ENERGY EXPENDITURE BY

ACTIVE TRANSPORT MECHANISMS

II. FURTHER GENERALIZATIONS

CLIFFORD S. PATLAK

From the Section on Theoretical Statistics and Mathematics, National Institute of Mental Health, National Institutes of Health, Bethesda

ABSTRACT   A method which was derived in a previous paper for calculating the energy expended in an active transport system has been generalized to (1) linked transport system, (2) multiple pathways, and (3) electrically non-neutral particles. The results are then applied to data from sheep erythrocytes and from yeast cells.
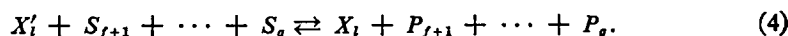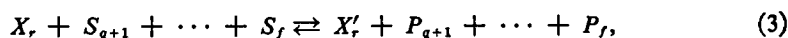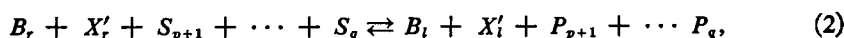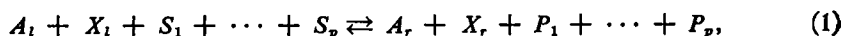
INTRODUCTION

In a previous paper (1), hereinafter referred to as I, a method for calculating a lower limit for the energy expenditure of an active transport system, by means of the concentration ratio and the unidirectional fluxes, was derived. For this derivation, a carrier model for the active transport was assumed to operate within a membrane which separated two homogeneous and ideally mixed fluid compartments on the left (l) and on the right (r). A single electrically neutral particle, A, was actively transported from compartment l to r via a single pathway with the help of an electrically neutral carrier, X, which was able to combine with A to form a complex. This complex was able to move across the membrane to interface r either by diffusion, rotation, rearrangement of the membrane, or by some other process. At interface r it either exchanged its A with other A in the compartment or unloaded its A. Then, through a series of further reactions and/or diffusion, the carrier returned, also via a single pathway, to interface l. It was further assumed that all the reactions involving A or the complex proceeded via unitary reactions which obeyed mass action kinetics and that all the constituents involved in the reactions were electrically neutral.

It is the purpose of this paper to see how the results of I are modified when three of the assumptions of I are either altered or eliminated to allow (1) a linked transport system, (2) multiple pathways, and (3) electrically non-neutral particles.

DOUBLE PUMP

The first assumption in I to be altered is that only a single species of particle is transported by the pump. This will be modified to consider the case of a double

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pump; *i.e.*, an active transport mechanism in which one molecule of species B is actively transported across the membrane for each molecule of species A that is transported in the opposite direction. We shall assume that this linked transport is accomplished *via* a single carrier system. (The type of pump discussed in I, in which only a single species of particle is transported by the active transport mechanism will be called a single pump.) Using the same assumptions and notation as in the previous paper, the reactions involved in this transport are as follows: the A will go through a series of reactions as listed in equations 1 through 3 in I; the B will also go through a completely analogous set of reactions except for the interchange of the subscripts l and r and the replacement of the symbol X by X', where X' represents the carrier in a possible different form; and finally, the carrier itself may proceed through a series of reactions which transform the X' to X on the left side and the X to X' on the right side. If S and P represent substrate and product respectively, the reactions for each of these four transformations may then be written as

$$A_l + X_l + S_1 + \cdots + S_p \rightleftarrows A_r + X_r + P_1 + \cdots + P_p, \tag{1}$$

$$B_r + X'_r + S_{p+1} + \cdots + S_q \rightleftarrows B_l + X'_l + P_{p+1} + \cdots P_q, \tag{2}$$

$$X_r + S_{q+1} + \cdots + S_f \rightleftarrows X'_r + P_{q+1} + \cdots + P_f, \tag{3}$$

$$X'_l + S_{f+1} + \cdots + S_g \rightleftarrows X_l + P_{f+1} + \cdots + P_g. \tag{4}$$

Thus, the over-all reaction for the transport of one mole of A from left to right and one mole of B from right to left is

$$A_l + B_r + S_1 + \cdots + S_g \rightleftarrows A_r + B_l + P_1 + \cdots + P_g. \tag{5}$$
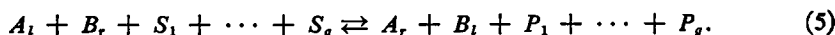
Let ΔF_A, ΔF_B, ΔF_X, and $\Delta F_{X'}$ represent the free energy change of equations 1, 2, 3, and 4 respectively. For the transport of one mole of A and B in the steady state, the change of free energy of the system equals the energy yielded by the system minus the energy put into the system. Thus, if W is the energy put into the system, then

$$\Delta F_T = \Delta F_A + \Delta F_B + \Delta F_X + \Delta F_{X'} = RT\left(\ln \frac{A_r}{A_l} + \ln \frac{B_l}{B_r}\right) - W, \tag{6}$$

where R is the gas constant, T is the absolute temperature, and $\ln$ is the natural logarithm.

As in I, we shall assume that the reactions yielding equations 1 and 2 proceed *via* unitary reactions which obey mass action kinetics. Then, by an argument completely analogous to that in I we have

$$\Delta F_A = RT \ln \frac{\overleftarrow{J_A}}{\overrightarrow{J_A}},$$

$$\Delta F_B = RT \ln \frac{\overleftarrow{J_B}}{\overrightarrow{J_B}}, \tag{7}$$

where the J's are the unidirectional fluxes through the pump. Since only experimental situations in which there is a net active transport of A from left to right are considered, then, if J represents the net active transport, $(J = \vec{J} - \overleftarrow{J})$,

$$J_A \geq 0, \qquad J_B \leq 0, \qquad \Delta F_X \leq 0, \quad \text{and} \quad \Delta F_{X'} \leq 0.$$

Therefore,

$$W \geq RT\left(\ln \frac{A_r}{A_l} + \ln \frac{B_l}{B_r} + \ln \frac{\vec{J}_A}{\overleftarrow{J}_A} + \ln \frac{\overleftarrow{J}_B}{\vec{J}_B}\right) \geq RT\left(\ln \frac{A_r}{A_l} + \ln \frac{B_l}{B_r}\right). \quad (8)$$

Equation 8 is the counterpart of equation 14 of I.

The above equation may be rewritten in the following manner. Define the unidirectional rate constants to be

$$\vec{k}_A = \frac{\vec{J}_A}{A_l} \qquad \overleftarrow{k}_A = \frac{\overleftarrow{J}_A}{A_r}$$

$$\vec{k}_B = \frac{\vec{J}_B}{B_l} \qquad \overleftarrow{k}_B = \frac{\overleftarrow{J}_B}{B_r}. \tag{9}$$

Equation 8 then reduces to

$$W \geq RT\left(\ln \frac{\vec{k}_A}{\overleftarrow{k}_A} + \ln \frac{\overleftarrow{k}_B}{\vec{k}_B}\right). \tag{10}$$

It must be emphasized that the k's are not constant but are functions of the external concentrations of both the A and B.

Implicit in the above derivation is the assumption that the A is freed from the carrier before the B can join the carrier and *vice versa*. This, of course, need not be so. Both the A and B may join the carrier to form an unstable complex which dissociates into a free B, plus a complex of A and carrier, or else a free A, plus a complex of B and carrier. If we modify our definitions of ΔF_A and ΔF_B so that they now represent the free energy change of the A and B reactions, including those portions involving the other molecular species, then equation 7 is still valid; but equation 6 is no longer correct since the complex formation reactions are common to the reactions involved in the ΔF_A and those in the ΔF_B. Thus, if ΔF_c is the free energy change of the reactions common to both A and B, and ΔF_X is the free energy change of the reactions of equations 3 or 4, then

$$\Delta F_T = \Delta F_A + \Delta F_B + \Delta F_X - \Delta F_c, \tag{11}$$

where ΔF_X is included, since this type of complexing might occur on only one side of the membrane. If we consider the same experimental situation discussed previously, in which there is a net transport of A from left to right, then $\Delta F_c \leq 0$, and thus equation 8 may not be valid for this case. However, since both $(\Delta F_A - \Delta F_c)$ and $(\Delta F_B - \Delta F_c)$ are non-positive, equation 8 may be replaced by the weaker state-

ment that

$$W \geq RT\left(\ln \frac{A_r}{A_l} + \ln \frac{B_l}{B_r} + \ln \frac{\vec{J}_A}{\overleftarrow{J}_A}\right) \geq RT\left(\ln \frac{A_r}{A_l} + \ln \frac{B_l}{B_r}\right),$$

$$W \geq RT\left(\ln \frac{A_r}{A_l} + \ln \frac{B_l}{B_r} + \ln \frac{\vec{J}_B}{\overleftarrow{J}_B}\right) \geq RT\left(\ln \frac{A_r}{A_l} + \ln \frac{B_l}{B_r}\right). \tag{12}$$

MULTIPLE PATHWAYS

The second assumption in I to be modified is that there is only one pathway by which the actively transported particle is actively transported across the membrane, and there is only one other pathway for the carrier to recross the membane. This assumption will be modified so that the actively transported particle and the carrier may cross the membrane *via* a number of different pathways which may have reactions in common with each other. For example, the actively transported particle may cross the membrane with the carrier *via* a number of energetic reactions; it may also cross with the same carrier but omit some or all of these reactions and perhaps have other reactions; it may cross with a different carrier and a different set of chemical reactions; or it might simply diffuse across. What can be measured *via* unidirectional tracer fluxes will usually be a composite of these separate unidirectional flows.

We shall first consider a single pump and will define the ith pathway to be the ith series of reactions that A and X undergo in order that A cross the membrane and X go through a complete cycle. The total number of pathways will be equal to the total number of possible ways that these series of reactions occur. Let $\vec{J}_{i_A}$ and $\overleftarrow{J}_{i_A}$ represent the unidirectional flux of A through the ith pathway, from left to right and from right to left, respectively. If $\vec{J}_A$ and $\overleftarrow{J}_A$ represent the net unidirectional flux through all these pathways from left to right and from right to left, respectively, then

$$\vec{J}_A = \sum_i \vec{J}_{i_A},$$

$$\overleftarrow{J}_A = \sum_i \overleftarrow{J}_{i_A}. \tag{13}$$

As stated above, only the $\vec{J}_A$ and $\overleftarrow{J}_A$ can be measured, and not the individual $\vec{J}_{i_A}$'s and $\overleftarrow{J}_{i_A}$'s. If the unidirectional fluxes for some of the pathways (such as the diffusion flux in the movement of glycine across the EMAT[1] cell membrane) can be individually measured, then these pathways will not be included in the summation in equation 13.

Define W_{app} as

$$W_{app} = RT \ln \frac{\vec{J}_A}{\overleftarrow{J}_A} + RT \ln \frac{A_r}{A_l}, \tag{14}$$

[1] Ehrlich mouse ascites tumor.

and consider the pathway, $i = m$, for which $\vec{J}_{m_A} / \bar{J}_{m_A}$ is the maximum, that is

$$\frac{\vec{J}_{m_A}}{\bar{J}_{m_A}} \geq \frac{\vec{J}_{i_A}}{\bar{J}_{i_A}}, \qquad (\text{all } i). \tag{15}$$

It is a well known algebraic theorem (2) that, given equations 13 and 15,

$$\frac{\vec{J}_A}{\bar{J}_A} \leq \frac{\vec{J}_{m_A}}{\bar{J}_{m_A}}. \tag{16}$$

Thus, if W_m is the energy input into the system for the mth pathway, then from equations 14, 16, and equation 14 of I,

$$W_{app} = RT \ln \frac{A_r}{A_l} + RT \ln \frac{\vec{J}_A}{\bar{J}_A} \leq RT \ln \frac{A_r}{A_l} + RT \ln \frac{\vec{J}_{m_A}}{\bar{J}_{m_A}} \leq W_m. \tag{17}$$

If W_{max} represents the maximum W_i, the $W_m \leq W_{max}$. Therefore, from equation 17 we find that

$$W_{app} = RT \ln \frac{A_r}{A_l} + RT \ln \frac{\vec{J}_A}{\bar{J}_A} \leq W_{max}. \tag{18}$$

In an actual system, there probably will be only one pathway which has energy supplied to it, and thus W_{app} will yield a lower limit for this energy input.

The derivation of the equation for the double pump when multiple pathways are allowed is similar to that used for the single pump. Assume that the carriers only combine with A or B separately and do not complex with both at the same time. Define the ith pathway to be the ith series of reactions that A, B, and X undergo for there to be a net exchange of A and B and for the carrier to go through a complete cycle. The number of pathways will be equal to the total number of possible ways for these to occur. Define $\vec{J}_{i_A}$, $\bar{J}_{i_B}$, etc. in a similar manner as before, where all the J's are positive. Thus,

$$\vec{J}_A = \sum_i \vec{J}_{i_A} \qquad \bar{J}_A = \sum_i \bar{J}_{i_A}$$
$$\vec{J}_B = \sum_i \vec{J}_{i_B} \qquad \bar{J}_B = \sum_i \bar{J}_{i_B}. \tag{19}$$

From the definition of the double pump and the term "pathway," it follows that in the steady state the net flux of A through any pathway is equal to the net flux of B in the opposite direction. Therefore,

$$\vec{J}_{i_A} - \bar{J}_{i_A} = \bar{J}_{i_B} - \vec{J}_{i_B}. \tag{20}$$

In analogy to the single pump analysis define W_{app} as

$$W_{app} = RT \ln \frac{A_r B_l}{A_l B_r} + RT \ln \frac{\vec{J}_A \bar{J}_B}{\bar{J}_A \vec{J}_B}. \tag{21}$$

If we now consider the pathway, $i = m$, for which

$$(\vec{J}_{m_A} \bar{J}_{m_B} / \bar{J}_{m_A} \vec{J}_{m_B})$$

is the maximum, that is

$$\frac{\vec{J}_{m_A}\vec{J}_{m_B}}{\overleftarrow{J}_{m_A}\overleftarrow{J}_{m_B}} \geq \frac{\vec{J}_{i_A}\vec{J}_{i_B}}{\overleftarrow{J}_{i_A}\overleftarrow{J}_{i_B}} \qquad \text{(all } i\text{)}, \tag{22}$$

then it may be shown (3) that when $J_A \geq 0$, equations 19, 20, and 22 yield

$$\frac{\vec{J}_{m_A}\vec{J}_{m_B}}{\overleftarrow{J}_{m_A}\overleftarrow{J}_{m_B}} \geq \frac{\vec{J}_A\vec{J}_B}{\overleftarrow{J}_A\overleftarrow{J}_B}. \tag{23}$$

Thus, if W_m is the energy put into the system in order to exchange one mole of A for one mole of B *via* the mth pathway, then from equations 8, 21, and 23 we have that

$$W_{\text{app}} = RT \ln \frac{A_r B_l}{A_l B_r} + RT \ln \frac{\vec{J}_A\vec{J}_B}{\overleftarrow{J}_A\overleftarrow{J}_B} \leq RT \ln \frac{A_r B_l}{A_l B_r} + RT \ln \frac{\vec{J}_{m_A}\vec{J}_{m_B}}{\overleftarrow{J}_{m_A}\overleftarrow{J}_{m_B}} \leq W_m. \tag{24}$$

If, as before, W_{max} represents the maximum W_i, then $W_m \leq W_{\text{max}}$. Therefore, equation 24 implies that

$$W_{\text{app}} = RT \ln \frac{A_r B_l}{A_l B_r} + RT \ln \frac{\vec{J}_A\vec{J}_B}{\overleftarrow{J}_A\overleftarrow{J}_B} \leq W_{\text{max}}. \tag{25}$$

Assume now that at least one of the pathways involves a complexing of the carrier with both A and B. Then by a completely similar approach to that used in the derivation of equation 18, we have that

$$W_{\text{app}_A} = RT \ln \frac{A_r B_l}{A_l B_r} + RT \ln \frac{\vec{J}_A}{\overleftarrow{J}_A} \leq W_{\text{max}},$$

$$W_{\text{app}_B} = RT \ln \frac{A_r B_l}{A_l B_r} + RT \ln \frac{\vec{J}_B}{\overleftarrow{J}_B} \leq W_{\text{max}}, \tag{26}$$

where W_{app_A} and W_{app_B} are defined by this equation.

Since only pathways in which there is a net exchange of one mole of A for one mole of B are considered, all pathways for A and B, in which their movement is not coupled, such as in a single pump, simple diffusion, or exchange diffusion, are not included in this derivation. However, under the conditions we are considering, $J_{i_A} < 0$ and $J_{i_B} > 0$ for simple diffusion while $J_{i_A} = J_{i_B} = 0$ for exchange diffusion. Thus, it is apparent that the inequality of equations 16 and 23 would not be altered if pathways for simple and exchange diffusion were included. Hence, equations 25 and 26 are applicable under the condition that simple and exchange diffusion pathways are also included in the unidirectional flux measurement.

In an experimental system involving neutral molecules in which the detailed mechanism of the active transport system is not known, it obviously cannot be ascertained whether or not there is more than one energetic pathway, or if both transported species in a double pump simultaneously complex with the carrier. Therefore, only equation 18 is applicable for these cases of active transport systems of

the single pump type, and the greater of the two inequalities in equation 26 is applicable for active transport systems of the double pump type.

CHARGED PARTICLES

The third assumption in I to be modified here is that the actively transported particles are electrically neutral. This assumption will be altered so that the actively transported particles may be charged. We shall first consider the single pump. Assume that the actively transported particle is completely ionized, with a valence of z. This problem, however, can be treated at present only with the addition of one further assumption. This is, assume that the reactions involving A are not influenced by the external electric field. The meaning and consequence of this assumption will now be discussed.

For the assumption of the non-interaction of the electric field and A to be valid for the series of reactions leading to A complexing with the carrier on the left and right sides of the membrane (equations 1 and 3 of I), it must be assumed that the A does not diffuse from one position in space to another. Thus, each one of these reactions would occur at a constant electric potential and is therefore unaffected by the electric field. Further, for this assumption to be valid, the complex between the carrier and A must be electrically neutral. Therefore, the carrier must have an opposite charge to that of A. Lastly, this assumption implies that the reaction of A joining onto or leaving the carrier is uninfluenced by the external field. If the carrier and the actively transported particle join together from random directions, this is obviously true. But, if the reaction is an oriented one, then the electric field may play a role. That is, due to the external electric field, there may be a potential gradient across the 1 or 2 A that the transported particle traverses to combine with the carrier between the start and the finish of their complexing reaction. This potential difference will probably be negligible compared to the total potential drop across the membrane, but for the assumption of non-interaction to be valid, it will be arbitrarily assumed that this potential difference is zero. However, it should be emphasized that this is only a plausible assumption with no concrete evidence that it is true.

The energy put into the pump minus the work derived from the transport is equal to the negative of the free energy change of the reactions involved in the transport. Let F be the Faraday constant and V be the electric potential difference between the solution on the right side and that on the left side of the membrane. Then,

$$\Delta F_T = \Delta F_A + \Delta F_X = W - \left(RT \ln \frac{A_r}{A_l} + zFV\right). \tag{27}$$

Since, by assumption, the reactions involving A are independent of V, equation 7 is still valid. Thus, under the same conditions as discussed in I,

$$W \geq RT \ln \frac{A_r}{A_l} + RT \ln \frac{\vec{J}_A}{\overleftarrow{J}_A} + zFV. \tag{28}$$

Except for the inequality sign, equation 28 is the same as found by Zerahn (4). Since equation 28 was derived under the assumption of the non-interaction of A and V no statement can be made as to the validity of this equation if this assumption is not true.

The analysis is completely analogous for the double pump. The assumption of the non-interaction of V with both A and B will be made as before. Since one molecule of A is transported for each molecule of B, we shall assume that $z_A = z_B$. Then, the derivation of equations 8 and 12 of this paper will be valid for this case, and therefore equations 8 and 12 still apply. Note that the value of V does not enter explicitly into the resultant inequality as it did in equation 28.

From the form of equation 28, and since equations 8 and 12 are still applicable, it is obvious that the conclusions of the section on multiple pathways are valid for the non-neutral cases discussed here.

APPLICATION

Some of the above results will now be applied to two biological systems: the $Na^+ - K^+$ exchange in red blood cells and the $K^+ - H^+$ exchange in yeast cells. These two systems have been chosen since they are virtually the only ones for which sufficient data have been gathered to allow a meaningful application of the above results.

Tosteson and Hoffman (5) have shown that there is a one to one exchange of K^+ for Na^+ *via* the active transport mechanism in the sheep erythrocyte. Only the high K^+ sheep erythrocyte data will be considered since they yield a better estimate of W, under the assumption that W is the same for both the high K^+ and the low K^+ sheep red cell. For the high K^+ sheep erythrocyte, the values of the concentrations are: external $Na^+ = 139$ mM, internal $Na^+ = 36.8$ mM, external $K^+ = 4.91$ mM, and internal $K^+ = 121$ mM. From an analysis of Tables III and IV of their article, we see that within experimental error the ratio of Na^+ pumped out to Na^+ pumped into the cell is at least 6:1 and similarly for the ratio of K^+ pumped in to K^+ pumped out of the cell. Inserting these values into equation 26, with $T = 310°K$, we find that

$$W \geq 615.8\left(\ln \frac{139}{36.8} + \ln \frac{121}{4.91} + \ln \frac{6}{1}\right) = 3895 \text{ cal/mole.}$$

In yeast cells, there appears to be a double pump for the active transport of K^+ and H^+ (6). Some values of external H^+ and K^+ for which there is no net movement of K^+ into the cells, when the internal values are $K^+ \geq 0.15$ M, $H^+ = 10^{-6.2}$ M are (7,8): $K^+ = 5 \times 10^{-5}$ M, $H^+ = 10^{-4.5}$ M; $K^+ = 5 \times 10^{-4}$ M, $H^+ = 10^{-3.5}$ M; $K^+ = 10^{-2}$ M, and $H^+ = 10^{-2.0}$ M. Inserting these values into equations 25 or 26 (which are identical for $J_K^+ = J_H^+ = 0$), we find that all three sets of values yield $W \geq 7100$ cal/mole.

The energy available from splitting ATP into ADP plus orthophosphate is of the order of 7000 cal/mole (9), the exact value depending upon the pH and the concentration of ATP and ADP. Thus, the results for both of the above active transport mechanisms are consistent with the thesis that the pump is driven by the breakdown of ATP. This is in agreement with the findings of Hoffman (10) that ATP is the immediate substrate of the pump in the red cell.

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