LETTER TO THE EDITOR

The Reversal Potential
for an Electrogenic Sodium Pump

A Method for Determining the Free Energy of ATP Breakdown?

Dear Sir:

In an earlier communication (Chapman, 1973) it was suggested that the free energy available from ATP breakdown might be estimated in the dialyzed squid axon (Brinley and Mullins, 1967) by measuring isotopically the opposing rates of the reversible sodium pump reaction. The free energy dissipated by the reaction was held to be given by
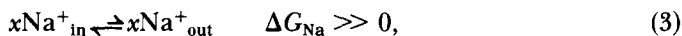
$$\Delta G_{dis} = -RT \ln(V_f/V_b), \tag{1}$$

where V_f and V_b are the velocities of the forward and back reactions respectively, and R and T have their usual meanings. As ΔG_{dis} corresponds to the amount of free energy available from ATP not conserved as free energy in the transported ions, knowledge of the transmembrane ionic free energy differences would allow calculation of the total free energy available from ATP.

Two shortcomings of this method are that (a) two simultaneous isotopic estimations of V_f and V_b for the sodium pump are required and (b) that Eq. 1 holds only if the rate-limiting steps of the transport ATPase reaction occur on a one-to-one molar basis per mole of overall reaction (Boudart, 1976). While this may very well be the case, one can avoid this unproven assumption and the difficulties of measuring simultaneous isotopic flux differences by making use of the current-carrying property of the electrogenic sodium pump.
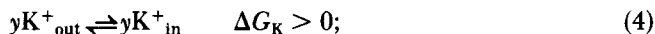
Although the concept of an electrogenic sodium pump has been widely accepted in a variety of tissues for many years now (Thomas, 1972 a), it is only recently that serious attempts have been made to conceive and measure the contribution that an electrogenic pump could make to the total membrane current-voltage relationship (Marmor, 1971; Isenberg and Trautwein, 1974; 1975). While most evidence in hand seems to suggest that the electrogenic pump operates independently of the electrical gradient over a wide range of membrane potentials, this result would not appear to be intuitively obvious.

The electrogenic sodium pump can be defined macroscopically in terms of three basic partial reactions:
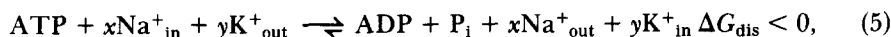
$$ATP \rightleftharpoons ADP + P_i \qquad \Delta G_{ATP} \lll 0, \tag{2}$$

$$x\mathrm{Na^+_{in}} \rightleftharpoons x\mathrm{Na^+_{out}} \qquad \Delta G_{\mathrm{Na}} \gg 0, \tag{3}$$

and

$$y\mathrm{K^+_{out}} \rightleftharpoons y\mathrm{K^+_{in}} \qquad \Delta G_{\mathrm{K}} > 0; \tag{4}$$

where ATP, ADP, and P_i refer to adenosine triphosphate, adenosine diphosphate, and inorganic phosphate, respectively, and $\mathrm{Na^+_{in}}$, $\mathrm{Na^+_{out}}$, $\mathrm{K^+_{in}}$, and $\mathrm{K^+_{out}}$ refer to the intra- and extracellular species of sodium and potassium ions, respectively. The ΔG's are the free energy changes involved in each partial reaction, these being negative for the spontaneous driving reaction (the splitting of ATP) and positive for the driven reactions (active extrusion of $\mathrm{Na^+}$ and active uptake of $\mathrm{K^+}$). The stoichiometric coefficients, x and y, in Reactions 3 and 4 will depend on interpretations of experimental results and may vary between preparations and conditions of measurement, but a widely (though not universally) accepted pump ratio for ATP:Na:K of 1:3:2 is thought to obtain for normal operation of some Na,K-ATPases found in cell membranes (Thomas, 1972 a).

The net pump reaction is a tightly coupled combination of the above three partial reactions:

$$\mathrm{ATP} + x\mathrm{Na^+_{in}} + y\mathrm{K^+_{out}} \rightleftharpoons \mathrm{ADP} + P_i + x\mathrm{Na^+_{out}} + y\mathrm{K^+_{in}} \quad \Delta G_{\mathrm{dis}} < 0, \tag{5}$$

where ΔG_{dis} is that part of the free energy liberated from the splitting of ATP which is not conserved in the electrochemical work of ion transport. This quantity is negative for a net spontaneous forward reaction and represents the free energy dissipated in producing an appreciable net reaction.

Any pump mechanism described by Reaction 5 is electrogenic in that net charge is passed across the cell membrane during the flux of chemical reaction, provided the stoichiometric coefficients, x and y, are unequal. As a consequence, the pump reaction contributes to membrane current and thereby membrane potential. Conversely, because membrane potential contributes to the total work done in moving ions across the membrane, it must influence the rate of the transporting reaction.

Without specifying any details as to the mechanism whereby ions and molecules interact with the ATPase enzyme, and without making any assumptions as to the nature of the electric field within the membrane, it is possible to draw up a free energy balance sheet for reactions 2 to 5:

$$\Delta G_{\mathrm{ATP}} = A, \tag{6}$$

$$\Delta G_{\mathrm{Na}} = xRT\ln\{[\mathrm{Na^+}]_{\mathrm{out}}/[\mathrm{Na^+}]_{\mathrm{in}}\} + xF \cdot E_m, \tag{7}$$

$$\Delta G_{\mathrm{K}} = yRT\ln\{[\mathrm{K^+}]_{\mathrm{in}}/[\mathrm{K^+}]_{\mathrm{out}}\} - yF \cdot E_m, \tag{8}$$

$$\Delta G_{\mathrm{dis}} = \Delta G_{\mathrm{ATP}} + \Delta G_{\mathrm{Na}} + \Delta G_{\mathrm{K}}$$

$$= A + xRT\ln\{[\mathrm{Na^+}]_{\mathrm{out}}/[\mathrm{Na^+}]_{\mathrm{in}}\} + yRT\ln\{[\mathrm{K^+}]_{\mathrm{in}}/[\mathrm{K^+}]_{\mathrm{out}}\} \tag{9}$$

$$+ \ ^{\mp}x - y - FE_m;$$

where R, T, and F have their usual meanings, and $[\mathrm{Na^+}]_{\mathrm{out}}$, etc. refer to

extracellular sodium ion concentration, etc. (activity coefficients will cancel and so will be ignored for present purposes), E_m is the membrane potential (*outside* with respect to *inside*).

According to the Second Law of Thermodynamics, Reaction 5 will proceed with a net forward rate for all negative values of ΔG_{dis} given by Eq. 9. When ΔG_{dis} is equal to zero there will be no net chemical change as Reaction 5 is in equilibrium. When ΔG_{dis} is positive, Reaction 5 will proceed with a net backward rate, i.e., ATP will be synthesized.

For an electrogenic pump with $x > y$, the same thermodynamic reasoning can be applied to the pump current. For $\Delta G_{dis} < 0$, there will be net outward current coupled to ATP breakdown; for $\Delta G_{dis} = 0$, there will be no net current and no chemical reaction; for $\Delta G_{dis} > 0$, there will be net inward current coupled to synthesis of ATP.

If an experiment were to be performed in which the activities of all the transported species in Reaction 5 were held constant while the membrane potential was varied then a current-voltage curve would be obtained showing a reversal potential, E_r, at which ΔG_{dis} of Eq. 9 would be equal to zero.[1] The reversal potential would thus be given by

$$E_r = \frac{RT}{(x-y)F} \left\{ x\ln\frac{[Na^+]_{in}}{[Na^+]_{out}} + y\ln\frac{[K^+]_{out}}{[K^+]_{in}} - A/RT \right\},$$ (10)

from which A, the free energy of ATP breakdown could be determined.

In practice, of course, electrogenic transport reactions take place in parallel with other ionic conductance pathways in intact membranes, and so the true current-voltage relationship of the electrogenic sodium pump could only be inferred by subtraction from the total membrane properties after pharmacological intervention.

In most cases where experiments of this sort have been reported in the literature, the estimated sodium pump current has shown little or no dependence on membrane potential (Marmor, 1971; Isenberg and Trautwein, 1974; Lambert et al., 1975; Thomas, 1972 *b*; see also Hodgkin and Keynes, 1955 and Brinley and Mullins, 1974 for related isotopic flux data).[2] These findings are not in conflict with the thermodynamic statements above relating the sign of ΔG_{dis} to the direction of the spontaneous reaction. The magnitude of the pump current will depend not only on the thermodynamic potential, including the membrane potential, but also on the kinetic properties of the transporting enzyme concerning which thermodynamics makes no statement. Nevertheless, reversal potentials for outwardly directed sodium pump currents are a thermodynamic necessity, and their absence from the literature is open to a number of

[1] In practice the pump is usually a subsystem within a more complex membrane system containing passive ionic conductances, although experimental measurement of E_r would require the input of external energy into the total membrane system which would not, of course, be in thermodynamic equilibrium. However, at E_r, the pump subsystem is at thermodynamic equilibrium.

[2] Contrary findings have been described by Kostyuk et al. (1972) and Kononenko and Kostyuk (1975), but these authors do not seem to have made a clear distinction between passive and active transport currents.

interpretations. Firstly, in mollusk neurones or cardiac muscle the detection of reversal potentials may require stronger hyperpolarizations than are commonly used in steady-state voltage clamp studies. However, some of the data published by Isenberg and Trautwein (1974; 1975) are suggestive of a possible reversal potential around 100 mV in Purkinje fiber strands.

Secondly, as the concept of a contribution to the membrane current-voltage relationship by the electrogenic sodium pump has not been explored experimentally in squid axons, there is no evidence relating to the estimation of the corresponding reversal potential. The evidence from isotope fluxes (Hodgkin and Keynes, 1955; Brinley and Mullins, 1974) does not relate directly to the net pump flux at the reversal potential which would be the zero resultant of opposing rates of reaction at a dynamic equilibrium. However, the apparent voltage-independence of the forward rate of the pump reaction (Na^+ efflux) suggests from Eq. 1 that there might be an exponential dependence of the reverse reaction rate on membrane potential.

Nevertheless, there are some indications that reversal potentials for the electrogenic sodium pump may exist fairly close to the normal resting potential in squid axon. If one accepts the validity of Eq. 1 applied to isotopic flux differences, a value for A of -7.9 kcal/mol can be calculated (Chapman, 1973) from the data of Brinley and Mullins (1968) and Mullins and Brinley (1969), assuming a pump stoichiometry of $x = 3$, $y = 1$. In this case, the reversal potential is 6.5 mV hyperpolarized relative to the resting potential. For a stoichiometry of $x = 3$, $y = 2$, the corresponding reversal potential is approximately 13 mV hyperpolarized relative to the resting potential. All these calculations are made on the assumption that the pump fluxes are properly defined by removal of ATP (Chapman, 1973; Brinley and Mullins, 1968).

Thus, the hyperpolarizations necessary to discover reversal potentials for the electrogenic sodium pump in squid axons may be well within the limits of feasibility in a preparation which, under the influence of a multiplicity of pharmacological, biochemical, and electrophysiological interventions, has disclosed so much fundamental knowledge concerning membrane permeability, electrical excitation, gating currents, and the active transport of ions. The dialyzed squid axon preparation (Brinley and Mullins, 1967) may offer unique possibilities for demonstrating the principle of the reversal potential and for studying the stoichiometric coefficients of Reaction 5 with different values of A applied by buffering the ATP:ADP ratio at various desired levels.

In the event that the normal reversal potential exceeds the limits of feasible hyperpolarization in the squid axon, then the principle of the reversal potential should still be demonstrable by dialyzing the high energy phosphate potential to an appropriately low level following the example set by Garrahan and Glynn (1967) in their original demonstration of pump reversal in red cells. If the predicted effects cannot be demonstrated at all, then a major rethinking of the mechanism and stoichiometry of the sodium pump may be required.

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