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Letters to the Editor

Cation Pump vs. Nernst Potential

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In a recent article, M. K. Jain *et al.* (M. K. Jain, F. P. White, A. Strickholm, E. Williams & E. H. Cordes, 1972. *J. Membrane Biol.* **8**:363) interpret their results as circumstantial evidence for reconstitution of an active cation pump across an artificial membrane. As the authors themselves point out, however, a high local proton concentration could develop as a result of ATP hydrolysis, producing a transmembrane electrochemical potential difference which could generate the observed short-circuit current in the absence of active transport. The purpose of this letter is to point out that a deeper analysis of their data strongly suggests that a transmembrane gradient is in fact responsible for the observed short-circuit current.

The effect of ouabain on the steady-state current-voltage relation (Fig. 7) is to lower the slope and shift the voltage intercept from a negative value toward zero. This strongly suggests a selective reduction in the conductance of an ionic species which has a negative Nernst potential. The accumulation of protons at the site of ATP hydrolysis would indeed produce a negative Nernst potential. Moreover, the fact that ouabain is most rapidly effective on the side opposite to the site of ATP hydrolysis is consistent with a model in which protons enter the membrane at the site of hydrolysis and are then prevented from leaving the opposite side by a ouabain-induced change in the cation exchange properties of the surface. The alternative interpretation of Fig. 7 in terms of active transport would require the net active transport current to vary linearly with voltage, and to reverse at voltages below -7 mV. Both of these requirements seem highly unlikely.

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Prior to publication we had given careful consideration to the hypothesis proposed by Dr. Kornacker (and several others —*personal communication*). However, this view is considered untenable because of the following observations:

(1) Development of short-circuit current or open-circuit potential is not observed when an acid such as hydrochloric acid is added to one of the compartments at concentrations 10 to 20 times that which could possibly be produced by hydrolysis of ATP under our experimental conditions. The possibility that most of the ATPase would be localized in the vicinity of the BLM was considered highly unlikely.

(2) Changing the buffer concentrations has little or no effect on generation of electrical asymmetry.

(3) Of various Na^+-K^+-ATPases tested for the "pump activity", only one preparation (rat brain synaptosomal fraction) was found to be active. Other ATPases with comparable specific activity had little or no effect; some of these inactive preparations dropped BLM resistance.

We believe that these observations argue very strongly against the "proton-leak" hypothesis. It is, however, conceivable (though very unlikely) that the observed electrical asymmetry arises from a membrane localized "proton-pump" which is ouabain-sensitive. Active transport current which develops open-circuit potential of the order of 2 to 10 mV is not very uncommon. In fact as reviewed by Thomas (*Physiol. Rev.*, **53:**563 [1972]), such small electronegativity is fairly common in nerve and muscles; large values observed in frog skin or toad bladder may be exceptions rather than the rule.

Consistently small values of OCP obtained in reconstituted BLM has led us to suggest that relatively large membrane fragments rather than only the membrane-bound ATPase is incorporated into the BLM.

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