

## PROPERTIES OF THE CHLORIDE CONDUCTANCE ASSOCIATED WITH TEMPERATURE ACCLIMATION IN MUSCLE FIBRES OF GREEN SUNFISH

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### SUMMARY

Characteristics of the anion conductance of muscle fibres from green sunfish have been determined. The membrane conductance of fibres from sunfish acclimated to 25 and 7 °C was linearly related to the extracellular chloride concentration. The chloride conductance exhibited a pH dependence which was adequately described by the titration of an acidic site with a  $pK_a$  of 5.3 in 25 °C-acclimated fibres and 6.4 in 7 °C-acclimated fibres.

The anion current-voltage (I-V) relationship of warm-acclimated fibres exhibited constant-field rectification, while the I-V relationship of cold-acclimated fibres was linear. In Ringer solutions containing elevated calcium concentrations (33 and 115 mmol l<sup>-1</sup>), the I-V relationship of warm-acclimated fibres was similar to the control situation. However, the I-V relationship of cold-acclimated fibres showed a calcium concentration-dependent curvature in the direction expected for constant-field rectification. The voltage-dependence of the time constant of chloride current inactivation was shifted along the voltage axis by about 40 mV in the negative direction in 7 °C-acclimated fibres as compared to 25 °C-acclimated fibres. The results can be adequately described by a model of constant-field rectification with the inclusion of a term for the membrane surface potential. A simple hypothesis which can qualitatively account for many of these observations is that temperature acclimation alters the density of fixed negative surface charges in the vicinity of chloride channels.

### INTRODUCTION

The preceding paper (Klein & Prosser, 1985) reported the effects of temperature acclimation on the conductive properties of skeletal muscle fibres of green sunfish. We showed that the ratio of resting conductances to chloride and potassium ions,  $g_{Cl}/g_K$ , was approximately 6 in 25 °C-acclimated sunfish fibres, and that this ratio fell to about 1.3 upon acclimation to 7 °C. The reduction in membrane conductance was found to be due to a six-fold decrease in  $g_{Cl}$ .

The present paper examines the characteristics of the Cl conductance in warm- and cold-acclimated sunfish fibres to elucidate the mechanism of alterations in  $g_{Cl}$  which accompany thermal acclimation. The results support a hypothesis that can

Key words: Chloride conductance, surface charge, temperature acclimation.

account qualitatively for many of the results described. The hypothesis considers that a negative surface potential plays a role in modulating the Cl conductance, and that the magnitude of the surface potential is altered by temperature acclimation.

Excitable membranes in general possess negative surface charges which influence the potential field near the membrane, and thus affect a variety of voltage-dependent phenomena (see McLaughlin, 1977 for a review). The precise location and identity of these surface charges remains obscure, however, their influence can be experimentally regulated by altering the concentration of divalent cations and pH in the bathing medium. This observation has been quantitated by applying the theory of the Gouy-Chapman diffuse double layer, as developed by Grahame (1947), to relate the surface potential to the ionic composition of the bathing solution (e.g. Gilbert & Ehrenstein, 1969). The main conclusions from these studies are that the surface potential can alter the gating of ion channels, and modify the local ionic concentrations by changing the potential which is 'seen' by the channel at the membrane-solution interface (McLaughlin, Szabo & Elsenman, 1971; Hille, Woodhull & Shapiro, 1975). The present study will show that certain voltage- and concentration-dependent parameters of the chloride conductance in sunfish muscle are altered during temperature acclimation in a manner which is consistent with a change in the surface potential of the membrane.

#### METHODS

The laboratory maintenance of green sunfish, muscle bundle dissection, experimental apparatus and electrical recording arrangements were as described in the preceding paper (Klein & Prosser, 1985). The solutions used in this study (Table 1) were designed to eliminate cationic conductances across the membrane. To this end, K was replaced on an equimolar basis by Rb. Rb reduces and linearizes the inwardly rectifying conductance in frog muscle fibres (Adrian, 1964; Hutter & Warner, 1972). Solution A is the same as was used previously except for the substitution of Rb, and was used in the earlier experiments of this study.

In other experiments the pH of the Ringer solution B (Table 1) was varied over the range of 3.3–10.3 by the addition of appropriate amounts of MES (2-[N-morpholino] ethanesulphonic acid) and Bis-tris propane (1,3-bis[tris(hydroxymethyl)-methylamino]-propane) to a final concentration of 5 mmol l<sup>-1</sup>. Solution C was used to depolarize the fibres. The measured membrane currents in normal Ringer were generally small, and while this was not a hindrance to the determination of steady-state currents, it did introduce significant error to the measurement of kinetic parameters. Fibres were soaked in solution C for about an hour before being transferred to solution D, in which Rb replaced K. Under these conditions, the fibres should be loaded with Cl, to a concentration of approximately 80 mmol l<sup>-1</sup> (Hodgkin & Horowicz, 1959). These solutions had the same ionic strength as normal Ringer, however, sucrose was added as an osmotic buffer to maintain an approximate Donnan equilibrium (Vaughan, McLarnon & Loo, 1980). Solution E contained elevated Ca concentration at constant extracellular [Cl]<sub>o</sub>, while in solution F methanesulphonate replaced Cl.

Table 1. *Solutions*

Solution	Na	K	Rb	Ca	Cl	MeSO <sub>3</sub>	Sucrose
A	100	—	2.5	2	106	—	—
B	124	—	2.5	2	130	—	—
C	—	126.5	—	2	130	—	100
D	—	—	126.5	2	130	—	100
E	—	—	2.5	115	130	102	—
F	124	—	2.5	2	—	130	—

Concentrations in mmol l<sup>-1</sup>. MeSO<sub>3</sub> is methanesulphonate. In addition, all solutions contain buffer (A, 25 mmol l<sup>-1</sup> HCO<sub>3</sub>; B, 5 mmol l<sup>-1</sup> MES/bis-tris propane; C-F, 5 mmol l<sup>-1</sup> HEPES) and 1 mmol l<sup>-1</sup> MgSO<sub>4</sub>. pH 7.3, except solution B.

Membrane current-voltage (I-V) relationships were measured by the three-electrode voltage clamp method (Adrian, Chandler & Hodgkin, 1970). Three electrodes were inserted at distances  $l$ ,  $2l$  and  $2l+l'$  from the end of a fibre. The two electrodes nearest the end recorded the membrane potential while the third electrode was used to pass current. Voltage control was taken from the electrode closest to the end of the fibre. The membrane current density,  $I_m$ , is approximated by (Adrian *et al.* 1970):

$$I_m = \frac{d\Delta V}{6R_i l^2},$$

where  $d$  is fibre diameter,  $\Delta V$  is the difference voltage recorded between the two electrodes closest to the fibre end,  $R_i$  is the internal resistivity and  $l$  has been previously defined. In this study distance  $l$  was set to 375  $\mu\text{m}$ , which should result in an error in the method of less than 5 % (Adrian *et al.* 1970).  $l'$  ranged from 50 to 100  $\mu\text{m}$ , and values for  $R_i$  were taken from data obtained previously (Klein & Prosser, 1985).

## RESULTS

### *The concentration dependence of $g_{\text{Cl}}$*

To characterize the Cl conductance of sunfish fibres the dependence of  $g_{\text{Cl}}$  on the extracellular Cl concentration,  $[\text{Cl}]_o$ , was measured.  $g_{\text{Cl}}$  was estimated by two methods, (1) by measuring cable parameters of many fibres (Klein & Prosser, 1985) after a period of equilibration in solutions containing various chloride concentrations (obtained by mixing appropriate amounts of solutions A and F), and (2) by inserting current passing and potential recording electrodes into the middle of a fibre, within 50  $\mu\text{m}$  of each other. Rectangular, hyperpolarizing current pulses were passed from one electrode, and the resulting potential change was recorded while solutions of different  $[\text{Cl}]_o$  flowed through the chamber. The experiments were performed at the temperature of acclimation. Under these conditions, the input conductance,  $g_{\text{in}}$ , is equal to  $(r_m \times r_i)^{1/2}$ , where  $r_m$  and  $r_i$  are the membrane resistance and internal resistance per unit length of fibre, respectively. If it is assumed that  $r_i$  is not

significantly altered in solutions of different  $[Cl]_o$ , then the square of the ratio of input conductances at normal (control) and at a test value of  $[Cl]_o$  is (Hagiwara & Takahashi, 1974):

$$\left(\frac{g_{in,t}}{g_{in,c}}\right)^2 = \frac{r_{m,c}}{r_{m,t}},$$

where subscripts c and t refer to control and test conditions.

Membrane potential is significantly reduced in solutions of lowered Cl concentration (Klein & Prosser, 1985) especially in 25°C-fibres; the validity of the normalized conductance values determined from the equation above is subject to the constraint that  $r_m$  is voltage-independent. Current-voltage relations were linear for depolarizations below the threshold of contracture in Rb-containing solutions. Low Cl solutions which were expected to cause a contracture were added slowly to reduce fibre movement to a minimum. Despite difficulties due to contractures, the method was useful in comparing directly the effects of altered  $[Cl]_o$  on membrane conductance in a single fibre, while avoiding the interfibre variation routinely encountered in cable measurements.

Fig. 1 shows the results of measuring membrane conductance in solutions of different  $[Cl]_o$ . Membrane conductance is indicative of the magnitude of  $g_{Cl}$ ; however, this assumption is less satisfactory in 7°C-fibres since the relative contribution of  $g_{Cl}$  to the total membrane conductance is lower than for 25°C-fibres. In Rb-containing Ringer,  $g_{cation}$  is reduced to about  $35 \mu S cm^{-2}$  in 7°C-fibres so the membrane is at least 2.2-fold more permeable to Cl than to cations. Membrane conductance in both 25°C- and 7°C-fibres was linearly related to the Cl concentration for  $[Cl]_o$  between 35 and 145 mmol  $l^{-1}$ . At lower concentrations the relationship approached zero slope, indicating that cations dominated the membrane conductance. No consistent difference was noted between 25°C- and 7°C-fibres in the value of  $[Cl]_o$  at which the concentration-conductance relationship became non-linear.

#### *Effects of pH on membrane conductance*

Ringer solution B was used to examine the effects of altered pH on membrane conductances in sunfish fibres. Normalized conductance was determined by method (2) above, since the preparation did not remain viable in solutions of low or high pH for the extended periods required for cable analysis. Also, the effects of pH on 7°C-fibres were evaluated at a bath temperature of 15°C because changes in membrane conductance with pH were too small to be recorded reliably at 7°C. Raising the temperature increased the relative contribution of Cl to the total membrane conductance (see Fig. 3 of the preceding paper, Klein & Prosser, 1985). While the magnitudes of the conductance changes were temperature sensitive, the pH dependence was found to be virtually insensitive to temperature. The data in Fig. 2 were obtained in Cl-containing and Cl-free Ringer (solutions B and F).

The results shown in Fig. 2 indicate that the Cl conductance is sensitive to external pH in fibres of sunfish from both acclimation groups,  $g_{Cl}$  approaching zero at low pH. The K conductance hardly varies over the range of 4.3 to 10.3. These

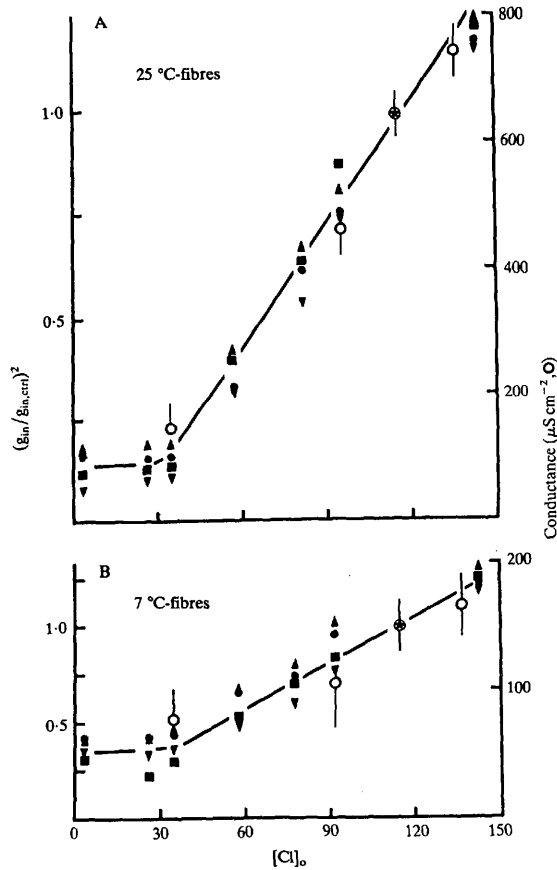


Fig. 1. The dependence of total membrane conductance on  $[Cl]_o$  in 25 °C- (A) and 7 °C-acclimated fibres (B). Solid symbols represent normalized membrane conductance from four different fibres, open symbol is the mean  $\pm$  S.E. of conductance values measured from cable properties in seven (A) and four (B) fibres. Temperature, 25 °C in A, 7 °C in B. Cl was replaced by methanesulphonate.  $[Cl]_o$  was increased to 145 mmol l<sup>-1</sup> by substituting 25 mmol l<sup>-1</sup> NaCl and 5 mmol l<sup>-1</sup> Tris-Cl for NaHCO<sub>3</sub> and NaH<sub>2</sub>PO<sub>4</sub>.

results are qualitatively similar to those reported in other vertebrate striated muscle preparations (Hutter & Warner, 1967; Hagiwara & Takahashi, 1974; Palade & Barchi, 1977). The membrane potential was not significantly altered by changes in

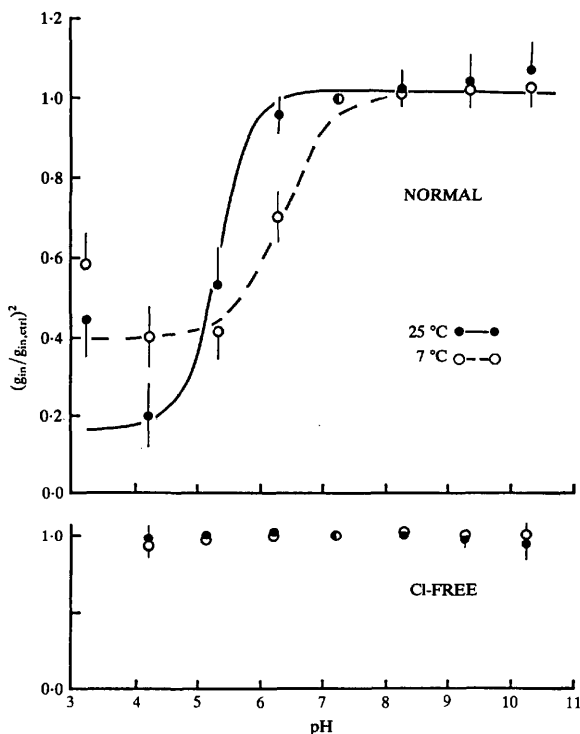


Fig. 2. The effect of pH on normalized membrane conductance. Upper, normal Ringer solution B. Points are means  $\pm$  s.e. for four ( $\bullet$ , 25°C) and three ( $\circ$ , 7°C) fibres. Curves represent the titration of a weak acid with  $pK_a$  of 5.3 (solid line) and 6.4 (dashed line), with the assumption that the Cl conductance comprises 88 % and 60 % of the total membrane conductance, respectively. They were fitted by eye. Data for 7°C-fibres were obtained at 15°C. Lower, Cl-free (methanesulphonate) solution. Same fibres as in above.

pH, indicating that the modulation of conductance by pH was independent of voltage. The increase in membrane conductance which occurred at pH 3.3 was probably due to an increase in  $g_{Cl}$  since it was not observed in Cl-free solutions (two fibres, in separate experiments from those shown in Fig. 2). The increase in conductance was completely reversed upon switching to a more alkaline solution. The data for  $g_{Cl}$  are reasonably described in 25°C-fibres by the titration of a weakly acidic group with a  $pK_a$  of 5.3, whereas the best-fit for 7°C-fibres is obtained for a group with a  $pK_a$  of 6.4. It should be noted that  $pK_a$  values obtained from binding isotherm data are independent of the concentration of Cl channel protein which binds hydrogen ions.

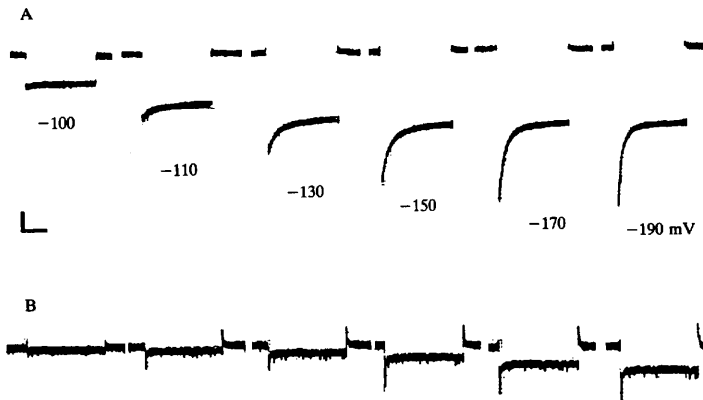


Fig. 3. Membrane currents from a 25°C-fibre in solution B (pH 7.3) (panel A) and a different fibre in solution F (panel B) voltage clamped to the potential indicated. Inward current is downward. Holding potential,  $V_h$ , -90 mV (A), -85 mV (B). Temperature, 14.3°C. Calibration: A,  $2 \mu\text{A cm}^{-2}$ , 50 ms; B,  $1 \mu\text{A cm}^{-2}$ , 50 ms.

#### Anion currents under voltage clamp

Membrane currents were examined under voltage clamp conditions. All of the experiments to be described were performed at an intermediate temperature of 15°C in order to compare directly currents from warm- and cold-acclimated sunfish.

Fig. 3A shows the current which flowed when the membrane potential of a 25°C-fibre was stepped from the holding potential,  $V_h$ , to several more hyperpolarized voltages. There was an instantaneous increase followed by a slower decay of current to a steady value. The decay was exponential, and its rate was voltage dependent (see below). These currents are qualitatively similar to those described by Warner (1972) in frog muscle. Fig. 3B shows currents recorded from a different fibre bathed in solution F. These records show little voltage or time dependence, so by inference we attribute the currents recorded in Fig. 3A to Cl ions.

Fig. 4A depicts the steady-state current-voltage (I-V) relationship of 25°C-fibres in solutions containing 130, 40 and 0 mmol  $\text{l}^{-1}$  Cl. In 130 mmol  $\text{l}^{-1}$  Cl solution the I-V relationship exhibits constant-field rectification in that the slope conductance decreased at increasingly larger hyperpolarized potentials. Reducing  $[\text{Cl}]_o$  by 70% resulted in a curve with a similar shape, although the relationship was less steep. The membrane current in 0-Cl solution F is assumed to be due to Rb, and it was linear over a wide range of membrane potentials. The mean Rb slope conductance of the fibres in Fig. 4A was about  $55 \mu\text{S cm}^{-2}$ . The constant Rb conductance has been subtracted from the anion relations plotted in the figure.

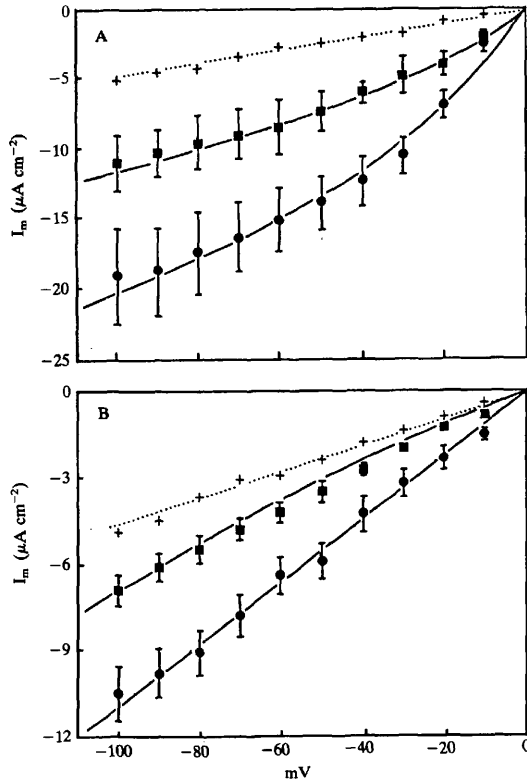


Fig. 4. Current-voltage (I-V) relations of sunfish fibres. Temperature, 15°C. The abscissa is the voltage displacement from the holding potential. (A) 25°C-fibres in normal (●), 40 mmol  $\text{l}^{-1}$  [Cl]<sub>o</sub> (■) and Cl-free (+) Ringer (solution F). The dashed line is taken as the Rb conductance of the membrane. The constant  $g_{\text{Rb}}$  has been subtracted from Cl I-V curves in this and subsequent figures. (●, ■) Mean  $\pm$  s.e. for eight fibres; (+) mean for two fibres.  $V_h$ , -90 mV (●), and -75 mV (■, +). (B) 7°C-fibres, same conditions as above for seven fibres,  $V_h$  -90 mV (●) and -80 mV (■), and mean for two fibres,  $V_h$  -80 mV (+).  $R$ , assumed to be 200  $\Omega$  cm, mean fibre diameter 60  $\mu\text{m}$ . Curves are calculated from equation 1 (see text) with values for  $P_{\text{Cl}}$  and  $V'$  as listed in Table 2.

The Cl conductance of frog fibres is well described by constant-field assumptions at neutral pH (Hodgkin & Horowicz, 1959; Hutter & Warner, 1972). Thus, the Cl conductance of warm-acclimated sunfish fibres shares many properties with those of frog.



However, Hutter & Warner (1972) showed that the agreement of  $g_{Cl}$  with constant-field theory at neutral pH was fortuitous since the membrane I-V relation did not conform to the predictions of the theory at high or low pH. Indeed, Fig. 4B shows that the I-V relationship of 7°C-fibres was linear over the entire range of membrane potentials and  $[Cl]_o$ . The  $g_{Rb}$  was approximately  $47 \mu S \text{ cm}^{-2}$ , so the Cl conductance was three-fold greater than the cation conductance at 15°C. These results indicate that the I-V relationships of 7°C-fibres are fundamentally different from those of 25°C-fibres.

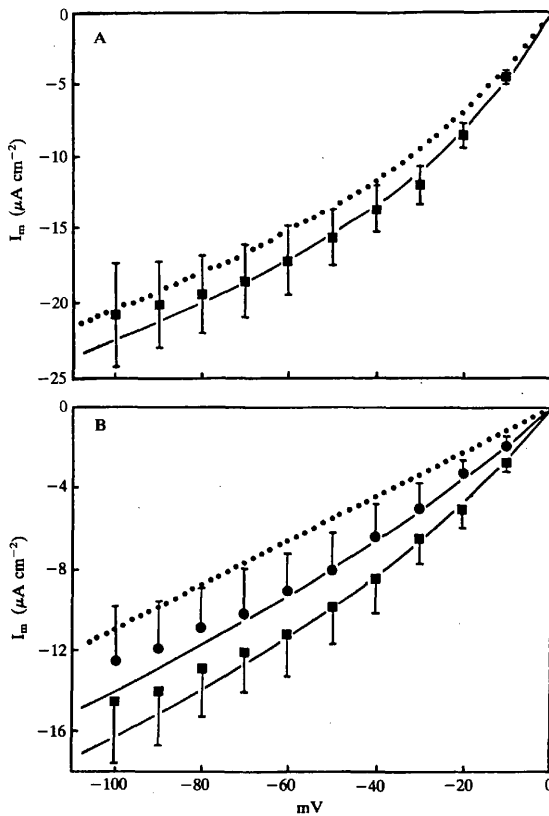


Fig. 5. I-V relationships of sunfish fibres in solutions of elevated  $[Ca]_o$ . Temperature, 15°C. The abscissa is the voltage displacement from the holding potential. (A) Mean  $\pm$  s.e. for six 25°C-fibres in  $115 \text{ mmol l}^{-1}$   $[Ca]$  Ringer (solution E). Dotted line is the same as the lowermost curve in Fig. 4A. (B) Data for six 7°C-fibres in  $33$  (●) and  $115 \text{ mmol l}^{-1}$  (■)  $[Ca]$  Ringer. Dotted line is the same as the lowermost curve in Fig. 4B.  $V_h$ ,  $-90 \text{ mV}$ ;  $R_i$ ,  $230 \Omega \text{ cm}$ ; mean fibre diameter  $55 \mu\text{m}$ . Curves are calculated from the model (see text) with parameters for  $P_{Cl}$  and  $V'$  given in Table 2.

*I-V relationships in elevated [Ca] solutions*

Fixed surface charges can impose a potential on a constant-field membrane (Frankenhaeuser, 1960; Hutter & Warner, 1972). To determine the possible influence of surface charges, I-V relationships were obtained from 25 °C- and 7 °C-fibres bathed in solutions of elevated [Ca]. Two solutions were used, one consisting of solution E, the other made by mixing solutions A and E in the ratio

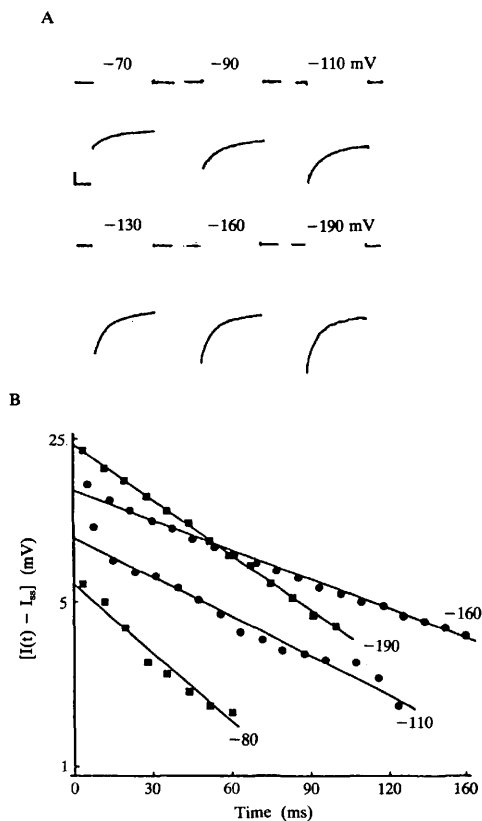


Fig. 6. (A) Membrane currents during hyperpolarizing voltage steps to the potentials indicated. 7 °C-fibre; temperature, 14.7 °C; solution D;  $V_{hh}$ , -20 mV. Calibration  $5 \mu A cm^{-2}$ , 60 ms. (B) Semi-logarithmic plots of the currents in A after subtraction of the steady-state current,  $I_{ss}$ . The lines are least-squares fits to the data. Current is given as  $\Delta V$  in mV.

2.5:1. These correspond to Ca concentrations of approximately 115 and 33 mmol l<sup>-1</sup>, respectively, but the [Cl]<sub>o</sub> of each solution was constant at 130 mmol l<sup>-1</sup>. These solutions were hypertonic to Ringer solution A. However, control experiments with 300 mmol l<sup>-1</sup> sucrose added to solution A gave results identical to those obtained in the absence of sucrose, after correction for the change in R<sub>i</sub> due to osmotic shrinkage.

Fig. 5A shows the effects of elevated Ca solution E in a 25 °C-fibres. The degree of rectification in the I-V relationship was increased slightly from the control situation. The I-V relationship in 33 mmol l<sup>-1</sup> Ca solution was virtually identical to the control, and is therefore not included in the figure.

For 7 °C-fibres in normal Ringer, the I-V relationship was linear, but in elevated Ca solutions the I-V curve exhibited a small degree of rectification in the direction expected for a constant-field membrane (Fig. 5B). The degree of rectification was increased with an increase in [Ca]<sub>o</sub>. The shape of the I-V curves in Fig. 5B was not statistically different from linearity. However, there was a consistent trend toward curvilinearity as the membrane potential was increased. At membrane potentials more negative than -160 mV the relationship appeared to exhibit a limiting slope conductance (Hutter & Warner, 1972).

The curves in Figs 4 and 5 represent theoretical I-V relationships calculated from constant-field theory, with the inclusion of a term for surface potential. In the model to be discussed below, the surface potential is the only adjustable parameter.

#### *Kinetic parameters of the chloride current*

As shown in Fig. 4, membrane currents in normal Ringer were small. To increase the magnitude of the current crossing the membrane, the fibres were loaded with Cl by the procedure outlined in the Methods. Fig. 6A shows currents recorded from a 7 °C-fibre bathed in solution D. The holding potential was -20 mV. The current decayed from an instantaneously large value to a smaller steady-state value, indicating that a portion of the Cl current inactivated with time. When the current is plotted on semi-logarithmic coordinates as the current at time *t*, *I*(*t*), minus the steady-state current, *I*<sub>ss</sub> (Fig. 6B), the relationship is linear with a time constant which is dependent on the membrane potential. The situation is somewhat analogous to the Hodgkin & Huxley (1952) parameter for Na channel inactivation, *h*, and the same terminology will be used here to describe the apparent inactivation of the chloride current. Fig. 7A plots the voltage dependence of the time constant of inactivation, *τ*<sub>h</sub>, for 25 °C-fibres, while Fig. 7B gives the corresponding relation for 7 °C-fibres. Both curves are bell-shaped, as is characteristic of the time dependent Hodgkin-Huxley gating parameters which underlie the action potential, but is in contrast to the *τ*<sub>h</sub> relationship of frog chloride current, which is a monotonic function of voltage (Warner, 1972). The voltage dependence of *τ*<sub>h</sub> in 7 °C-fibres was shifted by approximately -40 mV along the potential axis, and the time constants were roughly 1.3-fold smaller as compared to 25 °C-fibres.

To determine the voltage dependence of the steady-state inactivation parameter, *h*<sub>∞</sub>, the membrane was given a conditioning prepulse to a variable voltage, *V*<sub>c</sub>, followed by a step to a constant test voltage *V*<sub>t</sub>. The value plotted is the ratio of the initial current recorded after a step to *V*<sub>t</sub> with a prepulse to the same current without

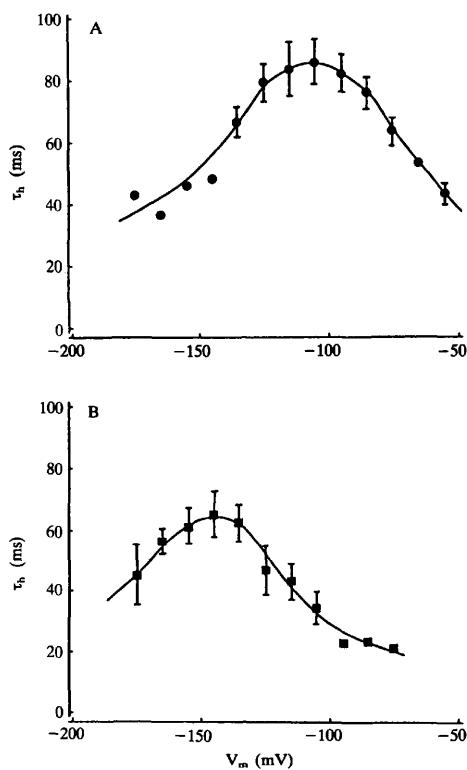


Fig. 7. The voltage dependence of kinetic parameters of the chloride conductance. (A),(B)  $\tau_h$ -voltage relations in 25°C- and 7°C-fibres, respectively. Points are mean  $\pm$  S.E. for five (A) and six (B) fibres, except points without error bars which represent the mean of two fibres.  $V_h$ , -20 to -25 mV; temperature, 15°C. Curves have no theoretical significance.

a prepulse. In Fig. 8A,  $h_\infty$  is plotted against  $V_c$  for 25°C-fibres, while Fig. 8B gives the corresponding relationship for 7°C-fibres. Both curves are sigmoidal, with half-inactivation occurring at -112 mV and -145 mV in 25°C- and 7°C-fibres, respectively. The relationship between  $\tau_h$  and  $h_\infty$  for a given acclimation group is qualitatively similar to voltage- and time-dependent currents in other preparations (e.g. Hodgkin & Huxley, 1952), indicating that inactivation of the chloride conductance of sunfish fibres could be described in terms of the Hodgkin-Huxley scheme for ion channel gating.

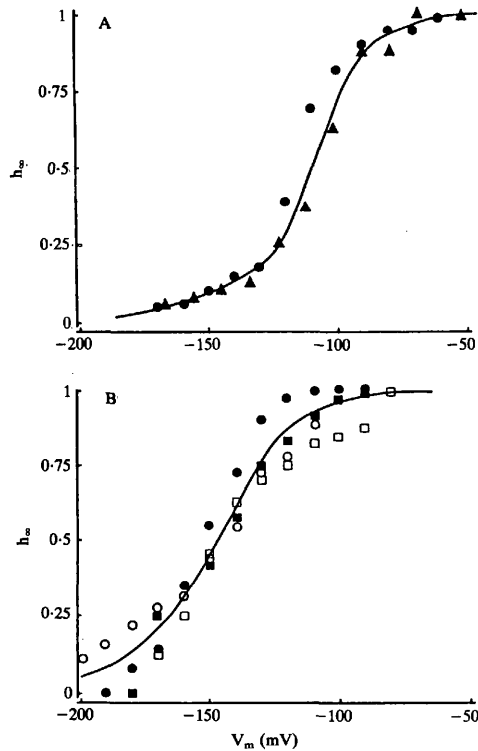


Fig. 8. The voltage dependence of the steady-state inactivation parameter  $h_{\infty}$ . (A), (B)  $h_{\infty}$ -voltage relationships in 25°C- and 7°C-fibres respectively. Each symbol represents a different fibre. Temperature, 15°C;  $V_h$ , -18 to -25 mV. Curves have no theoretical significance.

When the  $\tau_h$ -voltage relationship was determined from fibres bathed in solution E, the curve was translated along the voltage axis in the depolarizing direction by 10 mV in 25°C-fibres (three fibres) and 17 mV in 7°C-fibres (three fibres) (not shown).

#### DISCUSSION

The present results, taken together with those of the preceding paper (Klein & Prosser, 1985) indicate that the Cl conductance of sunfish muscle fibres can be altered by temperature acclimation. The Cl permeation pathway in 25°C-fibres is similar in many ways to that in frog fibres. Similarities include (1) the relatively large Cl permeability with respect to K, (2) the apparent distribution of  $P_{Cl}$  on the sarcolemmal membrane, (3) the shape of  $g_{Cl}$  vs pH relationship and (5) the

shape of the I-V relationship. Notable differences include the  $pK_a$  for  $P_{Cl}$ , and the voltage dependence of chloride current inactivation.

Hutter & Warner (1967) found that the  $pK_a$  of the Cl permeability in frog fibres was close to neutrality. The  $pK_a$  value reported here for 25°C-fibres (5.3) is similar to the values determined by Hagiwara & Takahashi (1974) in stingray fibres, and by Palade & Barchi (1977) in rat diaphragm. Interestingly, the permeability ratio  $P_{Cl}/P_K$  in these preparations varied from 6–10, a range which also encompasses the value reported for 25°C-sunfish fibres (Klein & Prosser, 1985).

The Cl conductance of fibres from 7°C-acclimated sunfish differs in the following ways from 25°C-fibres: (1) the magnitude to  $g_{Cl}$  is lower by approximately six-fold, (2) the  $pK_a$  is shifted slightly toward neutrality, (3) the anion I-V relationship is linear and (4) the voltage dependence of Cl current inactivation is shifted 40 mV in the hyperpolarizing direction.

The differences in  $g_{Cl}$  between warm- and cold-acclimated fibres suggest that thermal acclimation is accompanied by a significant alteration in the anion permeation pathway, and we speculated on some of the possible causes in the previous paper (Klein & Prosser, 1985). Basically, we considered that these alterations could result from a biochemical modification of the Cl channel (or carrier) protein. However, an effect on membrane lipid could not be excluded as a possible mechanism. The present results do not allow a clear choice to be made between the alternatives. One hypothesis which can qualitatively account for many of the results described here is that the negative surface potential in the vicinity of Cl channels in 7°C-fibres is greater (i.e. more negative) than in 25°C-fibres. It is of some interest to examine how far this idea can be taken toward explaining some of the reported differences.

We assume that the Cl conductance is described by constant-field theory (Goldman, 1943) with the inclusion of a term for the membrane surface potential. The relevant equation is given by (Frankenhaeuser, 1960).

$$I_{Cl} = P_{Cl} \cdot \frac{(V - V')F^2}{RT} \cdot \frac{[Cl]_o \exp(V'F/RT) - [Cl]_i \exp[-(V - V')F/RT]}{1 - \exp[-(V - V')F/RT]}, \quad (1)$$

where  $I_{Cl}$  is chloride current,  $P_{Cl}$  is permeability,  $V$  is the membrane potential,  $V'$  is the surface potential,  $[Cl]_o$  and  $[Cl]_i$  are extra- and intracellular Cl concentrations and  $R$ ,  $T$  and  $F$  have the usual thermodynamic meaning. The addition of the term for  $V'$  in equation 1 implies that the concentration of ions at the membrane-solution interface is determined by the Boltzmann distribution (Gilbert & Ehrenstein, 1969)

$$[Cl]_m = [Cl]_\infty \exp(V'F/RT), \quad (2)$$

where  $[Cl]_m$  and  $[Cl]_\infty$  are the Cl concentration at the membrane and in the bulk solution, respectively. Of course, this relationship applies for other ions as well. The surface potential reduces the  $[Cl]$  at the membrane, and thus reduces  $g_{Cl}$  through its concentration dependence (Fig. 1). Similarly, the pH at the membrane surface is lower than in the bulk solution. From Fig. 2, the difference in  $pK_a$  values between 25°C- and 7°C-fibres is 1.1 pH units, which indicates a surface potential difference of about 60 mV (7°C-fibres more negative).

Values for  $P_{Cl}$  at the resting potential of 25°C-fibres were calculated from the equation (e.g. Hodgkin & Horowicz, 1959):

$$P_{Cl} = g_{Cl} \frac{R^2 T^2}{F^3 V} \frac{[Cl]_i - [Cl]_o}{[Cl]_i [Cl]_o}, \quad (3)$$

with values for  $g_{Cl}$  obtained from experiments performed in the course of completing the previous study (Klein & Prosser, 1985). Hodgkin & Horowicz (1959) showed that the chloride permeability of frog fibres was virtually independent of Cl concentration and voltage. The solid lines in Fig. 4A were calculated from equation 1 with a surface potential of 0 mV, and a chloride permeability of  $9.0 \times 10^{-6} \text{ cm s}^{-1}$  (Table 2).  $[Cl]_i$  was chosen such that Cl was in equilibrium across the membrane at the holding potential of the fibres to which the data were fitted to equation 1. The calculated curves describe the data of Fig. 4A reasonably well, although a better fit would have been obtained with a surface potential of about +20 mV.

The effect of a negative surface potential can be seen in the curves of Fig. 4B. Here  $V'$  was set to -80 mV, but  $P_{Cl}$  was kept constant at  $9.0 \times 10^{-6} \text{ cm s}^{-1}$ , the same value as for 25°C-fibres (Table 2). The fit to the data is good in both the 130 and 40 mmol l<sup>-1</sup> Cl curves. At 40 mmol l<sup>-1</sup> Cl the theory gives an I-V relationship which rectifies slightly in the inward-going direction. The predicted effect is small, however, and was never observed in practice. These calculations suggest that the data of Figs 2 and 4 can be accounted for solely by an increase in the negative surface potential of 7°C-fibres, of the order of 60–80 mV.

What is the evidence for the existence of a surface potential difference of this magnitude? Figs 7 and 8 show that the voltage dependence of the  $\tau_h$  and  $h_\infty$  relationships are generally similar except that the curves for 7°C-fibres are shifted approximately 40 mV in the hyperpolarizing direction. These shifts along the voltage axis are reminiscent of the effects of elevated divalent cation concentrations on the voltage dependence of ion channel gating (e.g. Hille, 1968; Gilbert & Ehrenstein, 1969; McLaughlin, 1977). They have been ascribed to a reduction of the surface potential produced by the screening (or binding) of divalent cations to fixed surface charges. The difference in the voltage dependence of Cl current inactivation of 25°C- and 7°C-fibres is similarly taken as evidence that surface charges alter the potential gradient within the membrane, thus influencing the voltage dependence of the reactions. It is not clear why the kinetic parameters differ by only 40 mV, whereas the other evidence requires a surface potential shift of 60–80 mV between acclimation groups. It may be that the voltage-sensing portion of the Cl channel is buried within the membrane phase, and is therefore partially insulated from surface potential effects. Nevertheless, the hyperpolarizing shift along the voltage axis in 7°C-fibres is in the direction consistent with the main hypothesis.

The influence of Ca ions on the predicted I-V relationships can be seen in Fig. 5. The points more positive than -70 mV from the holding potential are adequately described by the model which assumes only a reduction of the surface potential with increasing  $[Ca]_o$  (Table 2). The deviation of the data from the calculated curves at potentials more negative than -80 mV from  $V_h$  could be due to saturation effects (Warner, 1972; Lindemann, 1982).

Table 2 summarizes the values of  $P_{Cl}$  and  $V'$  used in the calculated curves of Figs 4 and 5 under conditions of different  $[Cl]_o$  and  $[Ca]_o$  and gives an estimate of the goodness-of-fit to the data. The choice of  $V'$  for 25°C-fibres was arbitrary, and, in view of the results from other studies (McLaughlin, 1977), probably unrealistic. The point to be made is that the hypothesis requires only a *difference* in the surface potential of 25°C- and 7°C-fibres of 60–80 mV, but the absolute values could be large or small. Hille *et al.* (1975) suggest that a surface potential of –65 or –90 mV is consistent with their results on frog node of Ranvier. They indicate that raising  $[Ca]_o$  from 2 to 33 and 115 mmol l<sup>-1</sup> should reduce the surface potential by about 25 and 38 mV, respectively, assuming no binding of Ca to the surface charges. If the charge density of sunfish muscle is assumed to be similar, the values for  $V'$  in Table 2 indicate corresponding shifts of 20 and 42 mV, respectively. However, the comparison is not absolutely valid because Hille *et al.* (1975) maintained the monovalent ion concentration in their Ringer, while we replaced Na with Ca.

Table 2 also gives values for  $P_{Cl}$  which fit the data of 7°C-fibres in elevated Ca solutions, assuming  $V'$  is 0 mV. The fits are comparable to those for varying  $V'$ . However, we favour the interpretation that Ca influences the surface potential in a manner similar to that proposed for other preparations, and has little effect on  $P_{Cl}$  (cf. Hutter & Warner, 1967). When  $V'$  is 0 mV there is no value of  $P_{Cl}$  which adequately fits the data in normal Ca solution.

Hutter & Warner (1972) rejected the idea that pH modulates the Cl permeability of frog fibres *via* an effect on surface charge. There have been no reports of a modulatory role for surface charge on membrane conductance as suggested in this paper, although Ohmori & Yoshii (1977) have shown that alterations in the surface potential of tunicate eggs influence the I-V relationship of both Na and Ca permeation. Also, Satow & Kung (1981) have suggested that a similar reduction in

Table 2. *Values of  $V'$  used to model I-V relationships*

	$[Cl]_o$ (mmol l <sup>-1</sup> )	$[Ca]_o$ (mmol l <sup>-1</sup> )	$P_{Cl}$ ( $\times 10^6$ cm s <sup>-1</sup> )	$V'$ (mV)	$\chi^2$	$P$
25°C fibres	130	2	9.0	0	3.93	0.75
	40	2	9.0	0	0.77	>0.99
	130	33	9.0	+5	3.63	0.83
	130	115	9.0	+20	2.28	0.94
7°C-fibres	130	2	9.0	-80	1.46	0.98
	40	2	9.0	-80	2.61	0.94
	130	33	9.0	-60	2.08	0.96
	130	115	9.0	-38	2.78	0.91
	(130)	33	5.5	0	18.53	0.03)
	(130)	115	6.5	0	0.15	>0.99)

Ion concentrations refer to Ringer solutions used in the experiments of Figs 4 and 5.

The values of  $V'$  and  $[Cl]_o$  were used to calculate I-V curves from equation 1.

$[Cl]_o$  3 mmol l<sup>-1</sup> in 130 mmol l<sup>-1</sup> Cl<sub>o</sub>, and 1.8 mmol l<sup>-1</sup> in 40 mmol l<sup>-1</sup> Cl<sub>o</sub> solutions.

Temperature, 15°C.

$\chi^2$  is the chi-square goodness-of-fit statistic to the data of Figs 4 and 5, assuming the parent function is described by equation 1 with the parameters given above.

$P$  is the probability that a random set of data points would yield a value of  $\chi^2$  as large or larger when compared to the parent function (Bevington, 1969).

Parentetical entries are the parameters of fits to the data of Fig. 5B assuming that  $P_{Cl}$  changes while  $V'$  is 0 mV.



membrane surface charge underlies a positive shift in the resting potential and voltage dependence of the calcium conductance of *Paramecium* grown at high temperatures (22–34°C).

Indeed, this hypothesis cannot account for all the differences between fibres of sunfish acclimated to different temperatures. For example, it is unlikely that an alteration in surface charge would cause a large change in membrane capacitance (Klein & Prosser, 1985). Nevertheless, the hypothesis is a parsimonious one in that it is not necessary to suppose that cold acclimation induces the incorporation into the membrane of an entirely different Cl channel protein – perhaps one with only a few specific modifications. A surface potential of the required magnitude (60–80 mV) is reasonable for biological membranes which contain negatively charged phospholipids; however, it is more likely that the surface charges would be confined to the immediate vicinity of the Cl channels, since the Debye length in Ringer solution is only about 0.8 nm. From a consideration of the magnitude of the conductance change involved in thermal acclimation, it is probable that the membrane channel density is altered as well. It is concluded that while additional mechanisms should be considered to account for the overall membrane response to long-term temperature change, the simple surface charge hypothesis is sufficient to explain many of the reported observations.

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## REFERENCES

- ADRIAN, R. H. (1964). The rubidium and potassium permeability of frog muscle membrane. *J. Physiol., Lond.* **175**, 135–159.
- ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1970). Voltage clamp experiments in striated muscle fibres. *J. Physiol., Lond.* **208**, 607–644.
- BEVINGTON, P. R. (1969). *Data Reduction and Error Analysis for the Physical Sciences*. New York: McGraw-Hill.
- FRANKENHAEUSER, B. (1960). Sodium permeability in toad nerve and in squid nerve. *J. Physiol., Lond.* **238**, 109–127.
- GILBERT, D. L. & EHRENSTEIN, G. (1969). Effect of divalent cations on potassium conductance of squid axons; determination of surface charge. *Biophys. J.* **9**, 447–463.
- GOLDMAN, D. E. (1943). Potential, impedance and rectification in membranes. *J. gen. Physiol.* **7**, 37–60.
- GRAHAME, D. C. (1947). The electrical double layer and the theory of electrocapillarity. *Chem. Rev.* **41**, 441–501.
- HAGIWARA, H. & TAKAHASHI, K. (1974). Mechanism of anion permeation through the muscle fibre membrane of an elasmobranch fish, *Taeniura lymma*. *J. Physiol., Lond.* **238**, 109–127.
- HILLE, B. (1968). Charges and potentials at the nerve surface. Divalent ions and pH. *J. gen. Physiol.* **51**, 221–236.
- HILLE, B., WOODHULL, A. M. & SHAPIRO, B. I. (1975). Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. *Phil. Trans. R. Soc. Ser. B.* **270**, 301–318.
- HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol., Lond.* **148**, 127–160.
- HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol., Lond.* **117**, 500–544.
- HUTTER, O. F. & WARNER, A. E. (1967). The pH sensitivity of the chloride conductance of frog skeletal muscle. *J. Physiol., Lond.* **189**, 403–425.
- HUTTER, O. F. & WARNER, A. E. (1972). The voltage dependence of the chloride conductance of frog muscle. *J. Physiol., Lond.* **227**, 275–290.

- KLEIN, M. G. & PROSSER, C. L. (1985). The effects of temperature acclimation on the resting membrane of skeletal muscle fibres from green sunfish. *J. exp. Biol.* **114**, 563–579.
- LINDEMANN, B. (1982). Dependence of ion flow through channels on the density of fixed charges at the channel opening. *Biophys. J.* **39**, 15–22.
- McLAUGHLIN, S. (1977). Electrostatic potentials at membrane-solution interfaces. *Curr. Top. Memb. Trans.* **9**, 71–144.
- McLAUGHLIN, S. G. A., SZABO, G. & EISENMAN, G. (1971). Divalent ions and the surface potential of charged phospholipid membranes. *J. gen. Physiol.* **58**, 667–687.
- OHMORI, H. & YOSHII, M. (1977). Surface potential reflected in both gating and permeation mechanisms of sodium and calcium channels of the tunicate egg cells membrane. *J. Physiol., Lond.* **267**, 429–463.
- PALADE, P. T. & BARCHI, R. L. (1977). Characteristics of the chloride conductance in muscle fibers of the rat diaphragm. *J. gen. Physiol.* **69**, 325–342.
- SATOW, Y. & KUNG, C. (1981). Possible reduction of surface charge by a mutation in *Paramecium tetraurelia*. *J. Membrane Biol.* **59**, 179–190.
- VAUGHAN, P. C., McLARNON, J. G. & LOO, D. D. F. (1980). Voltage dependence of chloride current through *Xenopus* muscle membrane in alkaline solutions. *Can. J. Physiol. Pharmacol.* **58**, 999–1010.
- WARNER, A. E. (1972). Kinetic properties of the chloride conductance of frog muscle. *J. Physiol., Lond.* **227**, 291–312.