PRELIMINARY NOTES

PN 41036

Relation of amino acid transport to sodium-ion concentration

The dependence of amino acid transport on the presence and the distribution of the alkali metals was first noted in 1952[1]. In 1958 RIGGS, WALKER AND CHRISTENSEN showed that the ability of the Ehrlich cell to accumulate glycine decreased in proportion to the extent that Na^+ replaces cellular K^+ (ref. 2). The results of that research (Fig. 6 in ref. 2) effectively dissociated the ability of the cell to concentrate glycine from the distribution of K^+, and associated it with the distribution of Na^+, although the discussion failed to take note of that evidence in choosing potassium as perhaps the critical cation. Other authors have occasionally disregarded both the alternate hypotheses and the evidence by citing only the tentative preference. Subsequently, KROMPHARDT *et al.* showed that glycine is principally sensitive to Na^+ as the external alkali metal[3], a finding that has been repeatedly confirmed. In the meantime a similar finding for monosaccharide transport by the intestine also called attention to the sodium ion.

Recently VIDAVER has shown that the net direction of a mediated transport for glycine in the pigeon erythrocyte depends strictly on the direction of the Na^+ gradient between the exterior and the interior of the cell[4]. VIDAVER observed further that the rate of glycine uptake increased with the Na^+ concentration according to

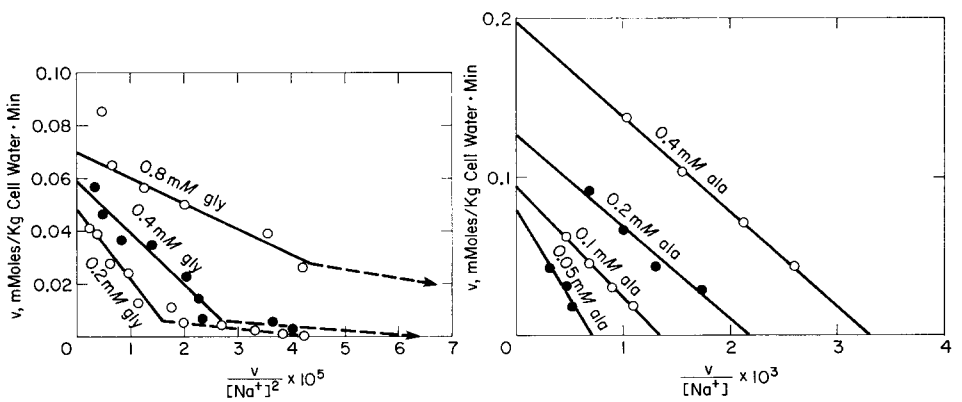

Fig. 1. Rate of uptake of glycine (left) and alanine (right) by pigeon erythrocytes at various external Na^+ levels. Uptake was measured by incubating at 37°, 10 min for [1-^{14}C]glycine, 3 min for L-[1-^{14}C]alanine, in solutions containing from 0 to 134 mM NaCl, 1.2 mM $CaCl_2$, 0.6 mM $MgSO_4$, 8.0 mM K_2HPO_4, 2.0 mM KH_2PO_4, and enough choline chloride to substitute for NaCl. Uptake was terminated by pouring into an equal volume of buffered ice-cold choline chloride, and centrifuging 3 min at 0°. After removing the supernatant solution (the last portion by capillarity into paper strips) the cells were extracted with 10 vol. 5 % trichloroacetic acid, and radioactivity determined both in the extract and the external medium by liquid-scintillation counting. The uptake rate observed in the absence of Na^+ (small except in the case of glycine) has been deducted in all cases. Under the conditions followed the radioactivity can be recovered in the form of the unchanged amino acid. For glycine (left) the Augustinsson plot is linear over a greater range when one introduced $[Na^+]^2$ (as shown) rather than $[Na^+]$. (Data of EAVENSON.)

the Michaelis–Menten equation, when the second-power rather than first-power of the Na^+ concentration was introduced as $[S]$ into that equation[5].

The present communication reports confirmation of the finding of VIDAVER that better correspondence is obtained with the square rather than the first power of $[Na^+]$ at Na^+ levels above 20 mM, although at lower Na^+ levels this relationship is no longer followed (Fig. 1, left). In contrast, the rate of uptake of L-alanine by a different mediating system in the same cell (Fig. 1, right), also the rate of its mediated uptake into the rabbit reticulocyte (Fig. 2), and the rate of the saturable uptake of α-aminoisobutyric acid by the Ehrlich cell (Fig. 3, left), all follow the Michaelis–Menten equation if Na^+ is introduced as its first power. The same result was obtained for

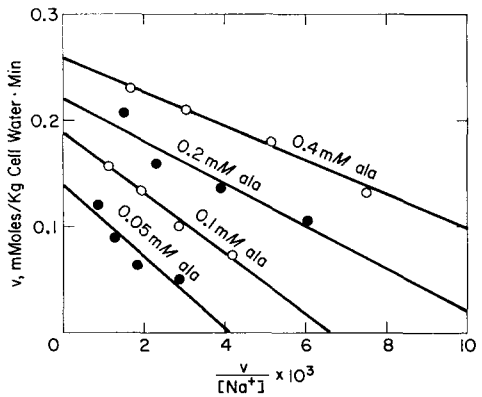

Fig. 2. Rate of alanine uptake by reticulocytes at various levels of Na^+. Rabbit red cells (61 % reticulocytes) were washed twice with 0.154 M choline chloride; then 0.2-ml samples were held 2 min at 20° in 5 ml medium containing 1 mM $MgSO_4$, 14 mM Tris–HCl (pH 7.6), and various levels of L-alanine and Na^+ as shown, choline chloride being substituted for NaCl to obtain iso-osmolarity. Other conditions as for Fig. 1, except uptake was terminated by adding 5 ml of ice-cold 0.154 M KCl, followed by 2 min of centrifugation at 0°. (Data of WHEELER.)

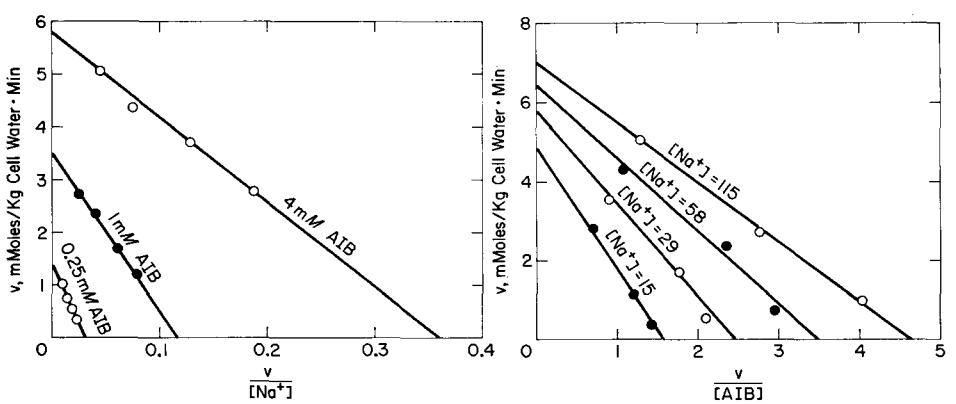

Fig. 3. Uptake rate of α-amino[1-^{14}C]isobutyric acid (AIB) by the Ehrlich ascites tumor cell at various concentrations of Na^+. Uptake was measured during 1 min at 37° in Krebs–Ringer bicarbonate medium modified to contain 26 mM K^+, various levels of NaCl as shown, and choline chloride to obtain iso-osmolarity. Other conditions approximately as in Fig. 1. Left, correspondence to Michaelis–Menten equation when $[Na^+]$ (rather than $[Na^+]^2$) is introduced. Right, same data replotted to show Na^+ effect on both V_{max} (shift in intercept on vertical axis) and K_m (change in slope) for α-aminoisobutyric acid uptake. (Data of INUI; results typical of those obtained with reticulocytes and pigeon erythrocytes.)

the Na^+-sensitive portion of the uptake of L-methionine by the Ehrlich cell. Furthermore, the influence of changing Na^+ in these several cases is to change K_m as well as V_{max}, as is illustrated in Fig. 3, right. That result is in contrast with an observation by CRANE, FORSTNER AND EICHHOLZ[6] that the effect of changing Na^+ levels on glucose transport by the hamster small intestine can be described by changes in the K_m only, for glucose. Changes in both K_m and V_{max} can be shown a theoretical expectation, however, when Na^+ and an amino acid enter as co-substrates according to the usual kinetic formulations (Y. INUI AND H. N. CHRISTENSEN, unpublished results).

The present results suggest that a single sodium ion, rather than two, frequently enters a rate-limiting event in amino acid transport.

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Photoassimilation of organic compounds by autotrophic blue-green algae

A number of photosynthetic microorganisms including eucaryotic algae, blue-green algae and photosynthetic bacteria appear to be strict photoautotrophs, *i.e.*, they grow only in the light with carbon dioxide as sole source of cell carbon. The reason for the inability of such strict photoautotrophs to grow on organic compounds is not clear. Studies on the strictly photoautotrophic green sulphur bacteria of the genus Chlorobium have established that these organisms can assimilate certain organic compounds into cell constituents[1,2]. Since many blue-green algae are reported to be unable to grow heterotrophically in the dark, it was of interest to determine whether such strictly photosynthetic blue-green algae are able to assimilate simple organic compounds in the light. It has been found that *Anacystis nidulans* is able to assimilate acetate in a light-dependent process, and that acetate is incorporated into a limited