Letters to the Editor

Comments on "The Role of Electrogenic Pump in *Chara corallina*"

In a recent paper of this title, Kishimoto, Kami-ike and Takeuchi (1980) have interpreted their measurements of membrane $PD(\psi_m)$ and conductance (g_m) in terms of the emf (ψ_p) and conductance (g_p) of the (proton) pump in the plasma membrane. They give plots of ψ_p against time for 60 min exposures of cells to 200 μM dinitrophenol (DNP) and to 2 μM triphenyltin (TPC). Both plots offer two interesting features (*see also* Kishimoto, 1980):

(i) that at long times ψ_p asymptotically approaches ψ_d, the PD of the diffusive pathways in parallel with the pump, and

(ii) that at shorter times (20–40 min) ψ_p is much more negative (-300 to -400 mV) than before exposure to inhibitor (about -250 mV).

If these interpretations could be sustained, (i) might be an interesting coincidence, while (ii) might be an exciting indication of the change in pump stoichiometry suggested by Warnke and Slayman (1980). It is here concluded that both are artefacts produced by assumptions made in interpreting the data.

To avoid the difficulty that their model has four unknown parameters while only two were measured, Kishimoto et al. assume:

(a) that g_d and ψ_d remain constant during the 60 min of exposure to 2 μM TPC, and

(b) that such exposure reduces g_p to exactly zero.

The second assumption leads directly to the result that at long times $\psi_m = \psi_d$ and $g_m = g_d$; then algebraic equations for ψ_p and g_p can be solved for any other time at which ψ_m and g_m are known.

Neither (a) nor (b) is well founded in other knowledge. P.T. Smith (*see* Walker, 1980; Smith & Walker, 1981) has shown with perfused *Chara* that g_{K+}, a large component of g_d, increases as ψ_m becomes more positive, and there are many experiments indicating that this also happens in intact *Chara*. Since g_d represents a number of parallel pathways, a change of g_{K+} will in general change both g_d and ψ_d. The constancy of g_d and ψ_d is not assured by the showing that the action potential occurs after TPC exposure.

The tri-substituted organic tins affect mitochondria in two ways at low concentrations: they alter the matrix pH and cause energy-linked Cl^- uptake, by catalysing Cl^-/OH^- antiport, and they inhibit oxidative phosphorylation by an unknown mechanism; triphenyltin shows both actions at around 1 μM (Aldridge, Street & Skilleter, 1977). Both effects would lead to the fall in cytoplasmic ATP concentration, which Kishimoto et al. mention; and this fall would be expected to affect the proton pump, reducing both ψ_p and g_p (e.g., *see* Spanswick, 1980). There is no need to postulate a direct effect on the plasma membrane proton pump, and there is no direct evidence of such an effect. There is no reason expect that g_p falls exactly to zero, whatever the mechanism of inhibition. Incidentally TPC would seem likely to alter the pH of the cytoplasm by catalysing Cl^-/OH^- antiport in the plasma membrane: this would in general alter both p and d pathways.

If we relax somewhat assumptions (a) and (b). we find that many different time-courses for ψ_p can be produced from the data. Those shown in Fig. 1a–c show that the asymptotic approach of ψ_p to ψ_d is an artefact of assumption (b). Small differences between the assumed values of ψ_d and g_d, and the values of ψ_m and g_m at long
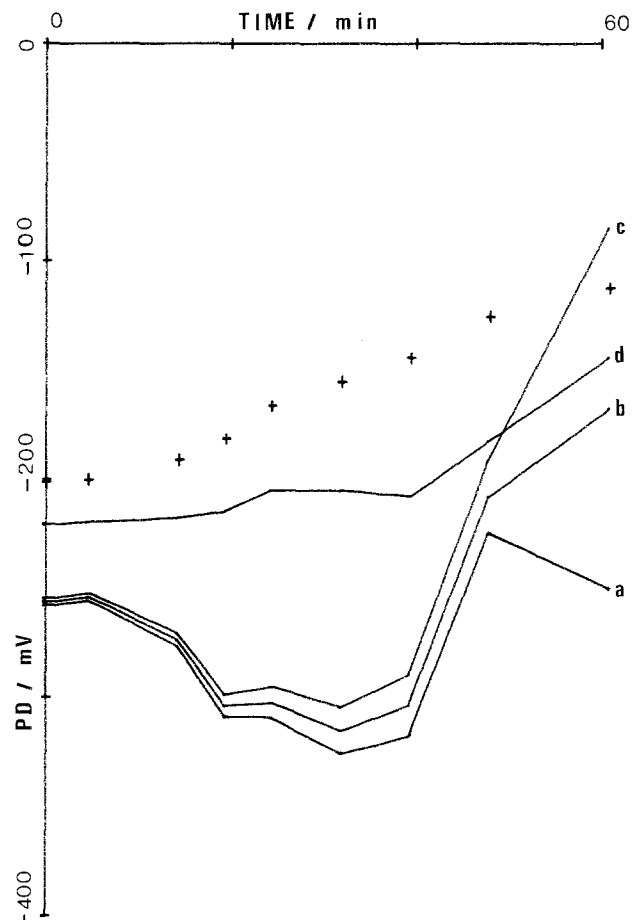

Fig. 1. Time course of membrane PD ($+$) replotted from Kishimoto et al. (1980), and time courses of ψ_p calculated using the parallel membrane model with (a): g_d, 0.55 S/m^2; ψ_d, -107 mV; (b): g_d, 0.55 S/m^2, ψ_d, -110 mV; (c): g_d, 0.55 S/m^2; ψ_d, -113 mV; (d): g_d given by Eq. (1) with: g_1, 0.4 S/m^2; g_2, 0.3 S/m^2; ψ_o, -150 mV; ψ_d, -110 mV

times, alter the late values of ψ_p in a very marked way. Similarly if we allow g_d to vary with ψ_m, the high negative values of ψ_p are no longer seen (Fig. 1(d)). Here the change in g_d is modelled by the equation:

$$g_d = g_1 + g_2 \tanh [Z(\psi_m - \psi_o)] \tag{1}$$

where g_1 and g_2 are constants, Z is F/RT, and ψ_o is the PD at which the change in g_d is half complete. This equation is discussed elsewhere; here it is used merely to model in a plausible way a rise of g_d when the cell is depolarized.

Figure 1 offers interpretations of the data which are at least as plausible as, but very different from, that of Kishimoto et al. It can be concluded that the original data will bear many different interpretations in terms of time courses of ψ_p and g_p, and that we need more information about ψ_d and g_d, together with inhibitors more specific than those currently used.

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References

Aldridge, W.N., Street, B.W., Skilleter, D.N. 1977. Halide-dependent and halide-independent effects of triorganotin and triorga-nolead compounds on mitochondrial functions. *Biochem. J.* **168**:353

Kishimoto, U. 1980. *Discussion in:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors, p. 364. Elsevier/North Holland, Amsterdam

Kishimoto, U., Kami-ike, N. Takeuchi, Y. 1980. The Role of Electrogenic Pump in *Chara corallina*. *J. Membrane Biol.* **55**:149

Smith, P.T., Walker, N.A. 1981. Studies on the perfused plasma-lemma of *Chara corallina*. I. Current-voltage curves: pH and potassium dependence. *J. Membrane Biol. (in press)*

Spanswick, R.M. 1980. Biophysical control of electrogenicity in the Characeae. Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editor. pp. 305–16. Elsevier/North Holland, Amsterdam

Walker, N.A. 1980. The transport systems of charophyte and chlorophyte giant algae and their integration into modes of behaviour. Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp. 287–304. Elsevier/North Holland, Amsterdam

Warnke, J., Slayman, C.L., 1981. Metabolic modulation of stoichiometry in a proton pump. *Biochim. Biophys. Acta* **591**:224