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An Electrogenic Sodium Pump as a Possible Factor Leading to the Concentration of Amino Acids by Mouse Ascites-Tumour Cells with Reversed Sodium Ion Concentration Gradients

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According to the so-called ion gradient hypothesis, the spontaneous movements of Na^+ and, possibly, K^+ across the plasmalemma of the mammalian cell provide the energy that is needed to concentrate various amino acids in the cellular phase (reviewed by Schultz & Curran, 1970). One important approach has been the demonstration that the ion gradients may apparently drive the amino acid pump in the absence of ATP (Eddy, 1968). Nevertheless, recent work both with intestinal preparations (Kimmich, 1970; Newey *et al.*, 1970) and with mouse ascites-tumour cells (Potashner & Johnstone, 1971; Schafer & Heinz, 1971) has been interpreted to mean that during respiration only a minor fraction of the driving force may be provided in that way. The tumour preparations used by Schafer & Heinz (1971) accumulated 2-aminoisobutyrate against its own concentration gradient, even when the combined concentration gradients of Na^+ and K^+ might have caused the amino acid to be expelled from the tumour cells. Schafer & Heinz (1971) estimated that a force of at least 4 kJ/mol, corresponding to a concentration factor of about 4.7-fold, acted in series with the gradients of Na^+ and K^+ driving the amino acid pump, and they suggested that the additional force might be provided by the direct coupling of amino acid transport to cellular metabolism. We have now re-examined the arguments leading to that conclusion with particular reference to the role of the membrane potential.

Let the extracellular phase be denoted by subscript 1 and the cellular phase by subscript 2, the relevant gradients of amino acid concentration and of Na^+ respectively being $[A]_2/[A]_1$ and $[Na^+]_1/[Na^+]_2$ (Eddy, 1968). Because Na^+ is a co-substrate with the amino acid, the uptake of the amino acid is at least potentially an electrogenic process. We shall use the ratio:

$$F = \frac{[A]_2[Na^+]_2}{[A]_1[Na^+]_1}$$

as a measure of the forces that act in series with the concentration gradient of Na^+ to maintain the gradient of amino acid concentration, with the assumption that 1 equiv. of Na^+ enters the cells with the amino acid (Eddy, 1968). The quantitative relationship between the ionic gradients and the amino acid gradient depends on how the movement of positive charge, due to the tight coupling between the flow of Na^+ and that of the amino acid, is neutralized. Under certain conditions, the movement of K^+ out of the tumour cells appears to be involved. Thus, when ATP was available, the amino acid gradient was at least partially coupled to the gradient of K^+ (Eddy, 1968; Eddy & Hogg, 1969; Reid & Eddy, 1971; Schafer & Heinz, 1971). In fact the last-named workers emphasized the possibility that the amino acid stimulated the uptake of 1 equiv. of Na^+ and the simultaneous loss of exactly 1 equiv. of K^+. The present work is concerned with the different possibility that the neutralization of charge during the absorption of the amino acid might be achieved, though only in certain special circumstances, largely by the ejection of Na^+ through the sodium pump. The latter process is potentially electrogenic, at least in the erythrocyte, in so far as the number of K^+ ions absorbed through the pump is probably smaller than the number of Na^+ ions that are simultaneously ejected (Post & Jolly, 1957). It seems possible that the sodium pump in the tumour cells behaves similarly. Further, the permeability of the tumour cells to Cl^- might be sufficiently small for the flow of positive charge out of the sodium pump to become electrically coupled to the flow of positive charge into the amino acid pump. The direct coupling between cellular metabolism and the amino acid pump that Schafer & Heinz (1971) envisage is thus replaced in our model by an indirect coupling dependent on a membrane potential generated by the sodium pump and varying with the rate of functioning of that system.

Preliminary work showed that L-methionine behaved with our mouse ascites-tumour cells like 2-aminoisobutyrate did in the experiments described by Schafer & Heinz (1971). Hence, to test the response of the amino acid pump to the membrane potential, we added the K^+-selective ionophore valinomycin to a system containing L-methionine. The permeability of the cell membrane to K^+ was expected to increase sufficiently for the membrane potential to be determined mainly by the K^+ diffusion potential. The methionine gradient might then become tightly coupled to the gradient of K^+, when F would approach the value of $[K^+]_2/[K^+]_1$ (Eddy, 1968). Two experimental approaches were used. (1) The tumour preparations were depleted of ATP (Eddy & Hogg, 1969) and put with 1 mM-methionine for 5–10 min in a Ringer solution containing 80 mequiv. of Na^+/l,

5 mequiv. of K^+/l, 2 mM-NaCN, 10 mM-deoxyglucose, 75 mM-choline chloride and 2 μg of valinomycin/mg dry wt. of cells. Cellular $[Na^+]$ was about 90 mequiv./l and cellular $[K^+]$ about 60 mequiv./l when the gradient of methionine concentration between the cellular and extracellular phases ($[Met]_2/[Met]_1$) reached a peak value of 12.1±2.9 S.E.M. (3). F was then 10.2±2.8, a value consistent with that of the ratio $[K^+]_2/[K^+]_1$, which was 13.7±3.1. The latter corresponds to a potential difference of about 70 mV. The controls without valinomycin, in which the coupling to K^+ was small (Reid & Eddy, 1971), reached a smaller mean peak ratio of 3.6±0.6 (3). In these circumstances F was 2.2±0.2 and $[K^+]_2/[K^+]_1$ was 19.6±5.9. The effect of valinomycin was apparently not mediated by ATP, the cellular content of which was 0.2–0.3 nmol/mg dry wt. both in the presence and in the absence of the ionophore. Similar effects with valinomycin were observed with L-leucine, glycine and L-lysine. The ionophore nigericin, which is a poor electrogenic agent, did not reproduce the action of valinomycin. (2) Similar behaviour towards valinomycin was observed during energy metabolism. The system contained 150 mequiv. of Na^+/l, about 5 mequiv. of K^+/l, 10 mM-glucose and 2 μg of valinomycin/mg. F was then 13.7±0.8 S.E.M. (6) and $[K^+]_2/[K^+]_1$ was 11.6±1.4 (6).

The above observations indicated that the amino acid pump probably responded to the membrane potential induced by valinomycin. The next question was whether a similar criterion could be used to show whether the accumulation of amino acid in the circumstances described by Schafer & Heinz (1971) was also related to the membrane potential. One situation of special interest in their work was where cellular $[Na^+]$ was initially larger than extracellular $[Na^+]$ and where $[K^+]$ was relatively large in both phases. Fig. 1 shows how our preparations then absorbed L-methionine. Despite the apparently unfavourable ionic gradients, a substantial gradient of methionine concentration formed within about 5 min. Fig. 2 (○) shows how the unexplained component of the amino acid gradient, represented by F, changed as Na^+ left the tumour cells. Because $[Met]_2/[Met]_1$ was almost constant, F necessarily decreased from 6 to below 3 as $[Na^+]_2$ fell. A similar dependence was observed in further work in which $[Na^+]_1$ was 80 mequiv./l.

Now according to our working hypothesis the accumulation of methionine under these conditions depended on the presence of an electrical potential varying up to a maximum of about 48 mV when F reached a peak value of 6. The hypothesis further required that the amino acid pump was not then coupled to the gradient of K^+. Because $[K^+]_2/[K^+]_1$ was about 1 the addition of valinomycin was expected to lower the membrane potential to a value near the Donnan potential. Fig. 1 shows that, in accordance with the hypothesis, valinomycin markedly lowered the accumulation of L-methionine. It also retarded the loss of cellular Na^+. The cellular ATP content fell to 57±3% S.E.M. (3) of the control values, although such a small change can hardly be the basis of the effect of valinomycin on methionine uptake. Indeed Fig. 2 shows that lowering of the cellular ATP content by

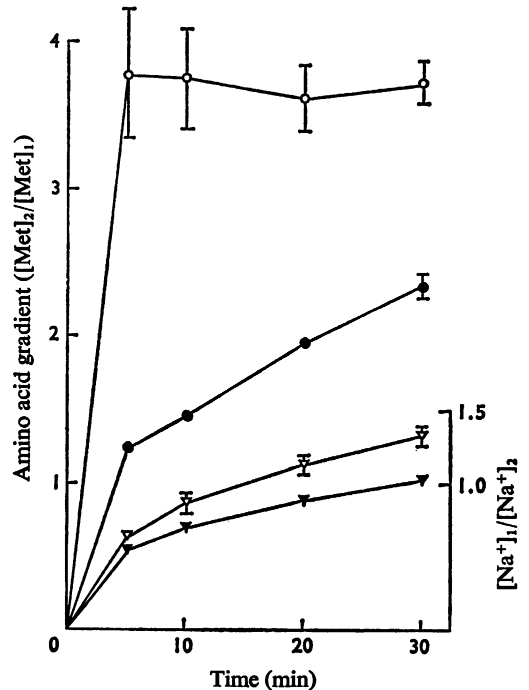

Fig. 1. *Effect of valinomycin on the distribution of Na^+ and of methionine between the cellular water of mouse ascites-tumour cells and the Ringer solution as a function of time*

Subscript 1 denotes the extracellular phase, subscript 2 the cellular phase. The mouse tumour cells were kept in the standard sodium Ringer solution at 37°C for 40 min. They were then transferred to a Ringer solution (33 ml) at 37°C containing 37 mequiv. of Na^+/l, 155 mequiv. of K^+/l, 10 mM-glucose, 1 mM-L-[*Me*-^{14}C]methionine (0.03 μCi/ml), 5–6 mg dry wt. of cells/ml and 0.8% (v/v) of ethanol. Four samples (5 ml each) were collected and processed as described by Reid & Eddy (1971). Valinomycin (10 μg/ml) was added in solution in ethanol. The ratio $[K^+]_2/[K^+]_1$ remained near unity (1.1–1.3). ● and ▼, Mean values (±S.E.M.) from four experiments with valinomycin present; ○ and ▽, mean values from seven experiments with valinomycin absent (controls). ▽ and, ▼ $[Na^+]_1/[Na^+]_2$; ○ and ●, $[Met]_2/[Met]_1$.

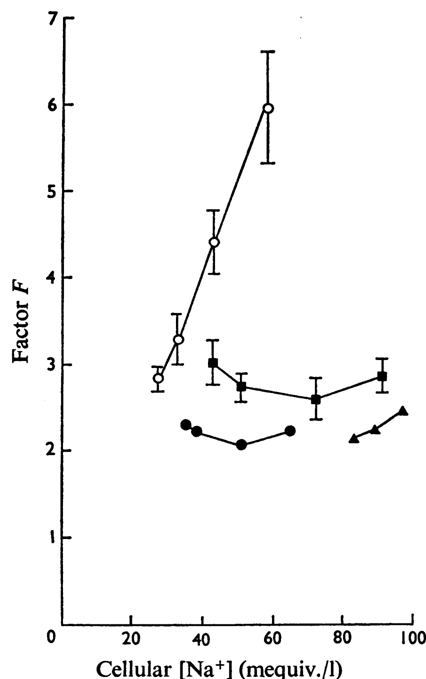

Fig. 2. *Effects of the metabolic inhibitors on the changes in the factor F with* $[Na^+]_2$ *with mouse ascites-tumour cells*

$F = [Met]_2[Na^+]_2/[Met]_1[Na^+]_1$ represents the amino acid accumulation that is not accounted for in terms of the concentration gradient of Na^+. $[K^+]_2/[K^+]_1$ was in the range 1.1–1.3. ●, Valinomycin present (results from Fig. 1); ○, control series (results from Fig. 1); ■, 0.3mM-ouabain present (mean values from three experiments); ▼, 10mM-2-deoxyglucose with 2mM-NaCN present and no glucose, the cells having been depleted of ATP (mean values from three experiments).

98%, by including both 2mM-NaCN and 10mM-deoxyglucose in the system (Eddy & Hogg, 1969), had a similar effect on methionine accumulation. The presence of 0.3mM-ouabain also slowed the uptake of methionine (Fig. 2, ■), in that case without significantly changing the cellular ATP content (9–21nmol/mg dry wt.). Heinz & Schafer (1971) observed that ouabain lowered the unexplained force of about 4kJ/mol to about 2.2kJ/mol. The latter corresponds to a factor of about 2.4-fold, like those we observed both in the presence of ouabain and when ATP was lacking.

Ouabain, valinomycin and the metabolic inhibitors thus each lowered F to ratios near 2.5. These correspond to the upper range of values that have been found for the normal membrane potential by inserting micro-electrodes into the mouse ascites-tumour cells (Lassen *et al.*, 1971) and that may represent a Donnan potential due to the fixed cellular anions. It seems likely that in the presence of the above compounds methionine was distributed roughly in equilibrium with the electrochemical gradient of Na^+ across the plasmalemma. We suggest that the dependence on ATP, the variation with cellular $[Na^+]$ and the inhibition by ouabain all point to a role for the sodium pump in the generation of the larger potential difference required to maintain the methionine gradient near a ratio of 4 when cellular $[Na^+]$ was relatively large. Unfortunately no relevant studies with micro-electrodes have been reported. Schafter & Heinz (1971), on the basis of a limited study of the cellular Cl^- content, tentatively assumed that the membrane potential in their preparations was always small. As noted above, they also favoured the view that the amino acid caused an influx of Na^+ that was neutralized by the efflux of K^+, a process that would be independent of the membrane potential. Both assumptions now seem questionable. However, we must emphasize that our model does not exclude the possibility of coupling between the amino acid gradient and the gradient of K^+ in other physiological circumstances. We believe there is good evidence for such coupling, which may involve either a K^+ diffusion potential or the direct interaction of K^+ with the amino acid carrier (Eddy, 1968). Finally, the notion that the sodium pump was in certain circumstances electrically coupled to the Na^+-dependent solute pump may help to interpret recent reports about the effect of ouabain on sugar absorption by intestinal cells (Kimmich, 1970) and about the role of cellular Na^+ in the absorption of methionine across the mucosal border of the intestine (Newey *et al.*, 1970).

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