Equilibrium and Steady-State Models of the Coupling between the Amino Acid Gradient and the Sodium Electrochemical Gradient in Mouse Ascites-Tumour Cells

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1. The tumour cells were incubated at 37°C in Ringer solutions containing glucose, 1 mM-methionine, various concentrations of Na⁺ and K⁺ and, in some instances, ouabain or valinomycin to lower the membrane potential generated by the Na⁺ pump. After about 30 min, when the system had reached a steady state, the ratio [extracellular Na⁺]/[cellular Na⁺] varied from about 0.6 to 3.2 with the ionic conditions. The membrane potential, determined by means of the fluorescent probe 3,3'-dipropylxadicarbocyanine, also varied systematically from about zero to -55 mV. 2. The ratio [cellular methionine]/[extracellular methionine] varied from about 1 to 35 in these circumstances. The Na⁺ electrochemical gradient, measured in the same units, varied from about 1 to 30. Its magnitude in 46 assays was almost directly proportional to, though slightly smaller than, the methionine gradient. 3. A mathematical model was used to define the relation between these two gradients, which were not in equilibrium, owing to the presence of a leak pathway for the amino acid. On the assumption that the values of [cellular Na⁺] were correct, the methionine gradient was about 1.8 times larger than this version of the gradient hypothesis predicted.

The development of a procedure for assaying the membrane potential of populations of mouse ascites-tumour cells, by means of the fluorescent probe 3,3'-dipropylxadicarbocyanine (Philo & Eddy, 1978), provided a basis for comparing the relative magnitudes of the amino acid gradients that these cells form, in various physiological circumstances, and the energy available from the electrochemical gradient of Na⁺ ions that the Na⁺ pump establishes across the plasmalemma. The problem of the adequacy of the relevant alkali-cation gradients to drive the amino acid pump has been investigated both in the present system (Colombini & Johnstone, 1974; Reid *et al.*, 1974; Heinz *et al.*, 1975; Schafer, 1977) and in others (Blaustein & King, 1976; Vidaver *et al.*, 1976; Lever, 1977), but an agreed solution to the problem has still to be found.

The pathways through which Na⁺, K⁺, Cl⁻ and the amino acid are likely to be transferred are illustrated in Fig. 1, which includes a representation of the electrogenic Na⁺ pump. The amino acid itself traverses the cell membrane either with n equivalents of Na⁺ through the amino acid pump or through the leak pathway represented by the permeability coefficient P_A (Morville *et al.*, 1973). It follows that, unless P_A were zero, the amino acid gradient and the Na⁺ gradient would necessarily fail to reach thermodynamic equilibrium. Thus the two main possibilities

are that the amino acid gradient and the Na⁺ gradient are (a) in equilibrium ($P_A = 0$) or (b) in a steady-state relationship ($P_A > 0$). (1) We have attempted to estimate the quantitative importance of the leak factor P_A in the present work by means of a mathematical model (Philo & Eddy, 1978). (2) The model is also used to interpret our previous experimental observations (Eddy, 1968*a*) bearing on the magnitude of the coefficient n of Fig. 1. (3) Finally, we have compared the relative magnitudes of the amino acid gradient and the Na⁺ electrochemical gradient formed by the tumour cells in certain critical circumstances where the validity of the gradient hypothesis has been most in doubt.

Materials and Methods

The assay of the membrane potential, the ionic composition and water content of the tumour cells and procedures used in handling the cell preparations were those described by Philo & Eddy (1978). Chemicals were also purchased from the same sources.

Mathematical procedures

The equations describing the transport of the amino acid in relation to the ionic gradients and the membrane potential are given in Philo & Eddy (1978).

Fig. 1. Model of the ionic flows associated with the amino acid pump

The Na^+ gradient is established by the electrogenic Na^+ pump expelling 3 equivalents of Na^+ and absorbing 2 equivalents of K^+ during the hydrolysis of 1 mol of ATP. The amino acid A is absorbed with n equivalents of Na^+ through the amino acid pump and also crosses the cell membrane through the leak pathway. The parameters V_m and K_A of the mathematical model (Philo & Eddy, 1978) refer to the former process and P_A to the latter process. The permeability coefficients P_{Na} , P_K and P_{Cl} refer to the diffusion of the corresponding ions, governed by their concentration gradients and the prevailing membrane potential, as described by the constant-field equations.

Of the 14 quantities involved in the model (Fig. 1), eight are solute concentrations amenable to direct measurement. $[\text{Cl}^-]_i$ was not measured but was assumed to be related initially to $[\text{Cl}^-]_o$ by $[\text{Cl}^-]_o/[\text{Cl}^-]_i = 2.5$, which appears to be approximately true in practice. Of the remaining six parameters, we have a direct measure only of P_A , the passive permeability coefficient for the amino acid (Morville *et al.*, 1973). P_A was assumed to be independent of the amino acid concentration. Assignment of values to the remaining parameters (K_A , V_m , P_K , P_{Na} , P_{Cl}) is problematical. By the use of an iterative procedure starting from a trial solution we found the values of the five parameters that simulated the five experimental quantities represented by (1) the membrane potential, (2) the change in E caused by the presence of an amino acid, (3) the rate of amino acid uptake, (4) the parameters of $\Delta\text{Na}/\Delta\text{Gly}$ and (5) $\Delta\text{K}/\Delta\text{Gly}$ describing the influx of Na^+ and the efflux of K^+ during the absorption of glycine by tumour cells depleted of ATP (Eddy, 1968a).

(1) Philo & Eddy (1978) measured the value of both $\alpha = V_m(P_{\text{Na}}[\text{Na}^+]_o + P_K[\text{K}^+]_o + P_{\text{Cl}}[\text{Cl}^-]_i)^{-1}$ and of K_A for both glycine and methionine. Deriving V_m from α thus requires a knowledge of the ionic permeability coefficients. These have not been determined directly. (2) However, appropriate trial values of P_K/P_{Na} and of $P_{\text{Cl}}/P_{\text{Na}}$ can be chosen to give the membrane potential near -50 mV that Philo & Eddy (1978) observed in the steady state maintained in the standard Ringer solution. These ratios are 14 and 1.4 respectively, from which we find that V_m/P_{Na} is approx. 16 for glycine. (3) Other trial solutions, based on the simulation of the observed rate of glycine uptake (Eddy *et al.*, 1967), indicated that P_{Na} might be 0.176 ml/s per kg and V_m for glycine 2.8 mmol/s per kg. To draw this conclusion the simulated rates of glycine uptake were fitted to the Michaelis-Menten equation. The 'simulated tracer uptake K_m ' found in this was 1.7 mM for glycine, which is to be compared with the observed value of 4 mM. The corresponding 'simulated tracer V_{max} ' of 0.52 mmol/s per kg was chosen to equal the observed V_{max} , which thus defined the value of V_m . (4) For methionine the values of P_K , P_{Na} , P_{Cl} were taken to be those derived from fitting the model to the glycine observations. K_A and V_m were derived from the fluorescence data summarized in Table 2 of Philo & Eddy (1978). These parameter values gave a 'simulated tracer K_m ' of 0.90 mM approximately equal to the observed value of 0.96 mM (Morville *et al.*, 1973). The 'simulated tracer V_{max} ' for methionine was 0.35 and the observed value 0.68 mmol/s per kg. (5) Given values for K_A , V_m , P_K , P_{Na} and P_{Cl} , we also simulated the assays Eddy (1968a) made to determine the number of equivalents of Na^+ that were absorbed along with glycine through the carrier complex. For this purpose P_K/P_{Na} was set at 4 to simulate the lowering of the membrane potential that appears to occur in the preparations depleted of ATP. The simulated stoichiometry, $\Delta\text{Na}/\Delta\text{Gly} = 0.90$ and $\Delta\text{K}/\Delta\text{Gly} = -0.62$ as defined by Eddy (1968a), equalled the observed values when the several parameters involved had the values shown in Table 1. The present analysis strengthens previous indications (Eddy, 1968a,b) that $n = 1$ (Fig. 1) in this system.

Results and Discussion

Preliminary work with the mathematical model

The general relations between the Na^+ gradient and the amino acid gradient illustrated in Fig. 1 lead to two specific questions about the energetics of the coupling mechanism. What is the effect of the leak pathway, represented by P_A , on (1) the amino acid accumulation ratio $[A]_i/[A]_o$ and (2) the apparent coupling between the net uptake of amino acid and the uptake of Na^+ ? Morville *et al.* (1973) determined

Table 1. Parameters of the mathematical model used to simulate the experimental observations and to predict the effect of P_A on the accumulation of methionine in various ionic conditions

The experimental observations indicate that the coefficient n of Fig. 1 is 1 for glycine. The same number was assumed to apply to methionine.

	P_{Na}	0.176 ml/s per kg
	P_K	2.46 ml/s per kg
	(metabolizing cells)	
	P_K	0.710 ml/s per kg
	(ATP-depleted cells)	
	P_{Cl}	0.240 ml/s per kg
Glycine		
	P_A	4.50 ml/s per kg
	K_A	$0.135 \times 10^{-2} \text{ mol}^2 \cdot \text{l}^{-2}$
	V_m	2.80 mmol/s per kg
L-Methionine		
	P_A	4.10 ml/s per kg
	K_A	$0.465 \times 10^{-3} \text{ mol}^2 \cdot \text{l}^{-2}$
	V_m	1.30 mmol/s per kg

P_A for various amino acids by measuring their influx or efflux in the absence of Na^+ . (1) The mathematical model predicts that such a leak pathway would lower the steady-state value of $[A]_i/[A]_o$ by a constant factor when $[A]_o$ was small and the ionic concentrations were fixed (Fig. 2). The accumulation ratio would fall progressively as $[A]_o$ increased, as would the membrane potential (Fig. 2). As illustrated in Fig. 2 the amino acid accumulation ratio is independent of $[A]_o$ when the Na^+ gradient and the amino acid gradient are at equilibrium (Eddy, 1968a,b). (2) Eddy (1968a) studied the net uptake of Na^+ and the net efflux of K^+ that accompanied the absorption of glycine, with the object of determining their stoichiometrical ratio relative to the glycine uptake. Such assays fail to provide a direct measure of n (Fig. 1), because they merely show how the amino acid changes the ionic composition of the system relative to the control (Eddy, 1968a; Jacquez, 1972). The computations illustrated in Fig. 3 show that when $n=1$, the value preferred by Eddy (1968a), the apparent stoichiometry for Na^+ would vary from 0.75 to about 1 dependent on the relative magnitudes of the parameters P_A and V_m . The interpretation involves two opposing effects. (1) The absorption of Na^+ outside the amino acid pump, represented by P_{Na} , is smaller in the presence of glycine than in the controls both because of the depolarization caused by glycine (Eddy, 1977) and because the amino acid raises the cellular content of Na^+ . These effects lower the apparent increase in Na^+ uptake caused by the uptake of glycine. (2) The existence of the leak P_A means that, whereas Na^+ absorbed with glycine

Fig. 2. Predicted effect of varying the extracellular amino acid concentration $[A]_o$ on the steady-state amino acid accumulation ratio $[A]_i/[A]_o$ and the membrane potential, either in the presence or in the absence of a leak pathway

The parameters of the mathematical model were similar to those shown in Table 1. The Na^+ electrochemical gradient in the absence of the amino acid was about 20-fold with $[\text{Na}^+]_o$, $[\text{K}^+]_o$, $[\text{Cl}^-]_o$, $[\text{Na}^+]_i$, $[\text{K}^+]_i$ and $[\text{Cl}^-]_i$ constant at 150mM, 8mM, 130mM, 50mM, 150mM and 50mM respectively. V_m was 0.9mmol/s per kg, K_A was $0.15 \times 10^{-2} \text{ mol}^2 \cdot \text{l}^{-2}$ and $n=1$. The mathematical model was used to compute the value of $[A]_i$ and the corresponding value of E when the amino acid reached a steady state at a given value of $[A]_o$. In curves (a) and (c) the leak was negligible ($P_A = 1 \times 10^{-9} \text{ l/s per kg}$), whereas in curves (b) and (d) $P_A = 4.5 \text{ ml/s per kg}$.

might be retained in the cells, some of the glycine would not be retained. This effect increases the net amount of Na^+ that is apparently absorbed with the amino acid. Similar factors influence the stoichiometry for K^+ (Fig. 3). The further question arose of the probable value of the parameter V_m , which is one factor governing the amplitude of the depolarization caused by the amino acid (Philo & Eddy, 1978). V_m is also related to the maximum rate of uptake of glycine by the Na^+ -dependent route (V_{max}). Eddy *et al.* (1967) estimated this by determining the uptake rate as a function of [glycine]. Because the amino acid depolarizes the cell preparation, $V_{max} < V_m$, to an extent that we determined by using the mathematical model. Inspection of eqn. (7) in Philo & Eddy (1978) shows moreover that the velocity of amino acid uptake would not be expected to be an exact hyperbolic function of the amino acid concentration. However, we note that the small deviations from linearity expected in conventional double-reciprocal plots have not yet been detected in this system.

Consideration of all the above factors, by means of the procedure outlined in the Materials and Methods

Fig. 3. V_m and P_A and the computed apparent stoichiometry of the net uptake of Na^+ and efflux of K^+ during the uptake of 10 mM-glycine for 10 min

The conditions simulated those studied by Eddy (1968a). The test system absorbed glycine to give a 26 mM solution in the cellular water after 10 min in the presence of 10 mM-glycine. The control lacked glycine. Initially $[\text{Na}^+]_o$, $[\text{K}^+]_o$, $[\text{Cl}^-]_o$, $[\text{Na}^+]_i$, $[\text{K}^+]_i$ and $[\text{Cl}^-]_i$ were respectively 150 mM, 8 mM, 130 mM, 50 mM, 150 mM and 50 mM. After 10 min in the presence of glycine $[\text{Na}^+]_i$, $[\text{K}^+]_i$ and $[\text{Cl}^-]_i$ were computed as 91 mM, 110 mM and 55 mM. P_{Na} , P_{K} and P_{Cl} were respectively 0.3, 1.8 and 0.54 ml/s per kg. These were selected to maintain a relatively low membrane potential, as required by the observations with cellular preparations depleted of ATP (Eddy, 1968b). The equations of the model (Philo & Eddy, 1978) were solved to show how $[\text{Na}^+]_i$ and $[\text{K}^+]_i$ increased with time, either in the presence or in the absence of glycine. The apparent stoichiometry for Na^+ was computed (Eddy, 1968a) as (the increase in cellular Na^+ content relative to the control)/(equivalents of glycine absorbed) (curves a and b). A similar ratio was determined for the decrease in cellular K^+ (curves c and d). The computations assumed that $n=1$ (Fig. 1) and $K_A=0.15 \times 10^{-2} \text{ mol}^2 \cdot \text{l}^{-2}$. V_m varied as shown. For curves (a) and (c), $P_A=4.5 \text{ ml/s per kg}$. For curves (b) and (d) P_A was negligible ($1 \times 10^{-9} \text{ l/s per kg}$).

section, led to the choice of parameters shown in Table 1. These provide a rough description, though certainly not an exact one, of the experimental observations of (1) the amino acid influx, (2) the net movements of Na^+ and K^+ relative to glycine and (3) the magnitude of the depolarization the amino acids caused. It is to be emphasized that the computations are based on the assumption that the prevailing membrane potential is solely due to ionic diffusion. The effect of the leak pathway on the functioning of the amino acid pump is independent, in the first instance, of the origin of the membrane potential according to the scheme illustrated in Fig. 1. We used the data summarized in Table 1 to estimate the accumulation of methionine that was expected to occur when a given Na^+ gradient became established during energy metabolism. These estimates were compared with the measured methionine gradients.

Methionine accumulation in various experimental conditions

Preliminary work indicated that under the conditions selected the methionine gradient and the conic gradients reached a steady state after incubation of the system at 37°C for 30 min. (1) When the tumour cells loaded with Na^+ were incubated in the so-called reversed gradient conditions, where $[\text{Na}^+]_o$ was small and $[\text{K}^+]_o$ was large, methionine and 2-aminobutyrate were each concentrated extensively within about 10 min (Schafer & Heinz, 1971; Gibb & Eddy, 1972). Gibb & Eddy (1972) suggested that, owing to the operation of the Na^+ pump under these conditions, the membrane potential might be sufficiently large to energize the amino acid pump through the electrochemical gradient of Na^+ . The further observations shown in rows 1–3 of Table 2 are broadly consistent with that suggestion. The membrane potential was about -90 mV after 8 min incubation (Table 2, row 1) and the Na^+ electrochemical gradient was equivalent to the ratio of about 23, or nearly 3 times larger than the prevailing methionine gradient. The mathematical model predicts that a Na^+ gradient of that magnitude would maintain a methionine gradient of about 12-fold. The fact that a smaller methionine gradient was actually formed may be due to the failure of the system to reach a steady state, since both the Na^+ content of the cells and the membrane potential fell during the next 20 min. The ratio 8.2/11.7 is listed in the last column of Table 2 as a measure of the discrepancy between the expected and observed ratios. The presence of either 0.3 mM-ouabain or of valinomycin ($4 \mu\text{g/ml}$) inhibited methionine accumulation (Gibb & Eddy, 1972) under these conditions (Table 2, rows 2 and 3). Both compounds lowered the membrane potential (Philo & Eddy, 1978) and thus lowered the magnitude of the Na^+ electrochemical gradient. Rows 4–6 of Table 2 summarize the corresponding observations made when the incubation had lasted 30 min and the methionine gradient was almost the same as that produced at 8 min. In the experiments summarized in row 4 of Table 2, Na^+ was expelled from the tumour cells and the membrane potential fell to about -25 mV . The methionine gradient was now about 2.1 times the predicted value. Similar factors were observed when valinomycin or ouabain was present. (2) Rows 7 and 8 of Table 2 refer to a situation in which work by Eddy (1968b) indicated that glycine accumulation during energy metabolism was enhanced when the ratio $[\text{K}^+]_i/[\text{K}^+]_o$ was made larger. Similar behaviour occurred with methionine (Table 2), the membrane potential being increased by about 50 mV when $[\text{K}^+]_o$ was lowered, so that the Na^+ electrochemical gradient increased by a factor of about 7.1/1.9. In these two series of experiments the observed methionine gradient again exceeded the predicted values by a factor of about 2.

Table 2. *Measured Na⁺ electrochemical gradient ($\Delta\mu_{Na}$ expressed as a ratio) and the observed and predicted methionine gradient in various ionic conditions during energy metabolism*

Each of the 61 assays involved a separate tumour-cell preparation (26 mg dry wt.), which was incubated at 37°C in the Ringer solution (80 ml) of the indicated composition with 1 mM-L-[¹⁴C]methionine. The system contained 60 μ M-2,4-dinitrophenol, as well as 6 ng of oligomycin, 1 μ g of antimycin and 2 mg of glucose per ml of cell suspension. After 3 min or about 30 min 80 μ g of 3,3'-dipropylloxadycarbonyl was added in 40 μ l of ethanol. A portion (4 ml) of the mixed suspension was placed in the fluorimeter cuvette. The fluorescence intensity at 620 nm was assayed at 37°C about 5 min after the addition of the dye and the membrane potential *E* inferred (Philo & Eddy, 1978). The remaining suspension was processed to determine the distribution of ¹⁴C, Na⁺, K⁺ and water, between the cellular and extracellular phases, at the specific time *E* was assayed (Philo & Eddy, 1978). Because the concentration of dye used appears not to affect the transport of Na⁺, K⁺ and the amino acid, the introduction of the dye was assumed not to affect their mutual relations. The predicted methionine gradient was computed from the mathematical model, describing the steady state with respect to the amino acid, when [Na⁺]_o, [Na⁺]_i, *E* and [A]_o had given values. The methionine gradient would equal $\Delta\mu_{Na}$ according to the alternative equilibrium model. Values are means $\pm$ s.e.m. for the numbers of observations given.

Row	Conditions	Time (min)	No. of observations	Observed [Met] _i /[Met] _o	[K ⁺] _i /[K ⁺] _o	[Na ⁺] _o /[Na ⁺] _i	- <i>E</i> (mV)	$\Delta\mu_{Na}$ (as ratio)	Predicted [Met] _i /[Met] _o	Observed gradient Predicted gradient
Series 1										
1	80 mM-Na ⁺ , 150 mM-K ⁺	8	25	8.17 $\pm$ 0.31	1.26 $\pm$ 0.03	0.70 $\pm$ 0.02	90.5 $\pm$ 2.9	23.3 $\pm$ 3.5	11.7 $\pm$ 0.6	0.7
2	80 mM-Na ⁺ , 150 mM-K ⁺ + 4 μ g of valinomycin/ml	8	3	2.31 $\pm$ 0.59	1.60 $\pm$ 0.13	0.76 $\pm$ 0.07	11 $\pm$ 8	1.27 $\pm$ 0.31	1.3 $\pm$ 0.3	1.8
3	80 mM-Na ⁺ , 150 mM-K ⁺ + 0.3 mM-ouabain	8	3	2.19 $\pm$ 0.39	0.91 $\pm$ 0.05	0.46 $\pm$ 0.04	36.4 $\pm$ 4.7	1.86 $\pm$ 0.25	1.8 $\pm$ 0.2	1.2
4	80 mM-Na ⁺ , 150 mM-K ⁺	30	9	8.98 $\pm$ 0.21	1.73 $\pm$ 0.05	1.72 $\pm$ 0.08	24.8 $\pm$ 3.6	4.68 $\pm$ 0.85	4.2 $\pm$ 0.7	2.1
5	80 mM-Na ⁺ , 150 mM-K ⁺ + 4 μ g of valinomycin/ml	35	3	6.41 $\pm$ 0.48	1.74 $\pm$ 0.13	1.76 $\pm$ 0.12	22.6 $\pm$ 6.2	4.30 $\pm$ 0.68	3.9 $\pm$ 0.6	1.6
6	80 mM-Na ⁺ , 150 mM-K ⁺ + 0.3 mM-ouabain	35	3	2.45 $\pm$ 0.04	1.33 $\pm$ 0.01	0.62 $\pm$ 0.03	26.4 $\pm$ 5.8	1.73 $\pm$ 0.13	1.7 $\pm$ 0.1	1.4
Series 2										
7	30 mM-Na ⁺ , 133 mM-K ⁺	30	4	4.32 $\pm$ 0.58	1.63 $\pm$ 0.07	1.55 $\pm$ 0.32	6.4 $\pm$ 4.9	1.91 $\pm$ 0.60	1.8 $\pm$ 0.5	2.4
8	30 mM-Na ⁺ , 8 mM-K ⁺ , 125 mM-choline chloride	30	3	11.0 $\pm$ 0.9	16.0 $\pm$ 1.4	0.80 $\pm$ 0.10	57.0 $\pm$ 6.3	7.07 $\pm$ 1.45	5.7 $\pm$ 0.9	1.9
Series 3										
9	155 mM-Na ⁺ , 8 mM-K ⁺	30	8	30.3 $\pm$ 1.4	23.5 $\pm$ 1.0	3.25 $\pm$ 0.32	55.0 $\pm$ 2.3	26.5 $\pm$ 3.5	15.6 $\pm$ 1.9	1.9

Fig. 4. Variation of the observed methionine gradient ($[Met]_i/[Met]_o$) with the measured magnitude of the Na^+ electrochemical gradient ($\Delta\mu_{Na}$) expressed in the same units

The results of a series of 46 assays of both parameters made with the tumour cells metabolizing glucose are illustrated (see the text). The interrupted line corresponds to equal values of the ordinate and abscissa. An initial methionine concentration of 1 mM was used in each instance. The Na^+ electrochemical gradient was computed as $[Na^+]_o e^{-EF/RT}/[Na^+]_i$. As explained in the text the observations were studied further by making a regression analysis of the observed values of the methionine gradient on (1) the Na^+ electrochemical gradient (equilibrium model) or (2) the value of the methionine gradient expected to be found at that Na^+ gradient when the amino acid leak operates (steady-state model).

(3) The mean methionine gradient formed during incubation of the system in the standard Ringer solution exceeded the value predicted by the steady-state model by a factor of about 1.9 (Table 2, row 9). Thus a discrepancy factor of from 1.4 to 2.4 between theory and experiment was observed in the several ionic conditions where the system is likely to have reached a steady state.

The above 30 assays in which the system was incubated for 30 min were combined with a similar set of 16 in which the methionine gradient varied from about 6- to 30-fold. For this purpose $[K^+]_o$ varied from 2.9 to 80 mM in seven steps (see Fig. 1 of Philo & Eddy, 1978) and $[Na^+]_o + [K^+]_o = 163$ mM. Fig. 4 shows that the methionine gradient observed in the 46 assays was roughly proportional to the corresponding Na^+ electrochemical gradient. The proportion-

ality factor deduced by a weighted fit of the observations was 1.140 ± 0.079 , which is not significantly different from 1 by Student's t test ($r = 0.909$; $P < 0.001$). The methionine gradient extrapolated to a value of 1.45 ± 0.37 at zero Na^+ gradient. Bearing in mind the small size of this factor we conclude that the observations roughly fit the equilibrium model. In contrast, the methionine gradient was 1.80 ± 0.11 times larger ($P < 0.001$), over the whole experimental range, than was predicted by the steady-state model ($r = 0.920$; $P < 0.001$). The methionine gradient extrapolated to a value of 0.14 ± 0.40 at zero Na^+ gradient in this instance. Clearly the steady-state model is unacceptable as it stands.

Conclusion

The direct comparison of the magnitudes of the Na^+ electrochemical gradient and the corresponding methionine gradient shows that these quantities are very roughly equal in several physiological conditions. However, the likelihood that there is a leak pathway by-passing the amino acid pump (Morville *et al.*, 1973; Jacquez, 1975) means that the measured Na^+ electrochemical gradient is probably just too small to maintain the methionine gradient, by a factor of 1.8 corresponding to about 15 mV. Although there is some evidence that the amino acid pump functions to some extent in the absence of Na^+ (Potashner & Johnstone, 1970; Morville *et al.*, 1973), our estimates of P_A made under these conditions, with K^+ as the predominant cation, are probably roughly correct. Reid *et al.* (1974) showed that the tumour cells depleted of ATP accumulated about as much methionine as did the metabolizing cell preparations, provided that the Na^+ electrochemical gradient was large. Hence it seems unlikely that the apparent energy deficiency, which is in any case small, represents a requirement for ATP. A different possibility is that our procedures somewhat underestimate the magnitude of the Na^+ gradient. We have assumed that the activity coefficients for Na^+ and K^+ respectively are both unity in computing the ratio $[Na^+]_o/[Na^+]_i$ and E . However, a decrease in $[Na^+]_i$, owing to the lowering of the activity coefficients for Na^+ in the cytosol, would be partly compensated by the corresponding fall in $[K^+]_i$ and therefore a diminished absolute value of E as estimated from $[K^+]_i/[K^+]_o$. An additional factor is that the Na^+ content of the cytosol might be lower than $[Na^+]_i$, due to the concentration of Na^+ in the nucleus (Pietrzyk & Heinz, 1974; Smith & Adams, 1977). These considerations indicate that it would be premature to reject the gradient hypothesis on the basis of the present observations. Indeed these establish that there is a direct relationship between the magnitude of the methionine gradient and its putative energy source.

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References

- Blaustein, M. P. & King, A. C. (1976) *J. Membr. Biol.* **30**, 153–173
- Colombini, M. & Johnstone, R. M. (1974) *J. Membr. Biol.* **18**, 315–337
- Eddy, A. A. (1968a) *Biochem. J.* **108**, 195–206
- Eddy, A. A. (1968b) *Biochem. J.* **108**, 489–498
- Eddy, A. A. (1977) in *Intestinal Permeation* (Kramer, M. & Lauterbach, F., eds.), pp. 332–349, Excerpta Medica, Amsterdam and Oxford
- Eddy, A. A., Mulcahy, M. F. & Thomson, P. J. (1967) *Biochem. J.* **103**, 863–876
- Gibb, L. E. & Eddy, A. A. (1972) *Biochem. J.* **129**, 979–981
- Heinz, E., Geck, P. & Pietrzyk, C. (1975) *Ann. N. Y. Acad. Sci.* **264**, 428–441
- Jacquez, J. (1972) *Math. Biosci.* **13**, 71–93
- Jacquez, J. A. (1975) *J. Gen. Physiol.* **65**, 57–83
- Lever, J. E. (1977) *Biochemistry* **16**, 4328–4334
- Morville, M., Reid, M. & Eddy, A. A. (1973) *Biochem. J.* **134**, 11–26
- Philo, R. & Eddy, A. A. (1978) *Biochem. J.* **174**, 801–810
- Pietrzyk, C. & Heinz, E. (1974) *Biochim. Biophys. Acta* **352**, 397–411
- Potashner, S. & Johnstone, R. M. (1970) *Biochim. Biophys. Acta* **203**, 445–456
- Reid, M., Gibb, L. E. & Eddy, A. A. (1974) *Biochem. J.* **140**, 383–393
- Schafer, J. A. (1977) *J. Gen. Physiol.* **69**, 681–704
- Schafer, J. A. & Heinz, E. (1971) *Biochim. Biophys. Acta* **249**, 15–33
- Smith, T. C. & Adams, R. (1977) *J. Membr. Biol.* **35**, 57–74
- Vidaver, G. A., Shepherd, S. L., Lagow, J. B. & Wiechelmann, K. J. (1976) *Biochim. Biophys. Acta* **443**, 494–514