where $dC_i/dt = 0$, the relation between C_i and C_0 is

$$C_i = \left\{\frac{V_{max}/k_D}{C_0 + K_m} + 1\right\} C_0 \qquad (2)$$

This can be transformed into 3 equivalent linear equations

$$1/(C_i - C_0) = k_D/V_{max} + K_m\, k_D/V_{max}\, C_0 \qquad (2a)$$

$$C_0/(C_i - C_0) = K_m\, k_D/V_{max} + C_0\, k_D/V_{max} \qquad (2b)$$

$$(C_i - C_0)/C_0 = V_{max}/k_D\, K_m - (C_i - C_0)/K_m \qquad (2c)$$

Published data for the steady-state concentrations of the non-metabolizable amino acid analogs α-amino*iso*-butyric acid and *cyclo*pentane-1-amino-1-carboxylic acid in mouse brain slices[2], and for L-histidine in rat small intestine[3] were tested for agreement with this model. Concentrations in brain tissue were reported as μmoles amino acid analog/ml total tissue water, assuming tissue dry weight to be 20% of final tissue wet weight. Before testing these data, tissue concentrations were converted to μmoles amino acid analog/ml intracellular water by the relation $C_i = (0.8\, C - 0.48\, C_0)/0.36$, where C_i is the concentration in intracellular water, and C is the reported concentration in total tissue water. This expression is based on 16% dry weight and 48% extracellular water ('inulin space') in wet rat brain slices[4]. The intracellular concentration is quite insensitive to moderate changes in the assumed % dry weight and extracellular water. If brain tissue is assumed to contain 20% dry weight and 58% extracellular water[5], the computed concentration in intracellular water will be uniformly decreased 7%. The published and revised concentrations are given in the Table. The values for rat intestine were used as published because the necessary information for corrections, if needed, was not available. Graphs of $1/(C_i - C_0)$ vs $1/C_0$, $C_0/(C_i - C_0)$ vs C_0, and $(C_i - C_0)/C_0$ vs $(C_i - C_0)$, which correspond to equations 2a, 2b, and 2c respectively, were constructed for all 3 sets of steady-state concentrations. From the graphs it was obvious that the model is not applicable. Surprisingly, graphs of $\log C_i$ vs $\log C_0$ for these 3 systems are linear with a slope of less than 1 (Figures 1 and 2), showing that the steady-state concentrations follow the well known FREUNDLICH adsorption

Steady-state concentration of amino acids in mouse brain slices[a]

Amino acid	C_0, μmole/ml medium water	C, μmole/ml tissue water	C_i, μmole/ml intracellular water
α-Amino*iso*butyric acid	0.23	3.34	7.12
	0.57	5.47	11.4
	1.09	11.8	24.8
	3.57	23.8	48.1
	7.52	42.0	83.3
	14.1	63.0	121.2
*Cyclo*pentane-1-amino-1-carboxylic acid	0.27	3.51	7.44
	0.63	4.57	9.32
	1.33	9.16	18.6
	3.48	22.7	45.8
	15.1	46.2	82.5

[a] C_i calculated from C_0 and C data by LAHIRI and LAJTHA[2].

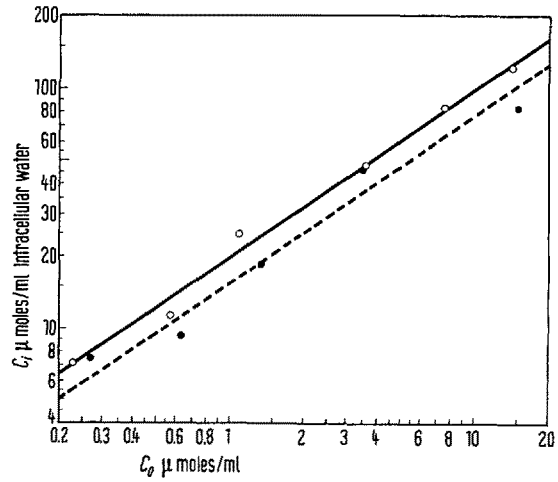

Fig. 1. Steady-state concentration of amino acid analogs in mouse brain slices (data from LAHIRI and LAJTHA[2]). o——o α-amino*iso*-butyric acid, ●------● *cyclo*pentane-1-amino-1-carboxylic acid.

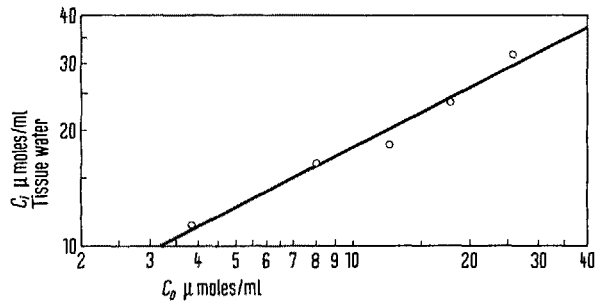

Fig. 2. Steady-state concentration of L-histidine in rat small intestine (data from AGAR, HIRD and SIDHU[3]).

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[3] W. T. AGAR, F. J. R. HIRD and G. S. SIDHU, Biochim. biophys. Acta *14*, 80 (1954).

[4] G. LEVI and A. LAJTHA, J. Neurochem. *12*, 639 (1965).

[5] R. BLASBERG and A. LAJTHA, Archs Biochem. Biophys. *112*, 361 (1965).

isotherm[6]. The 'FREUNDLICH' steady-state equations are: $C_i = 19\ C_0^{0.70}$ for α-amino*iso*butyric acid in mouse brain slices; $C_i = 15\ C_0^{0.70}$ for *cyclo*pentane-1-amino-1-carboxylic acid in mouse brain slices; and $C_i = 5.5\ C_0^{0.51}$ for L-histidine in rat small intestine, with all concentrations in μmoles/ml water. The agreement with the FREUNDLICH adsorption isotherm is almost certainly coincidental, as it is most unlikely that active transport has any fundamental physical resemblance to adsorption.

Although the 'FREUNDLICH' steady-state equation for active transport is strictly empirical and is not followed by all systems, when applicable it provides a convenient expression for the relation between internal and external steady-state concentrations. Furthermore, and equally important, the fact that certain systems obey this relation rather than equation 2 shows that the model of a combination of a Michaelis-Menten pump and simple diffusion is inadequate for some examples of active transport and suggests the possibility of regulatory mechanisms controlling uptake, exit, or both. Evidence for regulation of influx by the internal concentration has recently been found by RING and HEINZ in their study of the uptake of α-amino*iso*butyric acid by *Streptomyces hydrogenans*[7].

Zusammenfassung. Im aktiven Transport der Aminosäureanalogen (α-Aminoisobuttersäure und Cyclopentan-1-aminocarbonsäure) in Gehirnschnitten folgt das Verhältnis im stationären Zustand zwischen der intrazellulären Konzentration und der Lösungskonzentration der FREUNDLICH Adsorptionisotherme, $C_i = AC_0^n$. Dieses Verhältnis stimmt mit dem Modell einer aktiven Pumpe, die der Michaelis-Menten Kinetik folgt und mit einer passiven Ausdiffusion ausgeglichen ist, nicht überein. Es wird vermutet, dass eine Kontrolle des aktiven Transports durch intra- und extrazelluläre Konzentrationen reguliert wird.

S. R. COHEN

New York State Research Institute for Neurochemistry and Drug Addiction, Ward's Island, New York (New York 10035, USA), 3rd March 1967.

[6] H. FREUNDLICH, *Colloid and Capillary Chemistry* (translated from 3rd German edn by H. S. HATFIELD; E. P. Dutton and Co., New York 1926), p. 111.

[7] K. RING and E. HEINZ, Biochem. Z. *344*, 446 (1966).

Transplantation of Nuclei and Mitochondria of *Physarum polycephalum* by Plasmodial Coalescence[1]

The plasmodia of the coenocytic slime mold, *Physarum polycephalum*, when grown on semi-defined liquid medium on the surface of filter paper[2], are comparable, for experimental purposes, to giant, multinucleated 'cells' in which all nuclei divide in synchrony[3,4]. When plasmodia are brought into contact with one another, they coalesce spontaneously[3,5]. Once communication between them is established, plasmodial strands are formed which extend from one plasmodium into the other, and rapid exchange of components is brought about by the well-known protoplasmic streaming of this organism. This phenomenon can be employed for studies requiring transplantation of plasmodial components. Two prerequisites have to be fulfilled for such an experiment, namely, (1) that only a small amount of material from the donor is taken up by the host, and (2) that those components of the donor which we wish to study (e.g. nuclei, mitochondria) remain identifiable either by morphological criteria or otherwise, for some time after transplantation. If, for example, nuclei are transplanted at a stage in the mitotic cycle[6] which is different from that of the nuclei of the host, morphological criteria may be used for their identification during a short period after transplantation[4]. For longer periods, or if other plasmodial components, such as mitochondria, are involved, identification by label with a radioactive isotope might be necessary.

Method. Coalescence of 2 plasmodia can be conveniently obtained by sandwiching. For this purpose, the plasmodia are first placed, together with the underlying filter paper, on non-nutrient agar. Being deprived of nutrients, the plasmodia soon begin to rapidly spread over the agar. Approximately 30 min before coalescence is desired, a small piece (donor) of one plasmodium is placed, upside down (Figure 1), together with some of the adhering agar, on a considerably larger piece (host) of the other plasmodium. The small piece of agar adhering to the donor provides just enough pressure to promote close contact between the 2 plasmodial pieces.

When contact is established the beginning of coalescence can be easily determined by the gradual formation of plasmodial strands extending through donor and recipient (host) plasmodium. If both donor and host nuclei at the time of coalescence are at different stages

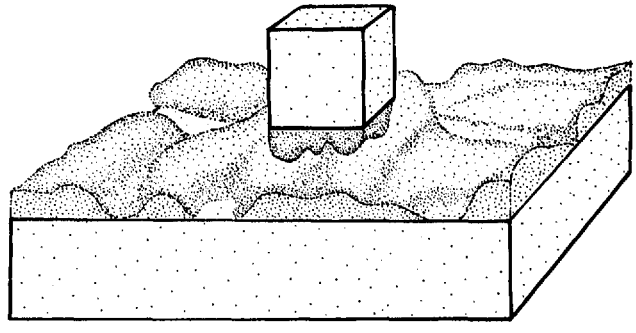

Fig. 1. Two plasmodial pieces sandwiched between agar. Both donor (upper) and host plasmodium (lower) are on non-nutrient agar.

[1] Supported by U.S.P.H.S. Grant No. 5-RO-1-GM 11949-04.

[2] J. W. DANIEL and H. H. BALDWIN, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), Vol. 1, p. 9.

[3] E. GUTTES and S. GUTTES, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), Vol. 1, p. 43.

[4] E. GUTTES, S. GUTTES and H. P. RUSCH, Devl Biol. *3*, 588 (1961).

[5] E. GUTTES, S. GUTTES and H. P. RUSCH, Fedn Proc. Fedn Am. Socs exp. Biol. *18*, 479 (1959).

[6] With this term we denote the time which elapses from any stage of mitosis to the same stage of the next mitosis.