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THE EFFECTS OF TEMPERATURE ACCLIMATION ON THE RESTING MEMBRANE OF SKELETAL MUSCLE FIBRES FROM GREEN SUNFISH

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SUMMARY

Conductive properties of muscle fibres from green sunfish (*Lepomis cyanellus*) acclimated to different temperatures were examined. The relative membrane permeability to chloride and potassium ions, P_{Cl}/P_K, measured at acclimation temperature, was approximately 7·0 after acclimation at 25 °C and 1·3 after acclimation at 7 °C. This difference was due to a six-fold reduction in the membrane chloride conductance upon acclimation to 7 °C as compared to 25 °C-acclimated fibres. Mean (±S.E.M.) values of the chloride conductance were $554 \pm 68\,\mu S\,cm^{-2}$ in warm-acclimated sunfish, and $75 \pm 9\,\mu S\,cm^{-2}$ in cold-acclimated sunfish. Membrane capacitance also differed significantly between the two acclimation groups.

When the temperature was varied acutely, the magnitude of the chloride conductance exhibited a maximum Q_{10} of only 1·9, compared with a Q_{10} of 3·0 associated with acclimation. Upon transferring 25 °C-acclimated sunfish to holding tanks at 7 °C, the total membrane resistance exhibited a sigmoidal increase over about 14 days, and a steady membrane capacitance was achieved in about 10 days. For 7 °C-acclimated sunfish, transferred to 25 °C, resistance showed a sigmoidal decrease over 10 days and capacitance was steady after 8 days. The results indicate that thermal acclimation of the muscle membrane involves cellular regulatory processes which underlie significant changes in the electrical properties of the fibre.

INTRODUCTION

Eurythermal organisms are able to maintain locomotor ability over a wide range of seasonal temperatures; this suggests that compensatory changes occur in the membrane properties of nerve and muscle cells that mediate motor activity. Since in skeletal muscle, the membrane potential determines the degree of contractile activation (e.g. Hodgkin & Horowicz, 1960), the regulation of active and passive membrane permeability is necessary for proper muscle function. Thus, the relative ion permeabilities of muscle cell membranes might be expected to remain somewhat constant as a result of acclimation to different temperatures.

Key words: Temperature acclimation, membrane conductance, green sunfish.

The resting muscle membrane is generally permeable to both potassium and chloride ions, but the relative contribution of these ions to the resting potential varies between species. The ratio of the permeability to chloride and to potassium, P_{Cl}/P_K, ranges from 8–10 in some elasmobranch muscle fibres (Hagiwara & Takahashi, 1974) to about 0·2 in barnacle (Hagiwara *et al.* 1968). Also, the effect of acute temperature change is different for K and Cl permeabilities. In most organisms studied, P_{Cl} alters with a Q_{10} of 1·7–2·0, whereas P_K exhibits a Q_{10} of 1·0–1·3 (Harris, 1958; Hagiwara *et al.* 1968; Hagiwara & Takahashi, 1974; Palade & Barchi, 1977).

No attempt has been made to correlate changes in muscle membrane component permeabilities (P_{Cl} and P_K) or conductances (g_{Cl} and g_K) with thermal acclimation. White (1983) showed that the specific membrane resistance, R_m, of crayfish walking leg muscle is about the same in animals acclimated to 10 or 25 °C, but that the effect of acute temperature change on the R_m of fibres from the two acclimation groups is different. Fibres from 10 °C-acclimated crayfish exhibited a steep relation between R_m and temperature, with a break point at about 13 °C. Identical results were obtained by Fischer & Florey (1981) from measurements of input resistance in *Astacus* fibres. Unlike cold-acclimated crayfish, fibres from warm-acclimated crayfish did not show a sharp break point in this relationship (White, 1983). The shape of the R_m-temperature relation is correlated with the effect of temperature on the amplitude (White, 1983) and time constant of decay (Fischer & Florey, 1981) of neurally-evoked excitatory junction potentials (see also Colton & Freeman, 1975; Harri & Florey, 1979; Stephens & Atwood, 1982).

In an attempt to explain the reported interfibre variation in the passive membrane properties of amphibian skeletal muscle, Dulhunty & Gage (1973) examined the cable properties of summer and winter toads. The R_m of muscles from summer toads was about two-fold greater than in muscles from winter toads, when measured at 20 °C. No attempt was made to determine the ionic basis of the differences in R_m, although Dulhunty & Gage (1973) commented that the chloride conductance was 'normal' in summer toads.

The present study reports the long- and short-term effects of temperature on the ionic permeability of skeletal muscle fibres. Green sunfish were used because studies from this laboratory on enzymes had previously demonstrated acclimatory compensation to temperature in this species (e.g. Sidell, 1977; Cossins, Kent & Prosser, 1980). It will be shown that temperature acclimation, but not acute temperature change, results in a significant modification of the conductive properties of the resting membrane, and that this modification involves a change in the absolute permeability to Cl. The next paper (Klein, 1985) examines some physical aspects of the chloride conductance in warm- and cold-acclimated sunfish fibres. An abstract of some of these results has appeared (Klein, 1983).

METHODS

Green sunfish, *Lepomis cyanellus*, were maintained in laboratory holding tanks at 25, 15 or 7 °C for at least 5 weeks. Only fish that ate regularly and remained active were used for experimentation.

A small strip of 'white' myotomal muscle was dissected from a region just anterior to the dorsal fin, and pinned in a small-volume (3 ml) tissue chamber. Ringer solution flowed through the chamber continuously, and was removed by aspiration. The bath temperature was controlled within ±0·5 °C over the range of 0–35 °C by a water conditioner. Temperature was monitored by a thermocouple probe placed close to the preparation.

Normal Ringer solution contained (mmol l^{-1}): NaCl, 100; KCl, 2·5; $CaCl_2$, 2·0; $NaHCO_3$, 25; NaH_2PO_4, 4·0; $MgSO_4$, 1·0. Solutions were bubbled with 95 % O_2-5 % CO_2 to a pH of 7·4. For ion substitution experiments, Cl was replaced on an equimolar basis by methanesulphonate or gluconate salts of Na, K and Ca. At the concentrations used, gluconic acid significantly reduces the free Ca activity (Christoffersen & Skibsted, 1975; Kenyon & Gibbon, 1977); however, control experiments in low Ca Ringer solution (0·1 mmol l^{-1}) gave identical results in the time required for the experiments to be completed.

Conventional microelectrode techniques were used for intracellular recording and stimulation. Microelectrodes for potential measurement were filled with 3 mol l^{-1} KCl, had resistances of 15–30 MΩ and tip potentials of less than 5 mV in normal Ringer solution. Current-passing electrodes were filled with 2 mol l^{-1} K-citrate and had resistances of 7–10 MΩ. The reference electrode was a 3 mol l^{-1} KCl-agar Ag/AgCl wire. Current was monitored by a virtual ground circuit. Junction potentials were avoided through the use of a balanced recording system similar to the one described by Dulhunty (1979).

Cable properties (Hodgkin & Rushton, 1946; Fatt & Katz, 1951) were determined using the semi-infinite cable equations. The lengths of fibres examined in this study ranged from 3 to 7 mm, and hence were too short to apply the infinite cable model. The semi-infinite equations gave results which were not significantly different from those obtained using a short cable model. The membrane time constant was taken as the time to reach 84 % of the change in membrane potential. Fibre diameter was measured with an ocular micrometer at three or four points along the fibre, with the assumption of a cylindrical fibre. While this assumption may overestimate membrane resistance and capacitance by 2 %, and internal resistivity by as much as 28 % (Dulhunty & Gage, 1973), it is not thought that this uncertainty will alter the significance of the results to be described.

RESULTS

When measured at the temperature of acclimation the mean resting membrane potentials from sunfish acclimated to 25 and 7 °C were significantly different. Fibres from 25 °C-acclimated sunfish (hereafter referred to as 25 °C-fibres) had a mean resting potential of −91·8 ± 2·4 mV (mean ± S.E.M., 68 fibres), while the corresponding value for 7 °C-acclimated sunfish fibres (7 °C-fibres) was −84·0 ± 2·8 mV ($N = 46$ fibres, Student's t-test, $P < 0·05$). However, this difference can be attributed to the measurement temperature since the resting potentials of fibres from the two acclimation groups were similar when measured at an intermediate temperature, 15 °C (25 °C-fibres, −87·1 ± 6·3 mV, $N = 12$; 7 °C-fibres, −89·4 ± 7·4 mV, $N = 15$; $P < 0·5$). Indeed, resting potentials of greater than

-100 mV were often recorded from 7 °C-fibres after warming up to 25 °C (not shown).

Cable parameters and component conductances

The passive cable parameters of muscle fibres from sunfish acclimated to 25, 15 and 7 °C are given in Table 1. Cable constants were determined in normal Ringer solution and in Cl-free Ringer, maintained at the temperature of acclimation. The magnitudes of all cable parameters were very different in fibres from different acclimation temperatures. Mean values of membrane resistance were 1546 Ωcm^2, 3052 Ωcm^2 and 7132 Ωcm^2 in 25 °C-, 15 °C- and 7 °C-fibres, respectively, in normal Ringer solution. The differences between the means were statistically significant (Table 1). Thus R_m increased 2·3-fold between 25 °C- and 15 °C-fibres, and 4·6-fold between 25 °C- and 7 °C-fibres. Internal resistivity (R_i) also increased with decreasing temperature. However, membrane capacitance, C_m, was reduced in 7 °C-fibres as compared with 25 °C-fibres ($P < 0{\cdot}01$), with mean values of 3·9 $\mu F\,cm^{-2}$ and 5·7 $\mu F\,cm^{-2}$.

To determine the absolute contributions of K and Cl to the membrane resistance, small bundles of fibres were soaked in Cl-free (methanesulphonate) Ringer solution either for several hours at the acclimation temperature, or overnight at 4 °C. No differences were detected in the results obtained by the two methods. Under these conditions the fibres should be depleted of Cl and only K should carry appreciable current across the resting membrane.

As can be seen from Table 1 the R_m of 25 °C-fibres in Cl-free Ringer was increased 7-fold over control values in normal Ringer, to a mean of 10889 Ωcm^2. The values for R_i and C_m were little changed in Cl-free solutions in any acclimation group.

The values for R_m may be expressed in terms of membrane conductance, g_M, the reciprocal of R_m. If it is assumed that the resting g_M is comprised of conductances to K and Cl such that $g_M = g_K + g_{Cl}$, it can be shown that g_{Cl} was approximately 554 $\mu S\,cm^{-2}$ and g_K was 92 $\mu S\,cm^{-2}$ in 25 °C-fibres. The corresponding values in 7 °C-fibres were 75 $\mu S\,cm^{-2}$ for g_{Cl} and 65 $\mu S\,cm^{-2}$ for g_K (Table 1). From these data the conductance ratio g_{Cl}/g_K was approximately 6 and 1·3 in 25 °C- and 7 °C-fibres, respectively. From the calculated values of g_{Cl} in the two acclimation groups, the Q_{10} of acclimation of this conductance was 3·0. The acclimation Q_{10} is operationally defined as the ratio of g_{Cl} (or g_K) in 25 °C-fibres to that in 7 °C-fibres, raised to the $[10/(25-7)]$th power. This definition is simply meant to provide an indication of the magnitude of the Q_{10} value required to account for the differences in ion conductances of warm- and cold-adapted sunfish.

It should be mentioned that fibres bathed in Cl-free solutions had resting potentials which were depolarized by 5–15 mV from the mean values in normal Ringer. Extrapolating the measured g_K to the normal resting potential would slightly underestimate its true value due to the existence of inward rectification. The error involved is probably less than 10%, and was ignored in the calculations.

In sunfish acclimated to 15 °C all cable constants had values which were intermediate between those of 25 °C- and 7 °C-fibres. The g_{Cl}/g_K for this group was 2·9.

Table 1. *Cable constants and electrical properties of muscle fibres from sunfish acclimated to 25, 15 and 7 °C*

Ringer solution	T_{accl} (°C)	N	d (μm)	λ (mm)	R_{in} (MΩ)	R_m (Ωcm^2)	R_i (Ωcm)	C_m ($\mu F\,cm^{-2}$)	τ (ms)	g_K ($\mu S\,cm^{-2}$)	g_{Cl} ($\mu S\,cm^{-2}$)
Normal	25	16	63 ± 2	1·25 ± 0·11	0·34 ± 0·03	1546 ± 210	155 ± 19	5·7 ± 0·3	8·6 ± 0·5		
Cl-free	25	7	57 ± 3	3·03 ± 0·35	0·98 ± 0·05	10889 ± 923	169 ± 27	5·8 ± 0·5	63·1 ± 3·8	92 ± 12	554 ± 68
Normal	15	9	58 ± 3	1·49 ± 0·09	0·56 ± 0·02†	3052 ± 460*	198 ± 33	5·2 ± 0·2	14·5 ± 1·5†		
Cl-free	15	4	55 ± 4	2·79 ± 0·07	1·21 ± 0·10	11659 ± 1216	205 ± 62	5·5 ± 0·3	67·1 ± 7·5	83 ± 7	247 ± 32*
Normal	7	15	57 ± 3	1·96 ± 0·14†	1·05 ± 0·08†	7132 ± 617†	263 ± 42	3·9 ± 0·4*	27·8 ± 4·1†		
Cl-free	7	7	60 ± 4	2·72 ± 0·10	1·29 ± 0·17	13340 ± 1497	269 ± 66	3·8 ± 0·5	50·7 ± 8·3	65 ± 8	75 ± 9†

Column headings: T_{accl}, acclimation temperature; N, number of fibres; d, fibre diameter; λ, space constant; R_{in}, input resistance; R_m, R_i, specific resistance of membrane and myoplasm, respectively; C_m, specific membrane capacitance; τ, time constant; g_K, g_{Cl}, specific membrane conductance to K and Cl. Values are means ± S.E.M.

*,† Indicate values significantly different from the corresponding value in 25 °C-fibres: *, $P < 0{\cdot}05$; †, $P < 0{\cdot}01$.

Permeability ratios are correlated with conductance ratios

The resting g_{Cl} was significantly greater than g_K in 25 °C-acclimated sunfish fibres (Table 1). This suggests that an alteration in the extracellular Cl concentration $[Cl]_o$, should cause a significant change in the membrane potential (Hodgkin & Horowicz, 1959), the magnitude of which will depend on the relative membrane permeability to Cl. Fig. 1 depicts the steady-state (approx. 10 min) values of membrane potential in solutions with reduced $[Cl]_o$ (gluconate substitution) and increased $[K]_o$ (Na reduction) from 25 °C-fibres, measured at 25 °C. The results indicate that the membrane behaves as a Cl electrode over the range of $[Cl]_o$ from 145 to 50 mmol l^{-1}, then deviates from linearity at lower concentrations. This deviation can be attributed to the cation permeability of the fibre.

When $[K]_o$ was increased, however, the membrane potential hardly changed until $[K]_o$ was about 8 mmol l^{-1}, then approaches a Nernstian slope at higher concentrations. These results indicate that normally the membrane is overwhelmingly permeable to Cl, and that in the region of physiological concentrations the Cl gradient across the membrane dominates the resting potential.

From the data in Fig. 1, the relative membrane permeability to Cl and K, P_{Cl}/P_K, can be calculated using equations derived by Hodgkin & Horowicz (1959), to be approximately 7, in fair agreement with the value determined from conductance measurements.

To assess the relative contribution of Na ions to the resting potential of 25 °C-fibres, $[Na]_o$ was reduced from 130 to 10 mmol l^{-1} (Tris-Cl substitution) and the resulting change in membrane potential was recorded. The addition of Tris-Ringer resulted in a reversible hyperpolarization of $2{\cdot}3 \pm 0{\cdot}3$ mV (five fibres). If the membrane potential is assumed to be linearly related to $[Na]_o$, then P_{Na}/P_K was approximately 0·03. This value is similar to the ratio reported by Hodgkin & Horowicz (1959) in frog semitendinosus; however, our result may not be very reliable since Cl was present in the solution, and P_{Cl} would be expected to shunt much of the change in membrane potential.

The Goldman-Hodgkin-Katz (GHK) equation (Goldman, 1943; Hodgkin & Katz, 1949), i.e.

$$V_m = \frac{RT}{F} \ln \frac{[K]_o + \alpha[Na]_o + \gamma[Cl]_i}{[K]_i + \alpha[Na]_i + \gamma[Cl]_o}, \quad (1)$$

has frequently been used to describe the steady-state membrane potential, V_m, in terms of the concentration and relative permeability of Na, K and Cl. Here α is P_{Na}/P_K, γ is P_{Cl}/P_K, subscripts i and o refer to intracellular and extracellular compartments, R, T, and F have their usual thermodynamic meaning, and the other symbols have been defined previously. The solid and dashed lines in Fig. 1 were determined from equation 1 with $\alpha = 0{\cdot}03$, $\gamma = 7{\cdot}0$, and other symbols having values indicated in the figure legend. For purposes of calculation, intracellular ion concentrations were assumed to remain constant. It is evident that the dependence of the membrane potential on Na, K and Cl can be adequately described by the GHK equation, in the physiological range of ion concentrations. The agreement

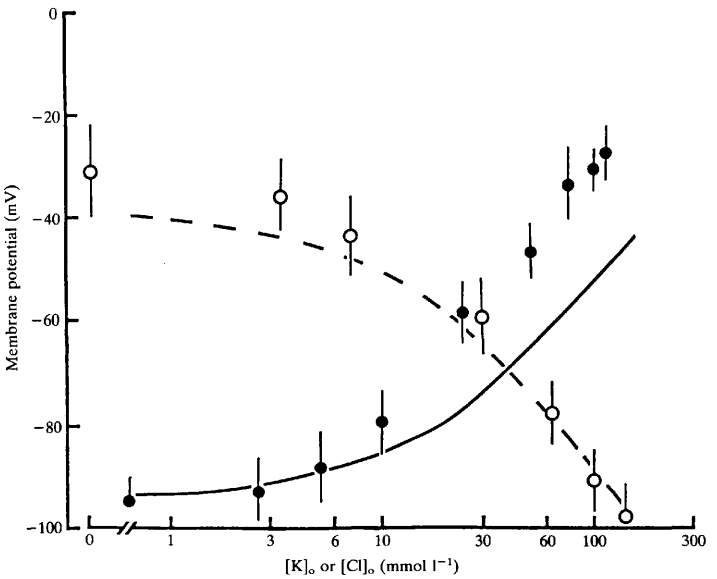

Fig. 1. The relationship between membrane potential and $[K]_o$ (●) or $[Cl]_o$ (○) in 25 °C-fibres. Semi-logarithmic scale. Points represent mean values ± S.E. from 8 (●) and 10 (○) fibres. All measurements made at 25 °C. Solid line, the graphic representation of the GHK equation (1) with the following constant parameters: $[K]_i = 125$ mmol l^{-1}, $[Na]_i = 10$ mmol l^{-1}, $[Cl]_i = 3$ mmol l^{-1}, $\alpha = 0{\cdot}03$, $\gamma = 7{\cdot}0$, T = 25 °C. The equation was solved for membrane potential at one of several values for $[K]_o$. $[Na]_o$ was such that $[K]_o + [Na]_o = 132{\cdot}5$ mmol l^{-1}. Dashed line, the GHK equation with the same values as above for intracellular concentrations and temperature. $[Na]_o = 130$ mmol l^{-1}, $[K]_o = 2{\cdot}5$ mmol l^{-1}, and membrane potential was calculated for several values of $[Cl]_o$. Intracellular concentrations were determined from analyses with ion-selective microelectrodes for K and Cl, and from Na-current reversal potentials under voltage-clamp (M. G. Klein & C. L. Prosser, unpublished results).

breaks down at less negative values of V_m, presumably due to the active cation permeability of the membrane.

Similar ion substitution experiments were performed on fibres from sunfish acclimated to 7 °C (Fig. 2). Reduction of $[Cl]_o$ caused the membrane potential to alter with a slope of 35 mV per 10-fold change in Cl concentration. Increase of $[K]_o$ depolarized the membrane with a slope of 25 mV per decade in the physiological region. The P_{Cl}/P_K is thus about 1·4 again in good agreement with conductance measurements. P_{Na}/P_K, determined as outlined above, is about 0·04 (mean hyperpolarization 3·6 ± 0·6 mV, six fibres).

The solid and dashed lines in Fig. 2 were determined from the GHK equation with $\alpha = 0{\cdot}04$, $\gamma = 1{\cdot}4$ and other parameters as indicated in the figure legend. The membrane potential at different ion concentrations is well fitted by equation 1; however, this agreement is subject to the validity of the simplifying assumptions given above.

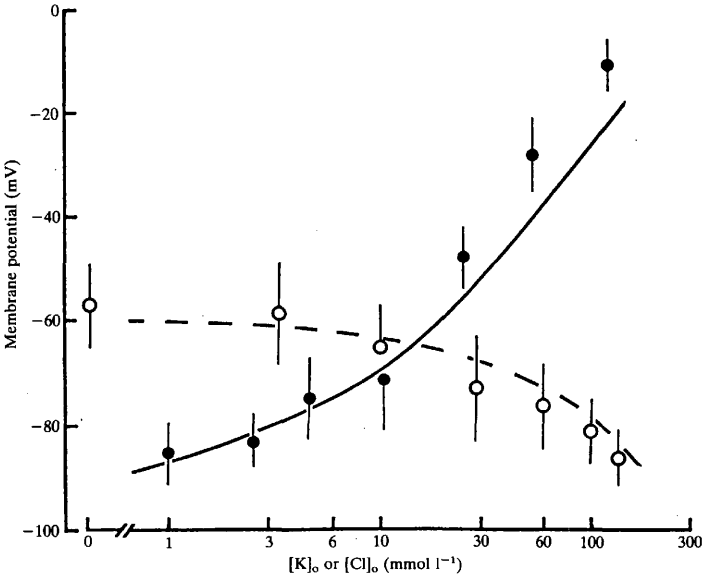

Fig. 2. The relationship between membrane potential and $[K]_o$ (●) or $[Cl]_o$ (○) in 7°C-fibres. Semi-logarithmic scale. Points are means ± S.E. from 7 (●) and 6 (○) fibres. Temperature, 7°C. Solid line, GHK equation with the following parameters: $[K]_i = 125$ mmol l^{-1}, $[Na]_i = 15$ mmol l^{-1}, $[Cl]_i = 5$ mmol l^{-1}, $\alpha = 0{\cdot}04$, $\gamma = 1{\cdot}4$, $T = 7$°C. $[K]_o$ and $[Na]_o$ were varied as described in the legend of Fig. 1. Dashed line, the GHK equation calculated from the above parameters with different values for $[Cl]_o$. Other details as in Fig. 1.

Acute effects of temperature on component conductances

The effect of temperature acclimation to 25 and 7 °C was to alter the magnitude of the Cl conductance, with an acclimation Q_{10} of 3·0, and the K conductance with an acclimation Q_{10} of 1·2. Fig. 3 and Table 2 present evidence to suggest that alterations in membrane conductance that accompany acclimation are not due to the acute effects of temperature change on g_{Cl}. Membrane conductance as a function of temperature was determined by the following methods. (1) Three electrodes were inserted into a fibre, one to pass hyperpolarizing current, and two to record the resulting change in potential, at about 200 μm and 2 mm distant from the current electrode. The bath temperature was slowly changed, and cable parameters were determined at each temperature. The procedure was then repeated after soaking the preparation in methanesulphonate-Ringer for several hours. It was desired to obtain data from any given fibre in both normal and Cl-free Ringer, but it proved difficult to obtain complete temperature curves since the fibre often deteriorated during an experiment. These results are therefore limited to three fibres from each group for which the analysis was completed. (2) Cable properties were determined from fibres bathed in normal and Cl-free Ringer, at several different temperatures. Variations in

electrode resistance and, more importantly, Ringer pH were found to be insignificant (range of pH 7·2–7·5 over 5–30 °C). Cl conductance of frog fibres is sensitive to pH (Hutter & Warner, 1967; see also Klein, 1985).

Fig. 3 shows that membrane conductance increased with acute changes of temperature, but by different amounts in each of the acclimation groups. These differences are mostly due to the temperature dependence of g_{Cl}, since g_K varied in a linear fashion in both 25 and 7 °C acclimation groups. Straight line segments can be drawn through the points representing g_{Cl} determined from a typical fibre, and there appear to be well-defined break points. 25 °C-fibres showed a distinct break at about 17 °C (mean of three fibres). The steepest portion of the g_{Cl} curves corresponds to a Q_{10} of 1·7 and 1·9 in 25 °C- and 7 °C-fibres, respectively. The g_K increased with a Q_{10} of 1·1 in fibres from both acclimation groups. Fig. 3 also shows the striking difference in the magnitude of g_{Cl} in fibres from sunfish acclimated to different temperatures.

Table 2 summarizes a number of similar experiments using cable analyses of several fibres at different temperatures. To facilitate comparison with Fig. 3, membrane data are given in units of conductance. The Q_{10} values were determined from least-squares fits to the data, and are in general agreement with the findings from single fibres. The Q_{10} of g_{Cl} were 1·46 and 1·63, while g_K exhibited Q_{10} values of 1·16 and 1·30 in 25 °C- and 7 °C-fibres, respectively. No attempt was made to fit the data to line segments as in Fig. 3. Membrane capacity and internal resistivity

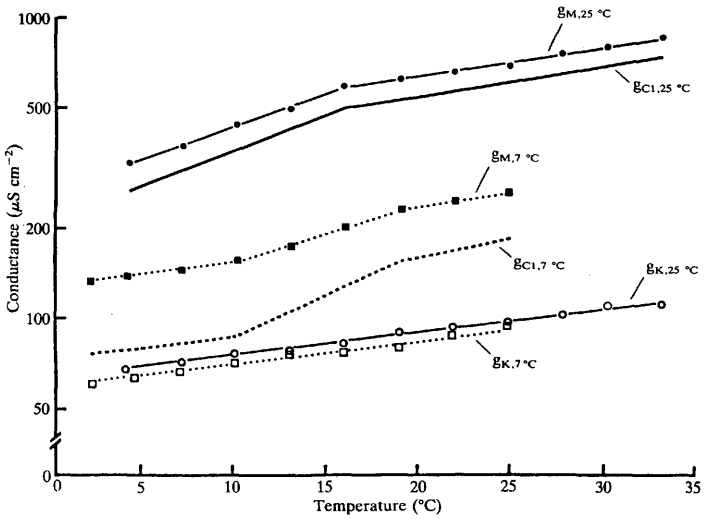

Fig. 3. The effects of acute temperature change on the component conductances in a typical 25 °C-fibre (circles, solid lines) and 7 °C-fibre (squares, dotted lines). g_K was measured in Cl-free (methanesulphonate) solution. Curves for g_{Cl} were obtained by subtracting g_K from g_M (see text).

exhibited Q_{10} values of about 1·06 and 1·40, respectively, in each acclimation group (Table 2).

Time course of the change in cable parameters

Since acute temperature change cannot account for the large change in g_{Cl} which occurs during temperature acclimation of sunfish fibres, it was of interest to examine the time course of the alteration in g_{Cl}. Holding tanks containing sunfish previously acclimated to 25 or to 7 °C were placed in constant temperature rooms maintained at 7 °C (for 25 °C-fish) and 25 °C (for 7 °C-fish). The change in water temperature was made gradually over 36 h to avoid temperature shock. Nevertheless, after about 2 weeks a few fish transferred to the lower temperature exhibited slight motor impairment, and a small percentage of their muscle fibres appeared to be vesiculated and broken. Data collection was limited to fibres with resting potentials greater than −80 mV. At several times after the transfer R_m, R_i and C_m were determined from cable measurements performed at the temperature to which the animal had been transferred. In Figs 4 and 5 the magnitudes of the initial (day zero) data points reflect the acute effects of temperature, especially evident in R_m and R_i curves (cf. Tables 1, 2).

Fig. 4 shows that membrane resistance (taken as a measure of the Cl conductance) increased sigmoidally over 14 days in a 25 °C-sunfish transferred to 7 °C. The change in total membrane capacitance displayed a faster time course than did R_m, and was essentially complete in 10 days. The internal resistivity increased rapidly to a value typical of 7 °C-fibres, and exhibited no other significant change over the next 14 days.

The rate of change of cable constants in 7 °C-fibres transferred to 25 °C was slightly faster than the 25 → 7 °C group (Fig. 5). This was probably due to the direct

Table 2. *Acute effects of temperature on cable parameters*

Temperature (°C)	N	g_M	g_K ($\mu S\,cm^{-2}$)	g_{Cl}	R_i (Ωcm)	C_m ($\mu F\,cm^{-2}$)
25 °C-fibres						
6	5	395 ± 48	70 ± 10	316 ± 81	294 ± 53	5·8 ± 1·2
11	7	410 ± 32	71 ± 9	329 ± 52	233 ± 41	5·7 ± 0·9
16	5	650 ± 89	83 ± 15	541 ± 89	202 ± 39	6·1 ± 0·8
21	5	605 ± 37	96 ± 26	527 ± 62	185 ± 46	6·3 ± 1·0
26	6	760 ± 58	90 ± 10	663 ± 97	150 ± 27	7·2 ± 1·3
31	5	950 ± 74	100 ± 37	791 ± 121	127 ± 31	6·5 ± 1·2
Q_{10}		1·42	1·16	1·46	1·39	1·08
r		0·95	0·93	0·96	0·99	0·94
7 °C-fibres						
2	5	140 ± 38	54 ± 22	83 ± 33	315 ± 57	4·3 ± 0·9
7	5	145 ± 25	66 ± 26	78 ± 45	288 ± 36	4·7 ± 1·1
12	6	165 ± 28	73 ± 33	95 ± 47	247 ± 39	4·6 ± 0·8
17	5	240 ± 32	88 ± 28	143 ± 39	198 ± 46	5·2 ± 1·0
22	4	320 ± 48	96 ± 45	210 ± 65	159 ± 53	5·1 ± 1·3
Q_{10}		1·54	1·30	1·63	1·41	1·05
r		0·95	0·98	0·92	0·98	0·95

Column headings same as in Table 1.
Q_{10} values were calculated by least squares. r is the correlation coefficient of the fit.

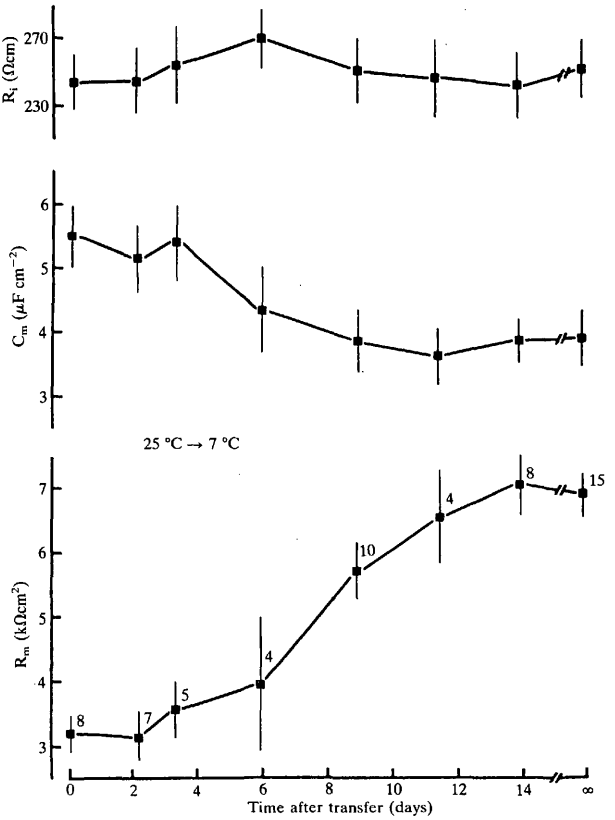

Fig. 4. The time course of changes in cable parameters upon transferring sunfish from 25 °C to 7 °C. Points are means ± S.E. for the number of fibres indicated on the R_m curve. Temperature, 7 °C.

effect of temperature on metabolism. Alterations in R_m required only 10 days to reach a steady value, while changes in C_m were complete after about 8 days of exposure to the higher temperature. As before, R_i attained a value characteristic of 25 °C-fibres in less than 1 day, then did not vary appreciably during the next 10 days.

Localization of the chloride conductance

We attempted to determine the magnitude of the Cl conductance that is localized in the transverse tubular system (TTS) of 25 °C-fibres by applying the glycerol-shock technique (Eisenberg & Gage, 1969; Eisenberg, Howell & Vaughan, 1971). Fibres with relatively large resting potentials (>−75 mV) were used to measure cable

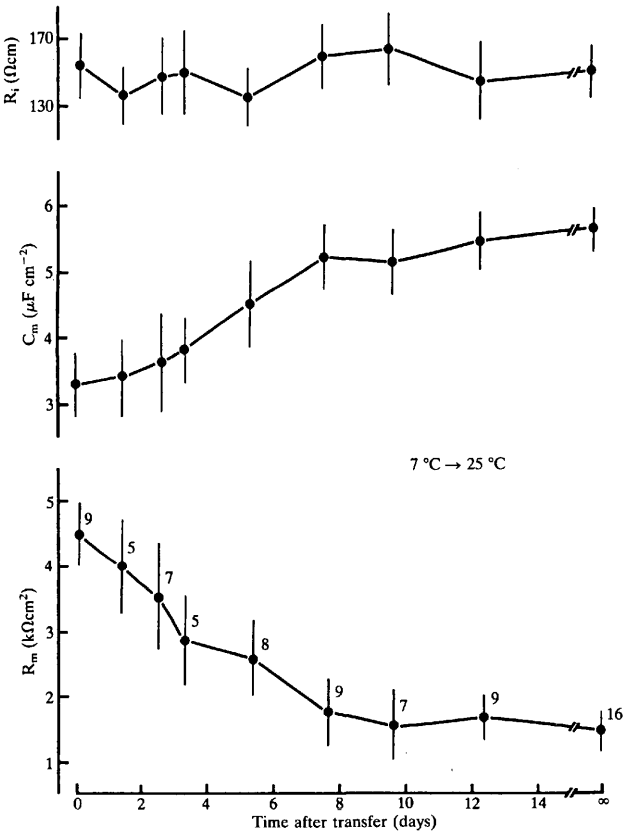

Fig. 5. The time course of changes in cable parameters upon transferring sunfish from 7 °C to 25 °C. Mean ± S.E. for the number of fibres indicated on the R_m curve. Temperature, 25 °C.

properties and to perform rapid ion-substitution experiments. Criteria used to assess the degree of uncoupling of the TTS from the surface membrane were: (1) a low membrane capacitance (less than 2·5 μF cm^{-2}), (2) the absence of a twitch in response to suprathreshold stimulation that elicited an action potential, and (3) the absence of a contracture in solutions that depolarized the membrane to less than −50 mV.

The results in Fig. 6 and Table 3 suggest that only a small proportion of the fibre g_{Cl} resides in the TTS. Fig. 6A shows a typical result of rapidly reducing the [Cl] in the Ringer (methanesulphonate substitution) in an untreated 25 °C-fibre. A prompt depolarization resulted in a contracture that dislodged the electrode. The response

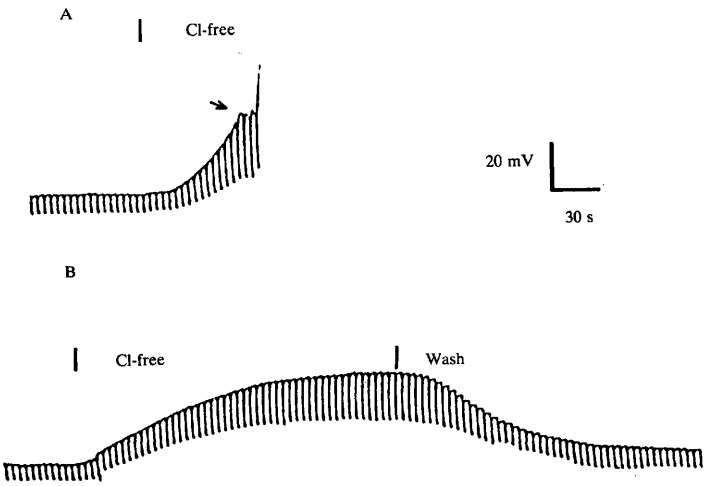

Fig. 6. The effects of Cl-free (methanesulphonate) solutions on the membrane potential in normal (A) and glycerol-treated (B) fibres. Downward deflections were caused by hyperpolarizing current pulses (10 nA) injected from another intracellular electrode located within 75 μm of the recording electrode. In A, depolarization to −55 mV resulted in a contracture which dislodged the electrode (arrow). 25 °C-fibres, temperature 25 °C. Initial membrane potentials are −85 mV in A, −81 mV in B.

of a detubulated fibre to Cl-free solution is shown in Fig. 6B. This fibre had an initial resting potential of −81 mV in normal Ringer, and exposure to Cl-free Ringer resulted in a slow, reversible depolarization to −40 mV. In six fibres from two glycerol-treated preparations the mean resting potential was −79·3 ± 5·8 mV, and the mean depolarization in Cl-free solutions was to −37·7 ± 6·3 mV. Detubulation by glycerol treatment had the effect of increasing R_m from a control value of 1643 Ωcm^2, to 2693 Ωcm^2. The internal resistivity was only slightly increased, while the membrane capacitance was reduced from 6·1 $\mu F\ cm^{-2}$ to 1·9 $\mu F\ cm^{-2}$ (Table 3). Thus, detubulation resulted in a fall in the contribution of g_{Cl} to the total membrane conductance, from 85 % (control) to 77 % after glycerol treatment.

DISCUSSION

The present results show that thermal acclimation in sunfish muscle fibres results in alterations in the membrane chloride conductance and the total membrane capacitance. In warm-acclimated sunfish Cl dominates the membrane conductance and the capacitance is relatively large. In cold-acclimated animals, both the Cl conductance and the membrane capacitance are reduced. Consequently, the muscle fibre input impedance is increased during cold acclimation approximately 2·5-fold, as calculated from cable equations.

Table 3. *Cable constants in normal and glycerol-treated 25 °C-fibres*

Ringer solution	N	d (μm)	λ (mm)	R_{in} (MΩ)	R_m (Ωcm^2)	R_i (Ωcm)	C_m (μF cm^{-2})	τ (ms)	g_K (μS cm^{-2})	g_{Cl} (μS cm^{-2})
Normal										
Cl Ringer	5	54 ± 2	1·17 ± 0·07	0·41 ± 0·02	1643 ± 151	162 ± 29	6·1 ± 0·5	10·9 ± 2·1	—	—
Glycerol-treated										
Cl Ringer	5	61 ± 5	1·53 ± 0·26	0·46 ± 0·09	2693 ± 427	175 ± 46	1·9 ± 0·4	5·1 ± 0·8	82 ± 10	289 ± 45
Cl-free	3	68 ± 4	3·35 ± 0·38	0·85 ± 0·08	12135 ± 1595	183 ± 58	2·1 ± 0·6	24·9 ± 3·6		

Abbreviations are the same as in Table 1.
Control group (Normal) was bathed in Cl-containing Ringer. Glycerol-treated fibres were analysed in both Cl-containing and Cl-free Ringer. Temperature, 25 °C.

The absolute values of g_M, g_K and g_{Cl} determined from cable measurements in the present study are well within the range of values reported in other species. In rat diaphragm, g_M is of the order of $2{\cdot}5\,mS\,cm^{-2}$, and g_{Cl}/g_K is about 6·2 (Palade & Barchi, 1977). At the other extreme, in frog rectus abdominus g_M is about $8{\cdot}9\,\mu S\,cm^{-2}$, almost all of which is attributable to g_K (Stefani & Steinbach, 1969). Perhaps a more meaningful comparison can be made between sunfish and frog sartorius, in which Sperelakis, Schneider & Harris (1967) found g_K to be $80\,\mu S\,cm^{-2}$, while g_{Cl} was $196\,\mu S\,cm^{-2}$. Recall that in sunfish fibres g_K ranged from $65–92\,\mu S\,cm^{-2}$ and g_{Cl} from $75–554\,\mu S\,cm^{-2}$, depending on the acclimation temperature.

Despite the large differences in membrane conductance from fibres of 25 °C- and 7 °C-sunfish, the mean resting potentials from the two groups were not significantly different when measured at an intermediate temperature. Resting potentials were, however, significantly different when measured at the respective acclimation temperatures. These observations imply that the membrane potential has a temperature sensitivity that is greater than is predicted by the GHK equation. This difference may result from stimulation of the electrogenic sodium pump at higher temperatures (M. G. Klein & C. L. Prosser, unpublished observations; see also Gorman & Marmor, 1970). The observations that 7 °C-fibres when warmed to 25 °C exhibited membrane potentials in excess of −100 mV, and that the GHK equation predicted with reasonable accuracy the resting potentials of fibres from both acclimation groups (Figs 1 and 2) suggest that the Na pump undergoes temperature acclimation as well (Merickel & Kater, 1974; but see Zecevic & Levitan, 1980).

The alterations in g_{Cl} and C_m which accompany acclimation are presumably the result of subcellular biochemical reactions that ultimately regulate the protein and lipid composition of the membrane. This supposition stems from the observation that acute temperature change has little or no effect on the magnitudes of g_{Cl} or C_m, whereas changes in these parameters require up to 14 days to be detected from electrical measurements (Figs 4, 5). The results shown in Figs 4 and 5 also indicate that acclimation-induced alterations in g_{Cl} and C_m are essentially simultaneous in the early stages (1–7 days) of the time course, and this suggests that the two might be correlated. One mechanism which would account for the simultaneous fall in g_{Cl} and C_m is a reduction in the membrane surface area. Most of the P_{Cl} is associated with the surface membrane, as in frog fibres (Eisenberg & Gage, 1969), so a likely location for such a mechanism would involve the surface membrane caveolae (Dulhunty & Franzini-Armstrong, 1975). Two ways by which the Cl conductance could be metabolically manipulated independently of a change in membrane surface area could be through (1) control of the number (density) of chloride channels in the membrane, or (2) the incorporation of a different type of Cl channel, e.g. one with a smaller conductance. Indeed, the next paper (Klein, 1985) provides evidence that certain properties of the Cl conductance in warm- and cold-acclimated sunfish fibres can be accounted for by a surface charge effect on the membrane anion permeability. The main conclusion of that paper is that both mechanisms (1) and (2) are likely to be important. Other ways by which the membrane capacitance could vary are a change in the membrane thickness or dielectric constant.

The reduction of g_{Cl} associated with cold acclimation may be adaptive to the

sunfish since it would tend to increase the electrical excitability of the fibre, resembling the condition of congenital myotonia (see, e.g. Bryant & Morales-Aguilera, 1971; Adrian & Bryant, 1974). The membrane impedance of warm-acclimated fibres is of sufficiently small magnitude to have a considerable effect in shunting the amplitude and duration of subthreshold voltage excursions such as the endplate potential (cf. Fischer & Florey, 1981; White, 1983), and the after-depolarization of muscle action potentials. Thus, the increase in membrane input impedance which accompanies cold acclimation may play a role in enhancing the probability of successful neuromuscular transmission at low environmental temperatures, where the synapse might otherwise fail.

In conclusion, it appears that adaptation to low temperatures in muscle fibres of eurythermal organisms involves an increase in passive membrane resistance that is larger than would be predicted on the basis of the Q_{10} of ionic diffusion. Sunfish require several days to develop this change in R_m (and C_m); the membranes of crayfish muscle (White, 1983) and *Helix* neurones (Zecevic & Levitan, 1980) seem to depend more on the steep inverse relationship between resistance and temperature. It is not known whether the temperature-sensitive alteration in membrane resistance is a property of the excitable cells of all eurythermal organisms which exhibit some kind of thermal acclimation. Further investigations with other species would be helpful in establishing the generality of this phenomenon.

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REFERENCES

ADRIAN, R. H. & BRYANT, S. H. (1974). On the repetitive discharges in myotonic muscle fibres. *J. Physiol., Lond.* **240**, 505–515.

BRYANT, S. H. & MORALES-AGUILERA, A. (1971). Chloride conductance in normal and myotonic muscle fibres and the action of monocarboxylic aromatic acids. *J. Physiol., Lond.* **219**, 367–383.

CHRISTOFFERSEN, G. R. J. & SKIBSTED, L. H. (1975). Calcium ion activity in physiological salt solutions: influence of anions substituted for chloride. *Comp. Biochem. Physiol.* **52A**, 317–322.

COLTON, C. K. & FREEMAN, A. R. (1975). Dual response of lobster muscle fibres to L-glutamate. *Comp. Biochem. Physiol.* **51C**, 275–284.

COSSINS, A. R., KENT, J. & PROSSER, C. L. (1980). A steady-state and differential polarized phase fluometric study of the liver microsomal and mitochondrial membranes of the thermally-acclimated green sunfish (*Lepomis cyanellus*). *Biochim. biophys. Acta* **599**, 341–358.

DULHUNTY, A. F. (1979). Distribution of potassium and chloride permeability over the surface and T-tubule membranes of mammalian skeletal muscle. *J. Membrane Biol.* **45**, 293–310.

DULHUNTY, A. F. & FRANZINI-ARMSTRONG, C. (1975). The relative contributions of the folds and caveolae to the surface membrane of frog skeletal muscle fibres. *J. Physiol., Lond.* **250**, 513–539.

DULHUNTY, A. F. & GAGE, P. W. (1973). Electrical properties of toad sartorius muscle fibres in summer and winter. *J. Physiol., Lond.* **230**, 619–641.

EISENBERG, R. S. & GAGE, P. W. (1969). Ionic conductances of the surface and transverse tubular membranes of frog sartorius fibers. *J. gen. Physiol.* **53**, 279–297.

EISENBERG, R. S., HOWELL, J. N. & VAUGHAN, P. C. (1971). The maintenance of resting potentials in glycerol-treated muscle fibres. *J. Physiol., Lond.* **215**, 95–107.

FATT, P. & KATZ, B. (1951). An analysis of the endplate potential recorded with an intracellular electrode. *J. Physiol., Lond.* **115**, 320–370.

FISCHER, L. & FLOREY, E. (1981). Temperature effects on neuromuscular transmission (opener muscle of crayfish, *Astacus leptodactylus*). *J. exp. Biol.* **94**, 251–268.

GOLDMAN, D. E. (1943). Potential, impedance and rectification in membranes. *J. gen. Physiol.* **27**, 37–60.

GORMAN, A. L. F. & MARMOR, M. F. (1970). Contributions of the sodium pump and ionic gradients to the membrane potential of a molluscan neurone. *J. Physiol., Lond.* **210**, 897–917.

HAGIWARA, S., GRUENER, R., HAYASHI, H., SAKATA, H. & GRINNELL, A. D. (1968). Effect of external and internal pH changes on K and Cl conductances in the muscle fiber membrane of a giant barnacle. *J. gen. Physiol.* **52**, 773–792.

HAGIWARA, S. & TAKAHASHI, K. (1974). Mechanism of anion permeation through the muscle fibre membrane of an elasmobranch fish, *Taeniura lymma*. *J. Physiol., Lond.* **238**, 109–127.

HARRI, M. & FLOREY, E. (1979). The effects of acclimation temperature on a neuromuscular system of the crayfish, *Astacus leptodacylus*. *J. exp. Biol.* **78**, 281–293.

HARRIS, E. J. (1958). Anion interaction in frog muscle. *J. Physiol., Lond.* **141**, 351–365.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol., Lond.* **146**, 127–160.

HODGKIN, A. L. & HOROWICZ, P. (1960). Potassium contractures in single muscle fibres. *J. Physiol., Lond.* **153**, 386–401.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol., Lond.* **108**, 37–77.

HODGKIN, A. L. & RUSHTON, W. A. H. (1946). The electrical constants of a crustacean nerve fibre. *Proc. R. Soc. B* **133**, 444–479.

HUTTER, O. F. & WARNER, A. E. (1967). The pH sensitivity of the chloride conductance of frog skeletal muscle. *J. Physiol., Lond.* **189**, 427–443.

KENYON, J. L. & GIBBON, W. R. (1977). Effects of low-chloride solutions on action potentials of sheep cardiac Purkinje fibers. *J. gen. Physiol.* **70**, 635–660.

KLEIN, M. (1983). Temperature acclimation modifies the chloride conductance of green sunfish muscle fibres. *Fedn Proc. Fedn Am. Socs exp. Biol.* **42**, 469a.

KLEIN, M. G. (1985). Properties of the chloride conductance associated with temperature acclimation in muscle fibres from green sunfish. *J. exp. Biol.* **114**, 581–598.

MERICKEL, M. & KATER, S. B. (1974). Neuronal change: compensatory acclimation of the contribution of an electrogenic pump to the resting potential. *J. comp. Physiol.* **94**, 195–206.

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.

SIDELL, B. D. (1977). Turnover of cytochrome C in skeletal muscle of Green sunfish (*Lepomis cyanellus*, R.) during thermal acclimation. *J. exp. Zool.* **199**, 233–250.

SPERELAKIS, N., SCHNEIDER, M. F. & HARRIS, E. J. (1967). Decreased K^+ conductance produced by Ba^{++} in frog sartorius fibers. *J. gen. Physiol.* **50**, 1565–1583.

STEFANI, E. & STEINBACH, A. B. (1969). Resting potential and electrical properties of frog slow muscle fibres. Effect of different external solutions. *J. Physiol., Lond.* **203**, 383–401.

STEPHENS, P. J. & ATWOOD, H. L. (1982). Thermal acclimation in a crustacean neuromuscular system. *J. exp. Biol.* **98**, 39–47.

WHITE, R. L. (1983). Effects of acute temperature change and acclimation temperature on neuromuscular function and lethality in crayfish. *Physiol. Zoöl.* **56**, 174–194.

ZECEVIC, D. & LEVITAN, H. (1980). Temperature acclimation effects on membrane physiology of identified neurons. *Am. J. Physiol.* **239**, C47–58.