LETTER TO THE EDITOR

[Brief letters to the Editor that make specific scientific reference to papers published previously in THE JOURNAL OF GENERAL PHYSIOLOGY are invited. Receipt of such letters will be acknowledged, and those containing pertinent scientific comments and scientific criticisms will be published.]

On the Reversibility of the Sodium Pump in Dialyzed Squid Axons

A Method for Determining the Free Energy of ATP Breakdown?

Dear Sir:

In an elegant series of experiments Brinley and Mullins (1967, 1968) have examined the active and passive fluxes of sodium ions in segments of internally dialyzed squid axons. These authors claim that their technique allows ion fluxes to be studied in preparations having relatively normal permeability properties. It is therefore interesting to note that there is a significant portion of the Na^+ influx which depends upon the presence of internal ATP (Brinley and Mullins, 1968). Evidence is presented which makes it highly unlikely that this component of Na^+ influx is due to a coupled Na^+:Na^+-exchange diffusion. The authors conclude that this component of Na^+ influx can be "formally described as a changed Na^+ permeability," the change being induced by the depletion of internal ATP to 1 μM. The authors clearly recognize that "these observations underline the difficulty in discriminating between pumped and passive ion movements against an electrochemical gradient," and that "the presently accepted theoretical framework is inadequate to explain the observations properly since the changes observed are undoubtedly closely connected with Na pumping."

It is evident that our definitions of Na^+ permeability on the one hand, or the Na^+ pump on the other, are greatly dependent on the pharmacological techniques used to resolve the components of tracer-determined ion fluxes. From the work of Brinley and Mullins (1968) it would appear that strophanthidin induces a constant rate of Na^+ efflux regardless of the presence of ATP (above 1 μM), whereas it has little or no effect on Na^+ influx (Mullins and Brinley, 1969). These effects, together with the effects of small uncontrolled alterations of membrane potential on the K^+ fluxes (cf. Mullins and Brinley, 1969), make the interpretation of tracer fluxes a very difficult undertaking.

However, there is one possible explanation of the effect of ATP depletion on the Na^+ influx which has not been suggested in relation to dialyzed axons and which is

worth considering, not because it is especially favored by the evidence available, but because it leads to some conclusions of general interest to people working in various aspects of bioenergetics.

It is possible that the ATP-dependent component of Na^+ influx in dialyzed axons is due to the reverse running of the Na^+-K^+ pump and that the observed component of Na^+ influx is coupled to a corresponding K^+ efflux. The available evidence does not preclude this possibility because of the difficulty in evaluating K^+ flux data when membrane potential is not controlled (Mullins and Brinley, 1969). Hence the sodium-potassium pump could be represented by a reversible chemical reaction of fixed stoichiometry (cf. Thomas, 1972).

$$ATP + 3Na_I^+ + K_O^+ \rightleftharpoons ADP + P_i + 3Na_O^+ + K_I^+. \quad (1)$$

When such a system is in equilibrium then the rates of the forward and back reactions are equal and the net free energy change is zero; that is, all of the free energy of ATP breakdown (including side-buffering reactions) is equal to the free energy conferred onto the Na and K ions in translocating them through their respective electrochemical fields. When the system is not in equilibrium, then the net free energy change is given by the amount of free energy of ATP breakdown dissipated as heat in order to produce a net forward reaction. The amount of free energy dissipated in this way is given in the first approximation by

$$\Delta G = -RT \ln \frac{V_f}{V_b}, \quad (2)$$

where V_f and V_b are the velocities of the forward and back reactions, respectively. The amount of free energy conferred on the Na^+ and K^+ ions per mole of ATP is given by

$$\Delta G = 3F(E_{Na} + E_M) + F(E_K - E_M), \quad (3)$$

where F = Farady
E_M = membrane potential
E_K = potassium diffusion potential
E_{Na} = sodium diffusion potential
3 is the stoichiometric ration of Na^+/K^+ pumping for reaction 1.

This ratio is variable but has been assigned a value of 3 for the present argument in order to achieve consistency between the values of E_{Na} and E_K chosen (see below), and the flux ratios described by Mullins and Brinley (1969).

Hence if the forward and back reaction rates are known, together with the membrane and ionic diffusion potentials, the free energy of ATP breakdown in the dialyzed squid axon is given by

$$\Delta G_{ATP} = 3F(E_M + E_{Na}) + F(E_K - E_M) - RT \ln \frac{V_f}{V_b}. \quad (4)$$

This equation does not allow for the free energy dissipation intrinsic to the ATPase reaction itself. However, as this unknown quantity of free energy would never be

available for use in ATP-driven reactions, it may be ignored when considering the effective molar free energy available from ATP in intact cells. If the ratio V_f/V_b exceeds 10 then a significant departure from Eq. 4 may be expected because of effects due to the complex nature of the reaction mechanism which would result in reaction rates different from those predicted by the law of mass action. However for smaller values of the ratio V_f/V_b, the system is sufficiently close to equilibrium for Eq. 4 to be essentially independent of the reaction mechanism.

From the work of Brinley and Mullins (1968) it is possible to define an ATP-dependent Na^+ efflux of 47 pmol/cm^2 s, whereas the ATP-dependent Na^+ influx has a value of 28 pmol/cm^2 s. The values of $E_{Na} + E_M$ and $E_K - E_M$ will be taken as -100 mV and -30 mV, respectively (Brinley and Mullins, 1968; Mullins and Brinley, 1969). Substituting these values into Eq. 4 at 17°C gives

$$\Delta G_{ATP} = -7.6 \text{ kcal/mol} - RT \ln \frac{V_f}{V_b}$$

$$= -7.9 \text{ kcal/mol.}$$

This value is smaller in magnitude than those calculated by Caldwell (1968) on the basis of biochemical analysis.

It can be seen from the small contribution of the $RT \ln (V_f/V_b)$ term to the free energy (i.e. the relative dissipation of free energy) that the system described by reaction 1 is very close to thermodynamic equilibrium, given that the removal of ATP provides a satisfactory pharmacological definition of the Na^+-K^+ pump. It may be noted that Eq. 4 is independent of the *absolute* reaction rates which would depend in practice on the activity of the membrane ATPase. So long as the ATPase activity is sufficient to maintain an ionic steady state against the passive ion fluxes then Eq. 4, which depends only on the *ratio* of the reaction rates, will be valid. (The fact that isolated axons are never in a steady state does not significantly affect the arguments presented here.)

It is obvious that if one can arrive at a satisfactory pharmacological definition of the Na^+-K^+ pump, then one can arrive at a very exact figure (biologically speaking) for the effective free energy of ATP breakdown in situ. This value, determined for the Na^+-K^+ pump, must hold elsewhere throughout the cell, provided that the high energy phosphate potential of the cell is buffered via an appropriate phosphoryl transferase system. Thus one would be forced to conclude that the value of ΔG_{ATP} in vivo could not exceed -10 kcal/mol. Any higher value would require V_f/V_b in Eq. 4 to exceed 63:1; i.e., Na^+ influx due to the pump would be undetectable. The only alternative conclusion would be that it is impossible at present to reach a pharmacological definition of active transport that comes anywhere near to approximating the true situation.

From Eq. 4 it follows that, if the pump is not electrically neutral, an induced shift of membrane potential should affect the ratio V_f/V_b independently of any effect on passive ion movements. If the considerable problem of artifacts could be overcome this would lead to a fairly sensitive test of the stoichiometric coefficients of reaction 1. In experiments where ions are removed or drastically altered in concentration, the

possible catalytic effects of these ions should be allowed for in any interpretation of changes in rate as discussed recently by Beaugé and Ortíz (1971) and Chapman (1972).

In cases where the Na^+-K^+ pump is defined pharmacologically by the use of cardiac glycosides the situation is more difficult to interpret. As mentioned above, the results of Brinley and Mullins (1968) show that, while strophanthidin induces a constant rate of Na^+ efflux regardless of the presence of ATP, the ATP- and Na^+-dependent Na^+ influx does not appear to be sensitive to cardiac glycosides: i.e., Na^+ influx is apparently unaltered by the presence of strophanthidin. However, the apparent insensitivity of the Na^+ influx to strophanthidin may be due to two mutually canceling effects: there may be a genuine permeability increase for Na^+ induced by strophanthidin which masks the inhibitory action of the pump. In this connection it would be interesting to discover whether strophanthidin restores the Na^+ influx to normal levels in ATP-depleted axons.

In conclusion it may be noted that, although the explanations presented in this letter are purely speculative, the principle that active transport mechanisms can function reversibly under appropriate conditions has already been established in other situations, the most notable of which are in red blood cells (Garrahan and Glynn, 1967) and in isolated sarcoplasmic vesicles (Makinose, 1971).

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