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Energetic Problems of the Transport of Amino Acids in Ehrlich Cells*

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Since Christensen et al. [1] more than 25 years ago postulated that the (concentrating) uptake of amino acids in Ehrlich ascites cells is by active transport rather than by binding to intracellular sites, other workers have supplied evidence to support this view [11, 12]. This view is based on the assumption that the amino acids accumulated inside the cell are in "free" solution, which has not been definitely proved yet, but in view of abundant indirect evidence, most workers nowadays strongly believe that this is so and that the transport is active. More controversy was raised later by another question, namely whether this transport is primary active or secondary active, i.e., whether the immediate driving force is the affinity of a chemical reaction, such as the hydrolysis of energy rich phosphates, or whether it is the electrochemical potential gradient of Na ions (gradient hypothesis[1]). Christensen et al. had shown long ago that the accumulation of those amino acids depends on the presence of Na^+ in the medium and that the uptake of amino acids is associated with a temporary uptake of Na and loss of K ions [1]. Crane first devised and experimentally supported a plausible model showing how a concentration gradient of Na^+ and possibly of K^+ can be utilized directly to drive sugars uphill through the intestinal wall [2]. Attempts soon followed to extend this model to amino acid transport in Ehrlich cells and other systems. It could indeed be shown that during complete metabolic inhibition these cells are still able to concentrate amino acids to the extent that a gradient of Na ions is maintained [6, 27]. Under such conditions the direction of amino acid transport could be reversed upon inverting the Na gradient. As should be expected, the interaction between Na and amino acid were mutual: uphill movement of Na could be induced by an imposed gradient of excess amino acid [3, 5]. Later, however, other observations were reported that appeared to contradict the gradient hypothesis, at least to the extent that it claimed the electrochemical Na gradient to be the only energy source of active amino acid transport. So, it could be shown that at any Na^+ gradient the amino acid transport was about three times more powerful with metabolizing than with inhibited cells [4]. Still less compatible with the gradient hypothesis appeared to be the finding that in the presence of metabolic activity active transport of amino acids was still apparent when a Na^+ gradient was absent or even moderately inverted. In contrast to the behavior of metabolically inhibited cells, inward transport of amino acid into normal cells was stopped, or slightly reversed, only if both Na and K gradients were drastically inverted [26]. Such observations obviously pointed to an energy source other than the Na gradient of at least 4 kJ/mol, unless the electrochemical Na gradient was grossly underestimated under such conditions. The electrochemical potential gradient of Na^+ is not unlikely to be underestimated as it is based on at least two uncertain quantities: the activity of the cytoplasmic Na^+, which may be overestimated, and the electric PD across the cytoplasmic membrane, which may be underrated. As to the former, there is little doubt that the activity coefficient of Na^+ is in general lower inside a cell than outside [20]. But this difference appears to be small to account for a substantial error in the present context, apart from the difficulty to see how it could depend on metabolism. A somewhat more significant source of error in estimating the Na gradient could be the sequestration of Na^+ in the nucleus as has been inferred from cellular fractionation studies [25]. But the resulting overestimate of the cytoplasmic Na activity was not found to be sufficient under all cir-

* *This paper is dedicated to the memory of Walther Wilbrandt.*

[1] It should be mentioned that this applies only to a group of neutral amino acids, which has been characterized as the H-system [22] and of which the amino acids glycine and 2-aminoisobutyrate used in the present experiments appear to be typical examples.

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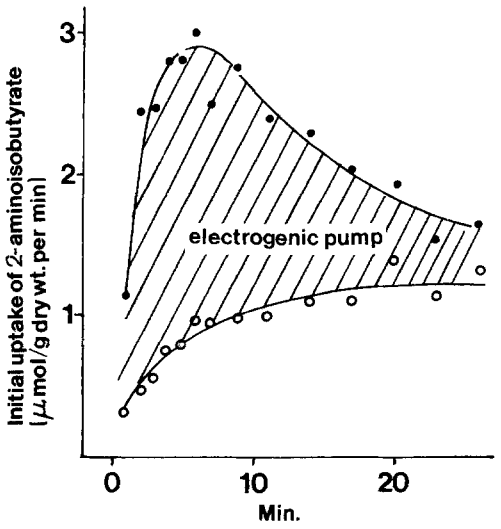

Fig. 1. Primary contribution of electrogenic Na pump to apparent driving force. Initial rate of 2-aminoisobutyrate net uptake under the influence of the electrogenic pump. To K^+-depleted and Na^+-rich cells, 13 mM K^+ was added at time 0 to each sample of two series in order to initiate the electrogenic pump. Subsequently the initial rate of 2-aminoisobutyrate net uptake was measured in the first series by the addition of 0.1 mM 2-aminoisobutyrate to the samples at varying time intervals after K^+ addition (●). In the parallel series the procedure was the same except that 3 mM ouabain was added together with the 2-aminoisobutyrate in order to test the uptake of this amino acid after the blocking of the electrogenic pump (○). The upper curve gives the initial 2-aminoisobutyrate uptake under the influence of the total electrical PD. The lower curve gives this uptake under the influence of merely the membrane diffusion PD remaining after the blockage of the electrogenic pump. The cross-hatched area between the two curves represents the primary contribution of the electrogenic pump to the initial 2-aminoisobutyrate uptake. *Ordinate:* initial uptake of 2-aminoisobutyrate (1 min) in μmol/g dry wt. *Abscissa:* time in minutes after the addition of K^+ to the suspension. Note that the initial uptake of 2-aminoisobutyrate is used here as a prompt monitor of the electrical PD. (From [15] with permission of *Biochim. Biophys. Acta.*)

cumstances either [16]. It may be mentioned that this sequestration has not yet been confirmed by the electron probe test (K. Thurau, *personal communication*). There remains the possibility that the electric PD is higher than it has been mostly reported to be. It should be especially high if the alkali ion gradients are inverted, at least 60 mV (inside negative) in order to outweigh the opposing Na chemical potential gradient. This latter postulate, however, would obviously disagree with the Goldman-Hodgkin-Katz equation, which predicts rather a decrease in electric PD when the cellular K^+ is replaced by Na^+, and also with the actual behavior of the Cl distribution, which actually seems to confirm this decrease [26]. Both these arguments against a rise of the electrical PD can, however, be challenged on two grounds: First, the Goldman-Hodgkin-Katz equation applies to a membrane diffusion PD only in the absence of an electrogenic pump. Second it has meanwhile turned out, as will be discussed later, that the Cl^- distribution is an unreliable or even misleading monitor for the electrical PD.

Whereas an electrogenic Na pump in Ehrlich cells, presumably related to the Na, K-ATPase, has been found and considered before [10, 21], its immediate effect on the electric PD, and its function in active amino acid transport had not been specifically investigated. An electrogenic pump affects the transmembrane PD in two ways: apart from an indirect (secondary) effect on the membrane diffusion PD, due to the translocation of ions, it may also raise the PD primarily by moving electrical charges through the membrane, thereby loading the membrane electric capacity. The (primary) effect is likely to be more rapid since it involves only negligible ion movements, whereas the indirect (secondary) contribution to the PD should be more sluggish since it involves major ion translocations. The magnitude, however, of the primary contribution depends on certain conditions concerning the concentrations and permeancies of other passive ions. The primary rise of the electrical potential upon initiation of the pump should be the stronger the smaller the (rheogenic) mobility and concentration of the permeant passive ions. As discussed further below, these conditions appear to be fulfilled in the Ehrlich cells as the rheogenic permeancies of K^+ and Cl^- appear to be very low.

That such a pump may under certain conditions have a strong and direct effect on the electric PD, not accounted for by membrane diffusion potentials, could indeed be shown using the lipophilic cation tetraphenylphosphonium [15] and under certain conditions later also by using fluorescent dyes [16, 19, 23] as a PD monitor. Whereas the ability of these monitors to indicate the absolute PD may be debatable, they rather reliably and proportionally respond to changes of this PD. The effect of the electrogenic pump on the electrical PD was most manifest with cells which were depleted of K^+ and enriched with Na^+, conditions rather similar to those with inverted Na^+ and K^+ gradients used before. The electrogenic pump in such cells depends on extracellular K^+ and is highly sensitive towards ouabain. This dependence can be demonstrated by the addition of K^+ to a K^+-free medium which causes a rapid and drastic rise of the distribution of TPP, as well as a rapid increase of Na^+ output and a rapid uptake of the amino acid 2-aminoisobutyrate. All three effects were absent if in the presence of ouabain or during metabolic inhibition. There is evidence that the electrogenic pump and its effect on the membrane PD is most powerful in K^+-depleted cells and gradually declines to a much lower level of activity (Fig. 1) as the composition of intracellular fluid approaches normality [24]. It seems that the pump is controlled by a regulatory mechanism whose adequate stimulus is either the rise of intracellular Na^+ or the drop of electric membrane

PD. A distinction between these two possibilities is not possible at the present time; hence we do not know whether the primary function of the stimulated Na pump is to restore the normal ion concentration inside the cell or the electrical PD. Even though the absolute value of the electrical potential across the Ehrlich cell membrane does not appear to be precisely known at the present time, the results obtained with different methods varying greatly [29], there seems to be nothing arguing *a priori* against the possibility that with inverted Na and K gradients, i.e. with K^+-depleted cells, the primary contribution of the electrogenic pump may at least temporarily be very high, easily high enough to provide an effective inwardly directed electrochemical potential gradient of Na ions, even with a moderately inverted concentration gradient. If owing to the electrogenic Na pump the driving force for inward amino acid transport may thus be adequate, even under seemingly adverse conditions, it appears that the coupling between the flows of Na and the amino acids is tight enough to utilize this driving force, whereas no direct coupling between amino acid flow and ATP hydrolysis could be detected [13, 27].

Active inward transport, taking place under such conditions, would then be directly driven by an electric PD, i.e., without a parallel concentration gradient. It could indeed be shown that with a rather constant concentration ratio of Na ions the rate of amino acid uptake is directly related to the electrical PD under a variety of conditions, as estimated by the TPP^+ distribution [16]. Similarly, it could be shown that by the addition of any amino acids whose transport is linked to sodium ions leads to a strong electrical depolarization of the cellular membrane, also detectable by a decrease in the PD before substantial amounts of Na^+ have been moved into the cell [24, 29]. It seems that the electrogenic pump is rather weak under normal conditions but becomes strongly stimulated upon altering the cellular ion concentration, probably as a means to most rapidly restore normal conditions. It therefore seems that the primary contribution of the electrogenic pump is crucially involved in driving amino acids uphill under seemingly adverse conditions. This crucial function of the electrogenic pump in amino acid transport would also explain the strong effect of metabolism of this transport at any Na concentration gradient.

As has been mentioned, the Cl^- distribution does not respond to the PD changes as one would expect for a passive, rheogenic ion. Meanwhile, it turned out that a major fraction of chloride moves "paradoxically" into the cell even if the electrochemical potential gradient, as estimated from the TPP distribution, should tend to move it out of the cell. This paradoxical chloride movement appears to be always associated with the parallel movement of K^+ into the cell [17]. Both these movements are insensitive towards

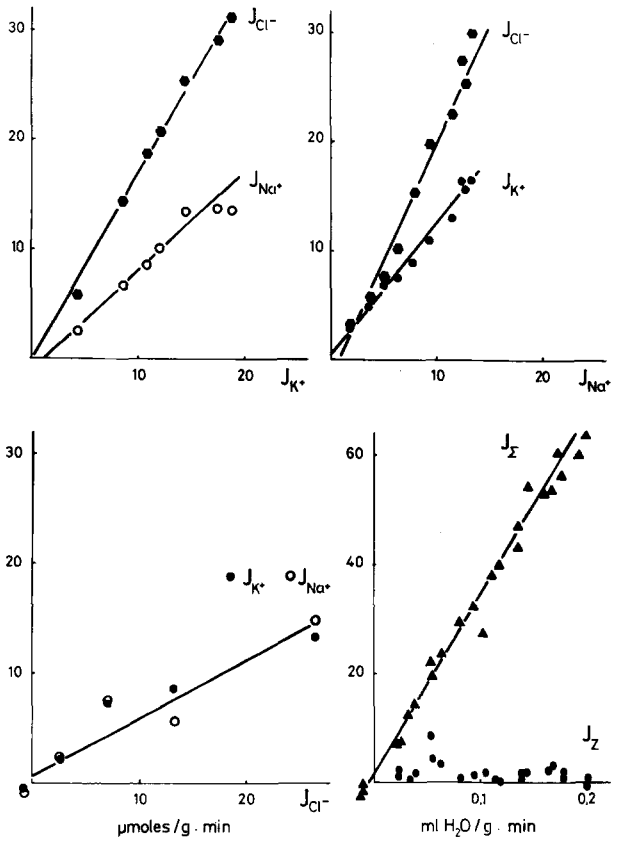

Fig. 2. Pulse-response experiments with Na^+, K^+ and Cl^-. Na^+-rich, K^+-depleted cells were incubated for 5 min at 37 °C in Krebs-Ringer phosphate buffers with varied Na^+ and Cl^- content, which were substituted by tetraethylammonium or gluconate, respectively. Furosemide-sensitive ion fluxes were initiated by addition of K^+. The furosemide-sensitive transport (µmol/g min) of the responding (constant) ion is plotted *vs.* that of the pulsed one. The forosemide-sensitive ion movements are defined as the uptake with 1 mM ouabain minus that with 1 mM ouabain and 2 mM furosemide. For the different types of experiments the following ion concentrations were kept constant: *Upper left:* 84 mM Na^+, 135 mM Cl^-. *Upper right:* 28 mM K^+, 135 mM Cl^-. *Lower Left:* 116 mM Na^+, 29 mM K^-. *Lower right:* The pooled results of all three types of experiments. The triangles show the sum of the three furosemide-sensitive ion fluxes ($J_{Na}+J_K+J_{Cl}$) (µmol/g min) plotted against the associated water movements (ml·g·min). The slope of the line indicating the osmolarity of the transported mixture in these three ion species is 321 ± 8 mM. The points give the flux of the electrical charge ($J_{Na}+J_K-J_{Cl}$) (µmol/g min). The slope of the regression line is not significantly different from zero (1 ± 5 mM). From [14] with permission of Ann. N.Y. Acad. Sci.

ouabain but strongly inhibited by furosemide and other "high ceiling" diuretics [8]. Evidence was found that this furosemide-sensitive movement of K^+ and Cl^- is electrically silent [29]. More detailed studies revealed that there seems to be a furosemide-sensitive cotransport of Na, K and Cl ions directed into the cell which is inhibited by furosemide and which is apparently energetically uphill but does not utilize ATP. As determined on the basis of nonequilibrium thermodynamics by the "pulse-response" method, the degree of coupling between the furosemide-sensitive parts of these three ion flows is high, approaching

the theoretical value of unity in each case, and the stochiometry of 1:1:2 for $Na^+/K^+/Cl^-$[14]. It seems that this electrically silent and furosemide-sensitive cotransport of the three ion species is related to the water content of the cell, as it tends to move water into the cell, thus counteracting the shrinking effect of the Na−K pump. Accordingly, this cotransport appears to be more active with shrunken cells and appears to decline accordingly as the water content of the cell rises. This Cl^- pathway is specific for Cl^- and K^+ and hence appears to be different from another, also electrically silent one, the $Cl^- - OH^-$ exchange, as the latter mechanism does not discriminate between Cl^- and NO_3^- whereas the former does. Also the K^+ site appears to be specific since K^+ can be replaced only by Rb^+ but not by other cations tested. Besides these two electrically silent pathways of Cl^- and K^+ there were at best only minor and slow rheogenic pathways for these ions, in line with the paradox behavior of the chloride and the powerful electrogenic activity of the Na pump under certain conditions.

Since some of the chloride moved into the cell by the furosemide-sensitive cotransport mechanism is likely to leave the cell in part subsequently via the $Cl^- - OH^-$ exchange [18], this mechanism may have also an indirect effect on the acid-base regulation of the cell, helping to remove H ions out of the cell.

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