ENERGY EXPENDITURE BY ACTIVE TRANSPORT MECHANISMS

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SUMMARY

The energy expended by an active transport process has been derived from the physico-chemical features of a hypothetical transport mechanism on the basis of a general "Carrier" model. For this purpose the transport system proper has been narrowly defined as including only those reactions in which the transported particle, or carrier, or both, directly participate. The total amount expended can be divided into three fractions, one of them being reversible and the other two irreversible. The reversible energy corresponds to the concentration work actually achieved. The irreversible fractions are connected with the transporting of the transported particle and with the continuous supply of active carrier, respectively. Of the last two only the first one is accessible to experimental measurement with the help of the unidirectional, carrier-bound fluxes of the transported particle. However, as the concentration of the transported particle approaches zero, the second irreversible energy loss also approaches zero and thus the energy expended by the transport process may be found. From various observations with the system of glycine transport into Ehrlich carcinoma cells, some general conclusions concerning the second irreversible fraction and the coupling between transport and metabolism have been tentatively drawn. The data has also been extrapolated to zero concentrations to yield the value of the energy expenditure. Possible mechanisms to account for the value found are then discussed.

INTRODUCTION

An active transport mechanism is able to perform osmotic work at the expense of metabolic energy. Knowledge of the magnitude of this expenditure would help to elucidate the mechanism and its linkage to metabolism. The "reversible" fraction of this energy, *i.e.*, that amount corresponding to the osmotic work actually achieved, can in many cases be estimated with fair accuracy. The total energy expenditure, however, which contains the "irreversible" fraction, *i.e.*, the dissipation indispensably associated with any real work, is hardly known for any system. Many attempts have been made to derive it from the increment of O_2 consumption by a transporting tissue due to an increment of transport activity. Such procedures, however, suffer from serious uncertainties, partly introduced by the dubious assumptions that the

basic metabolic rate of the tissue is the same in the resting and the working state and that none of the resting state energy is used by the transport system. Furthermore, the increment of energy consumption also includes energy dissipation by such reactions as phosphorylation, etc., which may be essentially linked to the transport process but cannot be considered an integral part of the mechanism proper. Highly conflicting results, *e.g.* those concerning the efficiency of gastric acid formation, seem to illustrate the shortcomings of this procedure[1].

The present paper deals with the estimate of the energy expenditure of a transport process on the basis of the chemical nature of the underlying mechanism. This procedure requires a clear and narrow definition of the system with respect to all of the reactions intrinsically involved. An ideal result would, of course, be possible only if all of these reactions and their free energies were known[2]. It is felt, however, that in the absence of such knowledge a useful estimate might be possible on the basis of a reasonable model, which is general enough to fit much of the experimental data available. For most systems of active transport, a simplified "carrier" model—the term "carrier" being used in its widest sense—seems to meet these requirements. A great variety of such models have been suggested by numerous authors, almost all of them having in common the following basic features: (a) the substrate A penetrates the transport region from left to right through interaction with one or more acceptors X; (b) the movement of A against the concentration gradient depends on continuous supply of active X on the left side and continuous inactivation of X on the right side; (c) either this supply of active X or the inactivation of X is energetically linked to an exergonic metabolic reaction. The narrowest definition of such a system in terms of the truly integral reactions concerned would include only those reactions in which at least one of either A or X directly participates.

An early attempt to estimate the minimum energy expenditure of such a system was made by HEINZ[3,4]. It was based on the realization that an indispensible amount of free energy is lost while the substrate carrier complex moves along its gradient within the transport region, and that the greater the rate of net transport the greater this gradient is. Under various simplifying assumptions (such as that the complex AX is virtually at equilibrium at both sides of the region and that A cannot pass the membrane except in combination with X) the energy loss per mole of transported substrate (ΔG_{irr}) was expressed by

$$\Delta G_{irr} = RT \ln \frac{\text{forward flux}}{\text{forward flux} - \text{net flux}}$$

where R and T are the Gas constant and the absolute Temperature, respectively, ln is the natural logarithm, and the term "forward flux" represents the unidirectional flux of A in the forward direction. The ratio is identical with the flux ratio. It can be shown that the assumption of virtual equilibrium between AX and A and X in the interfaces is not essential for the above formula to be valid. If, however, the membrane is to an appreciable extent permeable to free A in the uncombined form, the flux ratio has to be restricted to the "carrier-bound" fluxes, *i.e.*, to the fluxes of AX only.

A similar formula was used by ZERAHN[5] in order to estimate the energy necessary to overcome the "internal resistance" of the Na-pump in frog skin. However, no specification was made with respect to "carrier bound" and free diffusion fluxes.

These formula, though representing indisputable losses of energy and though applicable to experimentally accessible data, are deficient in one important respect. Since they consider only reactions in which A directly participates, and ignore reactions with X alone, they are based on too narrow a definition of the transport mechanism. Hence, they give an incomplete account of the energy budget, and the value they provide may fall considerably short of the real energy dissipation unless the substrate concentrations are vanishingly small[2].

In the following, the problem is approached in a more general way in order to assess the error involved in using the above formula. This approach is based on the view that both substrate and carrier are integral parts of the transport mechanism. It will be shown that the estimate as found by the above formula will always be less than the true value of the expended energy but will always be a better estimate than that found by simply taking the maximum concentration ratio that the system as a whole exhibits in a series of experiments.

DERIVATION

In the following derivation the carrier model for active transport will be assumed. The carrier is assumed to operate within a membrane which separates two homogeneous and ideally mixed fluid compartments on the left (l) and on the right (r). Across this membrane a single electrically neutral particle, A, is actively transported from compartment l to r with the help of a carrier X which is able to combine with a single A to form a complex and which is continuously supplied at the interface l, *i.e.*, between the membrane and compartment l. This complex is able to move across the membrane to interface r either by diffusion, rotation, rearrangement of the membrane texture, or some other process. At interface r it may exchange its A with other A in compartment r, or it may unload its A. Then through a series of reactions and/or diffusion the carrier will return to interface l. It will be further assumed that all of the reactions involving A or the complex proceed via unitary reactions which obey mass action kinetics and that all of the constituents involved in the reactions are electrically neutral. Since the resultant equation is valid under very general conditions, a general system will be chosen.

Before the A_l combines with the carrier, it may first react with other particles. These reactions may be written as

$$\begin{aligned} A_l + S_1 &\underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} A^{(1)} + P_1 \\ A^{(1)} + S_2 &\underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} A^{(2)} + P_2 \\ &\vdots \\ A^{(n-1)} + S_n &\underset{k_{-n}}{\overset{k_n}{\rightleftarrows}} A^{(n)} + P_n, \end{aligned} \tag{1}$$

where the k_i are the unidirectional rate coefficients, the positive subscripts referring to the forward direction and the negative ones referring to the reverse direction; the $A^{(i)}$ are intermediates containing the A; and the S's and P's represent substrates

and products respectively with all or none of the S's and P's present in an actual system. (The S's and P's may represent enzymes, or energy yielding reactants which "activate" the A, such as ATP and ADP respectively.)

The next step would be for the $A^{(n)}$ to combine with the X_l at the left interface and by a series of reactions and/or diffusion, X_r will appear at the right interface and the A will be liberated, perhaps in a form different from its free form. (Note that diffusion, under the assumption of no interaction between fluxes, may be represented by a first order reaction in which the forward and backward rate coefficients are the same and are equal to the permeability constant.) This series of reactions may be represented as

$$\begin{aligned} A^{(n)} + X_l + S_{n+1} &\underset{k_{-(n+1)}}{\overset{k_{n+1}}{\rightleftarrows}} A^{(n+1)} + P_{n+1} \\ A^{(n+1)} + S_{n+2} &\underset{k_{-(n+2)}}{\overset{k_{n+2}}{\rightleftarrows}} A^{(n+2)} + P_{n+2} \\ &\vdots \\ A^{(m-1)} + S_m &\underset{k_{-m}}{\overset{k_m}{\rightleftarrows}} A^{(m)} + P_m + X_r \end{aligned} \tag{2}$$

The $A^{(n+1)}, \ldots, A^{(m-1)}$ will all be complexes of the A, the X and perhaps other particles.

Finally, the $A^{(m)}$ will react with other substrates and/or enzymes until it becomes a free particle. These reactions are

$$\begin{aligned} A^{(m)} + S_{m+1} &\underset{k_{-(m+1)}}{\overset{k_{m+1}}{\rightleftarrows}} A^{(m+1)} + P_{m+1} \\ &\vdots \\ A^{(p-1)} + S_p &\underset{k_{-p}}{\overset{k_p}{\rightleftarrows}} A_r + P_p \end{aligned} \tag{3}$$

The overall reaction for the transport of the A and the carrier from compartment l to compartment r may then be written as

$$A_l + X_l + S_1 + S_2 + \ldots + S_p \rightleftharpoons A_r + X_r + P_1 + P_2 + \ldots + P_p \tag{4}$$

If it is assumed that the X_r will then return to compartment l through only one other pathway than that shown by Eqn. 4, it will go through a series of steps which may be summarized as

$$X_r + S_{p+1} + S_{p+2} + \ldots + S_q \rightleftharpoons X_l + P_{p+1} + P_{p+2} + \ldots + P_q \tag{5}$$

The overall reaction for the transport of one molecule of A from the left compartment to the right compartment may then be represented as

$$A_l + S_1 + \ldots + S_q \rightleftharpoons A_r + P_1 + \ldots + P_q \tag{6}$$

The free energy change of the overall reaction (Eqn. 6), ΔF_T, may be written as the sum of the ΔF's of the reactions shown in Eqns. 4 and 5.

Therefore

$$\Delta F_T = \Delta F_A + \Delta F_X, \tag{7}$$

where

$$\Delta F_A = \mathrm{RT}\ \ln \frac{A_r X_r P_1 \ldots P_p}{A_l X_l S_1 \ldots S_p} + \sum_{i=1}^{p} \mu_{0P_i} - \sum_{i=1}^{p} \mu_{0S_i} + \mu_{0X_r} - \mu_{0X_l}, \tag{8}$$

and

$$\Delta F_X = \mathrm{RT} \ln \frac{X_l P_{p+1} \ldots P_q}{X_r S_{p+1} \ldots S_q} + \sum_{i=p+1}^{q} \mu_{0P_i} - \sum_{i=p+1}^{q} \mu_{0S_i} + \mu_{0X_l} - \mu_{0X_r}, \tag{9}$$

where μ_0 is the standard chemical potential, the symbols A, X, S, and P represent concentrations, as well as representing the particle itself, and it is assumed that $\mu_{0A_l} = \mu_{0A_r}$. Since the sum of the standard chemical potentials of Eqn. 8 is equal to minus RT times the log of the equilibrium constant in the reaction of Eqn. 4, which in turn is equal to minus RT times the log of the product of the forward reaction rates, divided by the product of the backward reaction rates, Eqn. 8 may be rewritten as

$$\Delta F_A = \mathrm{RT} \ln \frac{A_r X_r P_1 \ldots P_p\, k_{-1} \ldots k_{-p}}{A_l X_l S_1 \ldots S_p\, k_1 \ldots k_p}. \tag{10}$$

If the pump is in the steady state, and if $\vec{J}$ and $\overleftarrow{J}$ are the unidirectional carrier fluxes of A_l towards the right compartment and A_r towards the left compartment respectively, then it can be easily shown[6], that for the reactions as shown in Eqns. 1, 2, and 3,

$$\frac{\overleftarrow{J}}{\vec{J}} = \frac{A_r X_r P_1 \ldots P_p\, k_{-1} \ldots k_{-p}}{A_l X_l S_1 \ldots S_p\, k_1 \ldots k v_p}. \tag{11}$$

It can be seen from Eqn. 6, that the total energy, W, put into the system in order to transport one mole of A from compartment l to r is, as discussed in ref. 2,

$$W = \mathrm{RT} \ln \frac{S_1 \ldots S_q}{P_1 \ldots P_q} + \sum_{i=1}^{q} \mu_{0S_i} - \sum_{i=1}^{q} \mu_{0P_i}. \tag{12}$$

It should be emphasized that W represents the energy expended by the reactions which are directly coupled to the active transport mechanism and not the energy expended by reactions which are only secondarily involved. For example, if the S's and P's represented ATP and its products, and if the ATP was regenerated by creatine phosphate breakdown, then the W would represent only the ATP reaction and not the creatine phosphate reaction. Thus, if the O_2 consumption method actually measured the O_2 required for the transport mechanism, it would yield an energy consumption which is greater than or equal to W since some of the O_2 might be involved in reactions which generate the S's.

The result of combining Eqns. 7 through 12 is then

$$W = \mathrm{RT} \ln \frac{A_r}{A_l} + \mathrm{RT} \ln \frac{\vec{J}}{\overleftarrow{J}} - \Delta F_X. \tag{13}$$

DISCUSSION

Eqn. 13 shows that the total energy fed into the transport system, as defined at the outset, consists of three fractions. The fraction represented by the term RT ln (A_r/A_l) is the osmotic work actually achieved by the system. The other two fractions, which are equal to $-\Delta F_T$, give the waste of energy, *i.e.*, the amount of energy irreversibly lost through the mechanism. The first of these terms, RT ln $(\vec{J}/\overleftarrow{J})$, or $-\Delta F_A$, covers only those reactions of the systems in which the substrate A directly participates. It is, as such, numerically identical with ΔG_{irr} in the early formula proposed by HEINZ[3,4] to represent the minimum loss of energy associated with any real work of the system. The last term, $-\Delta F_X$, gives the energy wasted by the series of processes involved in reactivating X at the expense of metabolic energy. This term, though referring to an integral part of the transport system proper, is, contrary to $-\Delta F_A$, not accessible experimentally.

We will now further assume that the concentrations of A and the orientation of the active transport mechanism are such that there is a net active transport from left to right, *i.e.*, $\vec{J} \geq \overleftarrow{J}$. Thus, the reactions of Eqns. 4 and 5 proceed from left to right, and therefore $\Delta F_X \leq 0$. Hence,

$$RT \ln \frac{A_r}{A_l} \leq RT \ln \frac{A_r}{A_l} + RT \ln \frac{\vec{J}}{\overleftarrow{J}} \leq W. \qquad (14)$$

As shown previously[2], under the above assumptions, (in particular that the X traverses the membrane via only two pathways, one with and one without the A), as the concentration of the A's approach zero, the inequality sign on the right side of Eqn. 14 may be replaced by the equality sign. That is, as the A's approach zero, the fluxes approach zero, the reaction of Eqn. 5 approaches equilibrium, and therefore ΔF_X approaches zero.

Eqns. 13 and 14 were derived under the following implicit assumptions: (a) The activity coefficient of the A's is unity. Otherwise the equations are valid only if A is considered to represent activities, since reaction kinetics actually involve activities and not concentrations alone[7]. (b) The standard chemical potential, μ_0, of A_l and A_r are identical. Otherwise the partition coefficient of the A's between the left and right compartments must be introduced into Eqns. 13 and 14.

One of our assumptions was that the l and r refer to the regions immediately adjacent to the carrier. However, the carrier may only operate within a subregion of the total membrane, as in gastric mucosa, frog skin, etc. Thus, l and r may not be the left and right solutions on either side of the membrane. If the A's now represent the activities times the partition coefficient, the ratio of the A's in the right and left solution will, under the assumptions listed above, be less than or greater than the ratio for the A's on either side of the carrier, dependent on whether the net flow (passive plus active) is from left to right or from right to left respectively. If the system is in pseudo-equilibrium, *i.e.*, the net flow is zero[8], or if the permeability of the regions on either side of the membrane is very large, then the ratios for the two regions will be equal and the l and r may refer to the left and right solutions on either side of the membrane.

W need not be a constant for any given system since the concentrations of the

S's and the P's may change during the course of an experiment. If there is an enzyme, it will appear in both the numerator and denominator of Eqn. 11 and will cancel out. Thus, variations in the concentrations of enzymes involved in the transport process will not alter the value of W. If there is a sufficient concentration of energy yielding substrates in the experimental environment, it is quite possible that the W may have a constant value independent of the rate of the pump or the concentration of A_l and A_r.

Although the carrier model for active transport was assumed, the same conclusions reached above are applicable for any individual type of active transport mechanism[8].

APPLICATION

The formulas derived in the foregoing section will now be applied to previously published experimental data concerning the accumulation of glycine in EMAT cells[9]. Here the relationship between the fluxes and the transport mechanism for glycine are fairly well understood[10]. Table I, which is derived from data given in the original paper, shows the accumulation ratios, the unidirectional carrier-bound fluxes, the

TABLE I

CONCENTRATION WORK (RT ln A_r/A_l) AND ΔF_A AT VARIOUS EXTRACELLULAR GLYCINE CONCENTRATIONS AND UNDER PSEUDO-EQUILIBRIUM DISTRIBUTIONS

The data given is derived from that presented in a previous paper[9]. The "carrier-bound" fluxes have been obtained by subtracting the contribution of free diffusion (leakage) from the total unidirectional fluxes of glycine. T has the value of 310° K.

Extracellular glycine (A_l) *mmoles*	*Pseudo-equilibrium distribution ratio of glycine* (A_r/A_l)	*"Carrier-bound" fluxes*		*Osmotic work* (RT ln A_r/A_l) *cal/mole*	ΔF_A (RT ln $\vec{J}/\overleftarrow{J}$) *cal/mole*	$W-\lvert\Delta F_X\rvert$ (RT ln A_r/A_l + RT ln $\vec{J}/\overleftarrow{J}$) *cal/mole*
		Influx ($\vec{J}$) *μmole/g·min*	*Efflux* ($\overleftarrow{J}$) *μmole/g·min*			
1.7	14.4	23.8	19.2	1645	129	1774
1.7	12.0	19.6	15.8	1528	129	1657
6.9	6.9	48.1	40.0	1189	111	1300
7.6	6.6	51.6	43.0	1164	111	1275
16.5	4.5	73.4	61.7	924	105	1029
16.5	5.0	82.2	69.0	992	105	1097
31.4	2.5	77.4	68.0	567	80	647
31.4	2.4	76.5	67.5	542	74	616

osmotic work, and ΔF_A calculated via Eqns. 10 and 11. If W, the work put into the system in order to transport one mole of A, is constant and independent of the concentration of A_l and A_r, then from Table I and Eqn. 13 we have to conclude that the system becomes less efficient with increasing A_l. Hence, either ΔF_A, ΔF_X, or both, have to increase in order to compensate for the decrease in A_r/A_l. Since ΔF_A, as derived from the experimental flux ratio (Table I), does not increase with the substrate concentration, the loss of efficiency has to be attributed to an increase of ΔF_X only. Such an increase must result from an increase of the ratio X_r/X_l. It can well be conceived that X_l decreases with an increase of A_l, but an increase of X_r/X_l would imply that the concomitant decrease of X_r is not as pronounced. It is interesting to recall that the carrier-bound efflux of glycine rises proportionately with A_r, *i.e.*, that the carrier-bound efflux coefficient h is independent of A_r over a wide range of values[9]. Hence, the carrier-bound efflux, being the reverse reaction

of Eqn. 4, is determined by A_r. This seems to indicate that under such conditions X_r is fairly constant, even at higher substrate concentrations, which is in agreement with the comments above.

To obtain W, according to Eqn. 13, ΔF_X has to be eliminated by extrapolating the experimental values to $A_l = A_r = 0$. Since both fluxes, $\vec{J}$ and $\overleftarrow{J}$, are required, Eqn. 1 of the paper concerned[9], has to be rewritten. We shall assume that the X influx through the carrier, $\vec{J}$, may be represented by a Michaelis-Menten relationship and that the outflux through the carrier, $\overleftarrow{J}$, is linear with A_r. Thus, if A_r and A_l represent the intracellular and extracellular concentrations of glycine, respectively, corresponding to a_c and a_f in the previous paper; K_L is the "leakage coefficient" (diffusion across cell membrane) determined to be about 0.2 ml/g·min; K_m is a figure analogous to the Michaelis constant; $\vec{J}_{max}$ is the maximum carrier flux; and h is the coefficient of the carrier-bound efflux, found to be independent of A_l over a wide range of values of A_r and to be about 0.8 ml/g·min[10], then in pseudo-equilibrium the above assumptions lead to

$$K_L(A_r - A_l) = \frac{\vec{J}_{max} A_l}{K_m + A_l} - hA_r. \tag{15}$$

As A_l approaches zero, $\vec{J}$ will approach the limiting value $(\vec{J}_{max}/K_m)\,A_l$ and $\overleftarrow{J}$ will be $h\,A_r$. Inserting these values into Eqn. 13, and using the fact that ΔF_X vanishes as A_l and A_r approach zero, we find that

$$W = \mathrm{RT} \ln \frac{\vec{J}_{max}}{K_m\, h}. \tag{16}$$

In order to find $\vec{J}_{max}/K_m$, Eqn. 15 may be rearranged to yield

$$\frac{5\,A_l}{5\,A_r - A_l} = \frac{A_l}{\vec{J}_{max}} + \frac{K_m}{\vec{J}_{max}}. \tag{17}$$

We obtain a straight line by plotting $5A_l/(5A_r - A_l)$ versus A_l with the data derived from Fig. 3 of ref. 9 (Fig. 1). The intercept yields a value of $\vec{J}_{max}/K_m = 16.8$ ml/g·min and we find from the slope that $\vec{J}_{max}$ has a value of 73.7 μmoles/g·min. As a check on the method, a slightly different approach may be used. Letting $\vec{J}$ equal the first term on the right side of Eqn. 15, we have, after elementary rearrangement,

$$\frac{A_l}{\vec{J}} = \frac{A_l}{\vec{J}_{max}} + \frac{K_m}{\vec{J}_{max}}. \tag{18}$$

We obtain a straight line by plotting $A_l/\vec{J}$ versus A_l with the data from Table I, (Fig. 2). The intercept of this line with the ordinate yields a value of $\vec{J}_{max}/K_m$ of 17.6 ml/g·min while the slope yields a value for $\vec{J}_{max}$ of 92.6 μmoles/g·min. The two approaches yield results which agree as well as could be expected. Taking $\vec{J}_{max}/K_m$

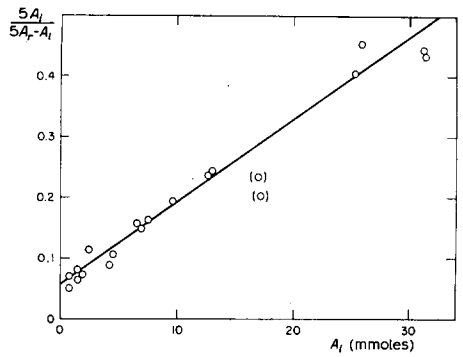

Fig. 1. Steady state distribution of glycine between intracellular fluid and medium at various extracellular concentrations, plotted according to Eqn. 17. The values are derived from those presented in Fig. 3 of a previous publication[9]. From the intercept of the regression line which has been obtained by the method of least squares, $K_m/\vec{J}_{max}$ can be computed to be about 16.8 ml/g·min.

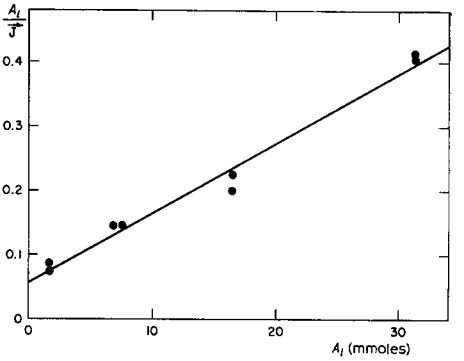

Fig. 2. Relationship between extracellular glycine concentration (A_l) and influx ($\vec{J}$) during the steady state at various levels of A_l plotted according to Eqn. 18. The values are derived from those presented in Table I of this paper and originally published in a previous paper[9]. From the intercept of the regression line, which has been obtained by the method of least squares, $K_m/\vec{J}_{max}$ can be computed to be about 17.6 ml/g·min.

to be 17.2 ml/g·min and h to be 0.8 ml/g·min, and inserting these values into Eqn. 16, we find that $W = 1{,}890$ cal/mole. In order to see how W varies as the above parameters are changed, we may consider some plausible extremum values. For $\vec{J}_{max}/K_m = 16$ ml/g·min and $h = 0.9$ ml/g·min, $W = 1{,}768$ cal/mole, while for $\vec{J}_{max}/K_m = 18$ ml/g·min and $h = 0.7$ ml/g·min, $W = 2{,}000$ cal/mole. Thus $W = 1{,}890 \pm 120$ cal/mole.

As stated before, we have assumed that the A can only cross the membrane in combination with the carrier via a single pathway. Thus, the A cannot also cross the membrane in conjunction with X via a second pathway which is not coupled with the energy yielding reactions. Though this latter possibility cannot be completely excluded by the experimental data, it may be considered to be a comparatively small effect if it exists at all. We have also assumed above that the influx is given by a Michaelis-Menten reaction and is independent of the intracellular concentration. Actually, the latter assumption is false as can be seen from Fig. 3 of ref. 10. However, since the fit of Eqns. 17 and 19 to the data is fairly good, since we are considering small values of A_l, and since in the limit the effect of the intracellular concentration on $\vec{J}$ should be negligible, this fact may not be too important. However, further experiments are needed. If the above effect of the intracellular concentration is non-negligible, then the values of $\vec{J}$ are larger than they would be for $A_r = 0$. Thus, the value of $\vec{J}_{max}/K_m$ would then be smaller than was actually found from Figs. 1 and 2, and hence the value of W would also be smaller.

The value of $W = 1{,}890$ cal/mole represents the free energy of reaction 6, which has been considered constant and independent of the transport rate. This figure gives the first reasonable estimate so far obtained for any real transport system on the

basis of experimental data. Some general conclusions as to the special features of the transport mechanism may now be drawn.

Evidence has been reported previously that energy-rich phosphates are most likely essential for the energy supply in this transport system[12]. This has also been postulated for almost every other system. The magnitude of W, however, seems to rule out that such phosphates interact directly with the transport mechanism. In other words, reaction 6 can hardly be considered as representing the exergonic splitting of ATP or related substances, because in that case W should be of the order of 7,000 to 8,000 cal/mole. It must rather be postulated that the coupling between energy-rich phosphates and the transport mechanism is through one or more energy transferring intermediates. The last of these intermediates would be among the S's of Eqn. 7, where energy-rich phosphates do not appear in this equation. In this connection the question arises as to the stoichiometric ratio of this coupling, *i.e.*, whether one or more glycine molecules are transported per phosphate bond.

The analogy to other reactions coupled with ATP splitting, such as the hexokinase reaction, or the activation of amino acids would suggest a one to one ratio. This would mean, however, that more than 5 kcal were dissipated with each reaction between ATP and S, *i.e.*, before the energy is transferred to the transport mechanism proper. If, on the other hand more than one glycine molecule is transported per phosphate bond, in analogy to the ratio assumed for the Na-transport across the frog skin, two possibilities may be conceived. First, the coupling ratio between phosphate and carrier is one, but more than one glycine molecule can be transported by each carrier. It is difficult to imagine a carrier of this kind in terms of organic chemistry. Also, it should be remembered that the influx and outflux data fit the kinetics of first order reactions. The other possibility would be that the total free energy liberated from the splitting of ATP be proportioned to more than one distinct reaction of lower energy. For this purpose the liberation of inorganic P would have to occur in several steps, each of which is coupled to an endergonic reaction such as activation of P_i to S_i in eqn. 6.

Such a mechanism would be analogous to that of the respiratory chain, where the transfer of one pair of electrons is step-wise coupled to the formation of several energy-rich phosphate bonds. No such step-wise coupling of the splitting of a phosphate bond is hitherto known for any biochemical system. It is therefore highly speculative to postulate this kind of mechanism for active transport. Since the maximum rate of glycine transport in Ehrlich cells is low enough to be compatible with a coupling ratio of one to one, it cannot be decided at the present time whether, and in which way, one or more glycine molecules are transported per phosphate bond.

Another possibility is that the transport of glycine is driven by the potassium ion gradient across the membrane[13,14]. If this were true, W should be equal to the osmotic work value of the potassium ion distribution. Assuming that the concentration of internal potassium is 134 mM, the concentration of external potassium is 4 mM, and that the electric potential across the membrane[15] is -15 mV, the value of the osmotic work is then 1810 cal/mole which is in excellent agreement with the value of 1890 ± 120 cal/mole found above.

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EFFECT OF SOME CONTRACTURE-PRODUCING AGENTS ON GLYCEROL-EXTRACTED MUSCLE FIBER RELAXED WITH RELAXING FACTOR

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SUMMARY

1. Caffeine, Ca ion, carnosine, DNP, nicotine and cyanide produced contraction of the fiber relaxed with the crude extract. Acetylcholine, choline, histamine and fluoride had no influence on the relaxed fiber.

2. In the absence of the relaxing factor, caffeine and carnosine had no marked influence on the tension development of the fiber in concentrations ranging from 2 m*M* to 10 m*M*. DNP lowered the tension development to about 50 % of the control value, but cyanide increased it to about 150 % at 10 m*M*. Nicotine increased it to 130 % at 5 m*M* and lowered it slightly at 10 m*M*.

3. These agents had no influence on the fiber relaxed with EDTA except Ca ion and nicotine.

4. Caffeine, DNP and carnosine did not contract the fibers relaxed with crude extract which were preincubated with ATP and Mg. However, Ca ion, nicotine and cyanide constantly produced contraction of the relaxed fibers.

5. On the basis of these findings, the production of relaxing substance by the granules in the presence of ATP and Mg and the significance of the granules in the excitation–contraction coupling were suggested.