

Slow Charge Movement in Mammalian Skeletal Muscle

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ABSTRACT Voltage-dependent charge movements were measured in the rat omohyoid muscle with the three-microelectrode voltage-clamp technique. Contraction was abolished with hypertonic sucrose. The standard (ON-OFF) protocol for eliciting charge movements was to depolarize the fiber from -90 mV to a variable test potential (V) and then repolarize the fiber to -90 mV. The quantity of charge moved saturated at test potentials of ~ 0 mV. The steady state dependence of the amount of charge that moves as a function of test potential could be well fitted by the Boltzmann relation: $Q = Q_{\max}/\{1 + \exp[-(V - \bar{V})/k]\}$, where Q_{\max} is the maximum charge that can be moved, \bar{V} is the potential at which half the charge moves, and k is a constant. At 15°C , these values were $Q_{\max} = 28.5$ nC/ μF , $\bar{V} = -34.2$ mV, and $k = 8.7$ mV. Q_{\max} , k , and \bar{V} exhibited little temperature dependence over the range 7 – 25°C . "Stepped OFF" charge movements were elicited by depolarizing the fiber from -90 mV to a fixed conditioning level that moved nearly all the mobile charge (0 mV), and then repolarizing the fiber to varying test potentials. The sum of the charge that moved when the fiber was depolarized directly from -90 mV to a given test potential and the stepped OFF charge that moved when the fiber was repolarized to the same test potential had at all test potentials a value close to Q_{\max} for that fiber. In nearly all cases, the decay phase of ON, OFF, and stepped OFF charge movements could be well fitted with a single exponential. The time constant, τ_{decay} , for an ON charge movement at a given test potential was comparable to τ_{decay} for a stepped OFF charge movement at the same test potential. τ_{decay} had a bell-shaped dependence on membrane potential: it was slowest at a potential near \bar{V} (the midpoint of the steady state charge distribution) and became symmetrically faster on either side of this potential. Raising the temperature from 7 to 15°C caused τ_{decay} to become faster by about the same proportion at all potentials, with a Q_{10} averaging 2.16 . Raising the temperature from 15 to 25°C caused τ_{decay} to become faster at potentials near \bar{V} , but not at potentials farther away. At the potentials where raising the temperature was without effect, τ_{decay} at both 15 and 25°C approached the value of the time constant of decay of the slow phase of the linear capacity transient. These observations can be explained by the hypothesis that the mobile charge is located in the t-system, and that at higher temperatures and potentials far from

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\bar{V} , propagation delays within the t-system become rate-limiting for the decay phase of charge movement.

INTRODUCTION

Excitation of contraction in a vertebrate skeletal muscle fiber is elicited by depolarization of the transverse-tubule (t-tubule) membrane. The mechanism that couples this depolarization to the release of calcium from the sarcoplasmic reticulum (SR) and to the subsequent activation of contractile proteins is as yet poorly understood. A voltage-dependent, nonlinear capacitative current thought to be associated with this mechanism has been observed in frog twitch (Chandler et al., 1976) and slow muscle fibers (Gilly and Hui, 1980), and in fast- and slow-twitch muscle from rat (Hollingworth and Marshall, 1981; Simon and Beam, 1983; Dulhunty and Gage, 1983). Since the stimulus for calcium release is a potential change across the t-tubule membrane, it has been hypothesized (Schneider and Chandler, 1973) that this capacitative current, which has been termed "charge movement," arises from the rearrangement of charged groups located within the membrane of the t-tubule system. In turn, this rearrangement of charge is thought to be the first step in the process that gates the release of calcium from the sarcoplasmic reticulum.

One of the aims of this study was to characterize the kinetics and steady state properties of charge movement in a mammalian muscle for comparison with those of charge movement in frog muscle, since any differences would be important in understanding the role of charge movement in excitation-contraction (E-C) coupling. Charge movements were elicited by a standard ON-OFF pulse sequence in which the fiber was depolarized from -90 mV to test potentials of -50 to $+50$ mV, and then repolarized to -90 mV. Also used was a stepped OFF pulse protocol in which potential was first stepped to 0 mV and then stepped back to test potentials from -10 to -90 mV. Thus, it was possible to study charge movements over the potential range from -90 to -50 mV, where little charge moves during an ON pulse.

Another aim of the study was to investigate the effect of elevating the temperature from 7 to 25°C . Because previous measurements of charge movements in rat muscle had been carried out at temperatures far below physiological, it was important to determine whether a temperature closer to physiological revealed any differences. In addition, in order to relate charge movement to its proposed role in governing the release of calcium from the SR, it is necessary to understand the molecular reaction scheme describing that movement. Inferring this reaction scheme from measured charge movements is complicated by the presumed tubular location of the mobile charge. Thus, it is difficult to know the extent to which the rising and falling phases of a charge movement reflect the underlying rate constants of charge redistribution and the extent to which they reflect the speed with which the voltage clamp is able to change potential within the t-system. Changing the temperature provides a powerful tool for investigating this problem.

We observed that the potential dependence of the steady state charge distribution was similar at 7, 15, and 25°C. This was not the case for the potential dependence of the time constant of charge movement decay. Although the dependence of the time constant on potential at 15°C was parallel to that at 7°C, the time constant at 25°C exhibited less voltage dependence. These observations have led us to the hypothesis that t-tubular delays influence the falling phase of charge movements at 25°C and must also influence the time course of the rising phase of charge movement at all temperatures. This hypothesis is examined quantitatively in the following paper (Simon and Beam, 1985).

Preliminary accounts of some of the results presented here have appeared previously (Simon and Beam, 1982, 1983).

METHODS

Charge movements were measured in the omohyoid, a fast-twitch skeletal muscle (Müntener et al., 1980), from male Sprague-Dawley rats weighing 400–500 g. Muscle fibers were voltage-clamped using the three-microelectrode voltage-clamp technique (Adrian et al., 1970). The omohyoid is especially suited to this technique because most of its fibers terminate on a tendon traversing the center of the muscle. In the three-microelectrode method, voltage-measuring electrodes V_1 and V_2 are inserted at distances of l and $2l$, respectively, from the tendon end of a fiber. The potential at electrode V_1 is controlled by passing current through a third electrode at a distance $2l + l'$ from the tendon end. The membrane current per unit fiber length is calculated according to:

$$i_m = 2\Delta V/3l^2r_i,$$

where $\Delta V = V_2 - V_1$, and r_i is the longitudinal resistance per unit fiber length (Adrian et al., 1970). In the experiments described here, l and l' were typically 210 and 70 μm , respectively. The voltage-measuring microelectrodes were filled with 3 M KCl, had resistances of 3–8 M Ω , and were coated with Q-dope (G.C. Electronics, Rockford, IL) to within 100 μm of the tip to decrease capacitance to bath ground. The current-passing microelectrodes were filled with 2 M K-citrate and had resistances of 5–12 M Ω . After amplification and electronic filtering (four-pole, low-pass Bessel, $f_{\text{cutoff}} = 4$ kHz), the signals ΔV , V_1 (the controlled voltage), and I (the current through the current-passing electrode) were sampled by an A/D converter at 4 kHz and stored in the memory of a PDP 11/03 computer (Digital Equipment Corp., Maynard, MA). Additional details of the voltage-clamp procedures are given elsewhere (Beam and Donaldson, 1983).

During the measurement of charge movements, the muscle was continuously perfused with an oxygenated solution that contained: 146 mM TEA-Br, 5 mM CsBr, 10 mM CaAc₂, 1 mM MgAc₂, 400 mM sucrose, 1 μM tetrodotoxin (TTX), and 10 mM HEPES, titrated to pH 7.4 with NaOH. Bromide and acetate were used as the extracellular anions to reduce the Cl⁻ conductance, which is known to be nonlinear and time dependent in mammalian skeletal muscle (Palade and Barchi, 1977).

Voltage-clamp command pulses were exponentially rounded with a time constant of 50–330 μs . Two pulse protocols were used. In a single sequence of the standard protocol, used to measure ON and OFF charge movements, four control steps 45 mV in amplitude were made from a base potential of -135 mV. These were followed by 31-ms test steps of increasing amplitude from the holding potential (-90 mV). The currents from the control steps were summed, appropriately scaled, and subtracted from each of the test currents. This individual sequence was repeated four times and the test currents at each

voltage were averaged. In the second protocol, which was used to measure stepped OFF (cf. Almers, 1975) and stepped ON charge movements, the test pulse consisted of a 31-ms step to 0 mV followed by a 31-ms step to test potentials of varying amplitude. The control steps and averaging procedure for the stepped OFF and stepped ON protocol were the same as for the standard protocol, except that the scaling and subtraction of the control current were done in two 31-ms segments appropriate for the test pulses in those intervals. Total charge moved was normalized by the total fiber capacitance and expressed in units of nanocoulombs per microfarad (nC/ μ F). This has the advantage of minimizing errors associated with leaks at the sites of electrode impalement (Schneider and Chandler, 1976). The total fiber capacitance was measured for a step in potential from -135 to -90 mV.

Exponentials were fitted to the decay phase of charge movements by a least-squares algorithm. The region of fit extended from the time at which the charge movement had decayed to 0.75 times its peak value, to the time at which it had decayed to 0.02 times its peak value. The criterion that the exponential $A \exp(-kt)$ be a least-squares fit to the N data points (y_i, t_i) requires that

$$P = \sum_1^N [y_i - A \exp(-kt_i)]^2$$

be minimized. For a given value of k , P is minimized when $\partial P/\partial A = 0$, which yields:

$$A = \frac{\sum_1^N y_i \exp(-kt_i)}{\sum_1^N \exp(-2kt_i)}.$$

Thus, P can be treated as a function of the single variable k . To determine the minimum of P , the zero of $f = \partial P/\partial k$ was found by the method of inverse linear interpolation (Abramowitz and Stegun, 1972, p. 18).

Numerical values given in the text are expressed as means \pm 1 SD.

RESULTS

ON and OFF Charge Movement

Fig. 1 illustrates uncorrected charge movements measured in rat omohyoid muscle fibers. The records in *A* were obtained from a fiber at 16.6°C and those in *B* from a fiber at 25°C . The charge movements were elicited by the standard ON-OFF protocol in which the fiber was depolarized for 31 ms from the holding potential (-90 mV) to the test potential indicated to the right of each trace, and then repolarized to the holding potential. For the records in *A*, there is good agreement between Q_{ON} , the charge that moves in response to the depolarizing pulse, and Q_{OFF} , the charge that moves back in response to the repolarizing pulse ($Q_{\text{OFF}}/Q_{\text{ON}}$ was 0.97 ± 0.06 , range 0.88–1.05, for test pulses of +10 mV or less, and 1.30 for the test pulse to +20 mV). The near equality of Q_{ON} and Q_{OFF} is consistent with the idea that these nonlinear currents reflect the movement of membrane-bound charges, and suggests that the records are relatively free of contamination by ionic current. As another indication of the absence of "ionic contamination," the ON transients decay back to a steady state level that is essentially flat and equal to the zero-current level. By contrast, the ON charge movements in Fig. 1*B* do not return to a horizontal baseline. Instead, there is a "pedestal" of current that follows the decay of the ON transient. The pedestal slopes upward for the test pulses to -30 , -20 , and -10 mV, and slopes downward for the test pulse to +10 mV.

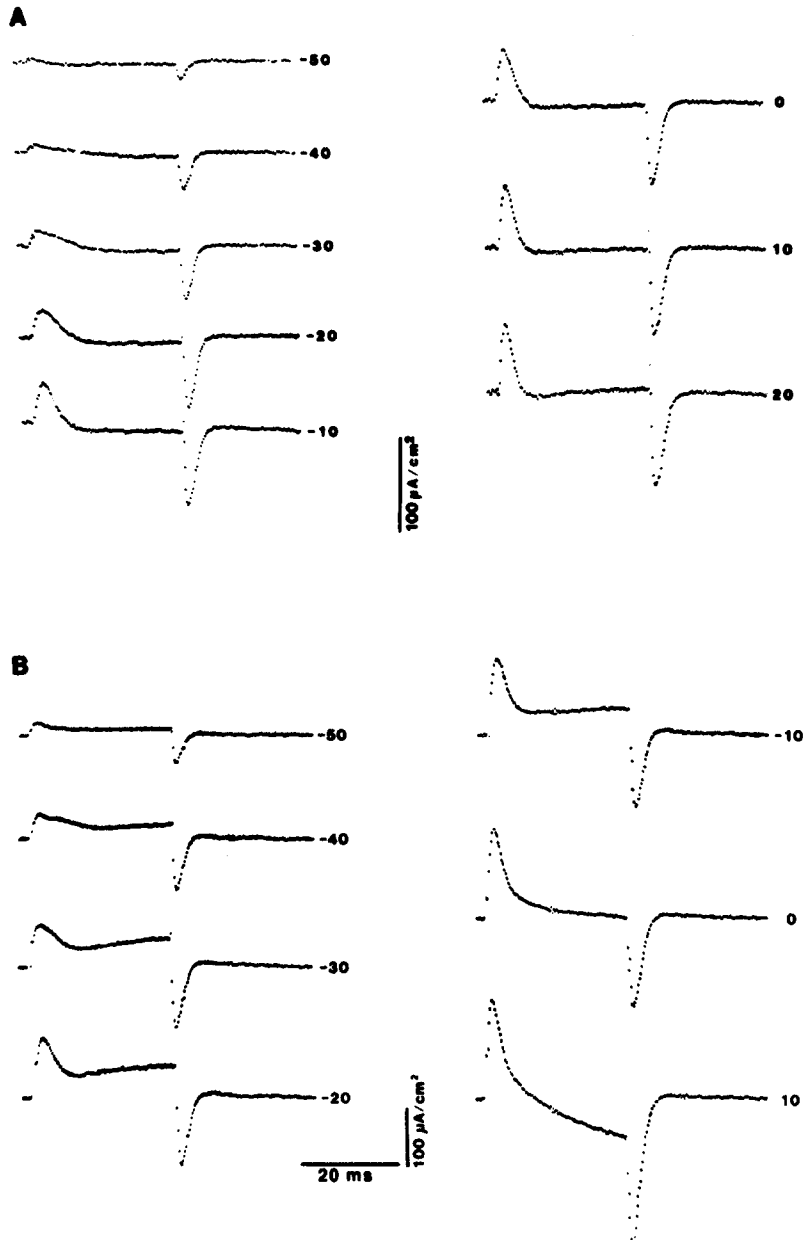


FIGURE 1. Uncorrected ON and OFF charge movements at 16.6 and 25°C. Depolarizing test steps, 31 ms in duration, were superimposed on the holding potential, -90 mV. The potential during the test step is indicated next to each trace. (A) Charge movements at 16.6°C. Muscle 101-5. (B) Charge movements at 25°C. Muscle 99-2.

The activation of a time-dependent, outward ionic current can account for the upwardly sloping pedestal. It was usually possible to correct for this kind of time-dependent nonlinearity by mathematically subtracting a sloping baseline as described by Horowicz and Schneider (1981). After the application of this correction to the records in Fig. 1*B* at -30 , -20 , and -10 mV, Q_{OFF} agreed with Q_{ON} to within $\pm 15\%$. (The appearance of the ON charge movements after correction is illustrated in Fig. 3 of the following paper [Simon and Beam, 1985]). The agreement between Q_{OFF} and Q_{ON} suggests that the ionic channels responsible for the upwardly sloping pedestal contribute little ionic "tail" current following repolarization to -90 mV.

Both its kinetics and its sensitivity to extracellular cadmium argue that the downwardly sloping pedestal represents ionic current through calcium channels that are present in the omohyoid muscle (Donaldson and Beam, 1983). Correction was difficult because there was often no clear-cut kinetic separation between the decay of the ON transient and the downward slope of the pedestal. Moreover, when a downwardly sloping pedestal was present, ionic contamination of the OFF charge movement also appeared to be present. For example, after application of the sloping baseline correction to the record at $+10$ mV in Fig. 1*B*, Q_{OFF} was twice as large as Q_{ON} . Similar ionic contamination of Q_{OFF} was also sometimes present even when the ON transient's pedestal showed no obvious downward slope (e.g., Q_{OFF} is $\sim 50\%$ larger than Q_{ON} for the record at 0 mV in Fig. 1*B*). It seems likely that under these circumstances the activation of inward calcium current is masked by the roughly simultaneous activation of outward current. Based on the presence of downwardly sloping pedestals and/or values of Q_{OFF} that greatly exceeded Q_{ON} , contamination by inward calcium currents was frequently present in records obtained at 25°C . The contamination by calcium currents could be reduced by the inclusion of 0.5 mM Cd^{2+} in the bathing medium, but since cadmium led to the more rapid deterioration of fibers, its routine use was avoided. In addition, most experiments were carried out at 15°C , where the calcium currents for test pulses less than $+20$ mV were sufficiently small and slow that they were not a problem.

The Amount of Charge Moved as a Function of Potential

The dependence of the amount of charge (Q) that moves as a function of test potential (V) is illustrated in Fig. 2. Fig. 2*A* presents the average of the normalized Q vs. V relationships obtained in eight fibers at 15°C . The solid line is a best fit to the data points of the Boltzmann expression:

$$Q/Q_{\text{max}} = 1/\{1 + \exp[-(V - \bar{V})/k]\},$$

where Q_{max} is the total amount of charge available to move, \bar{V} is the potential at which half the charge has moved, and k is a constant related to the steepness of the curve. For the eight fibers in Fig. 2*A*, Q_{max} was 28.5 ± 3.4 nC/ μF , and a linear regression analysis of $\ln(Q_{\text{max}}/Q - 1)$ vs. V yielded the values $\bar{V} = -34.2$ mV and $k = 8.7$ mV. These are similar to the values reported for frog muscle by Chandler et al. (1976) and, if experimental differences are taken into account (see Discussion), roughly the same as the values reported for rat extensor

digitorum longus muscle (EDL) by Hollingworth and Marshall (1981) and Dulhunty and Gage (1983).

In addition to measuring charge movements at 15°C, we also measured charge

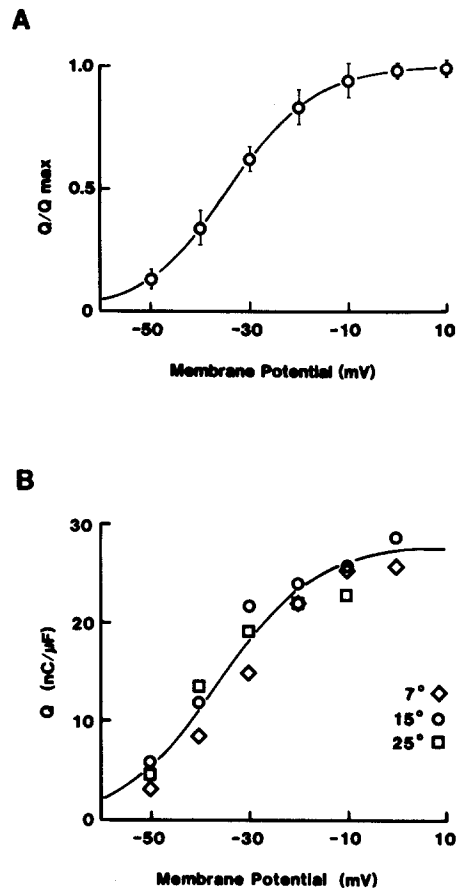


FIGURE 2. (A) Normalized steady state distribution of charge as a function of potential. Charge movements were measured in eight fibers at a temperature of 15°C, and corrected as necessary by subtraction of a sloping baseline. At each potential V , $Q(V)$ was taken to be the average of Q_{ON} and Q_{OFF} , which were obtained by integration of the ON and OFF transients. The $Q(V)$ values for a given fiber were normalized by the largest value of Q (Q_{max}) for that fiber and the normalized $Q(V)$ values were then averaged. The smooth curve represents $Q/Q_{max} = 1/\{1 + \exp[-(V - \bar{V})/k]\}$, where $\bar{V} = -34.2$ mV, $k = 8.7$ mV, and $Q_{max} = 28.3 \pm 3.4$ nC/ μ F. Error bars denote ± 1 SD. (B) Steady state distribution of the absolute amount of charge moved as a function of potential in a single fiber at 7, 15, and 25°C. The smooth curve is a visual fit of the Boltzmann expression to the average of the charge values at each test potential. Muscle 99-2.

movements at other temperatures ranging from 7 to 25°C. Comparing the Q vs. V relationship at these varying temperatures was difficult because at the higher temperatures the charge movements were contaminated by calcium currents for

test potentials ≥ 0 mV. This ionic contamination prevented the accurate determination of Q_{\max} and thus the normalization of charge. We therefore adopted the procedure illustrated in Fig. 2B, which plots the potential dependence of the absolute amount of charge moved in a single fiber at 7, 15, and 25°C. For the sake of comparison, the solid line represents the Boltzmann expression visually fit to the average of the three charge values at each potential. The steady state Q vs. V relationship is about the same at all three temperatures, although there appears to be a slight depolarizing shift and a decrease in steepness as the temperature is lowered from 15 to 7°C. This small shift and the change in steepness were also revealed by comparing averaged, normalized data at 7 and 15°C. A visual Boltzmann fit to the average of the normalized $Q(V)$ values in four fibers at 7°C yielded values of $k = 10.5$ mV, $\bar{V} = -32$ mV, and $Q_{\max} = 30.4 \pm 3.7$ nC/ μ F. It is not clear how much confidence should be placed in the differences between these values for k and \bar{V} and those at 15°C, however, because the small size and slow time course of charge movements at 7°C made the integration more difficult. The main conclusion from our data, therefore, is that temperature little affects the steady state charge vs. potential relationship. The same conclusion was reached by Hollingworth and Marshall (1981), who found that in both frog and rat muscle, raising the temperature from 2 to 15°C had no effect on the amount or steady state distribution of charge.

Saturation of Charge Moved at Large Positive Potentials

The usual procedure for comparing the equilibrium charge distribution measured in different laboratories and in different muscle types is to compare the parameters k , \bar{V} , and Q_{\max} obtained from fitting the Boltzmann expression to steady state Q vs. V data. The values for k and \bar{V} depend critically on the value of Q_{\max} , which can be accurately determined only if the Q vs. V relationship shows saturation and if the largest test depolarization employed is sufficient to move all the available charge. We did not routinely measure charge movements for test steps beyond +10 mV because stronger depolarizations often activated ionic currents, which made an accurate determination of Q_{ON} and Q_{OFF} difficult. However, in a few fibers where ionic contamination of the ON transient was small, Q_{ON} for depolarizations up to +50 mV was not significantly different from Q_{ON} at +10 mV. An example is illustrated in Fig. 3, which shows uncorrected charge movements for depolarizations to -10, +10, and +50 mV. At these three potentials, Q_{ON} was 26.3, 26.8, and 25.9 nC/ μ F, respectively, and Q_{OFF} was 25.6, 35.6, and 66.5 nC/ μ F. Presumably, Q_{OFF} fails to saturate because the test depolarizations to +10 and +50 mV activated calcium channels (see above) that contaminated the OFF transients with an inward ionic tail current. The activation of these channels interferes less with the resolution of Q_{ON} both because the driving force for calcium current is much smaller during the test potential and because the rate of activation at 15°C is slow compared with the ON transient. Thus, although Q_{OFF} cannot be determined for large depolarizations that activate ionic currents, Q_{ON} shows saturation for depolarizations to ~ 0 mV and no additional charge appears to move for further depolarizations to +50 mV.

Fig. 4 illustrates a second test of whether additional charge moves for potentials

greater than +10 mV. The fiber was first depolarized for 31 ms to +10 mV and then further depolarized to potentials varying from +20 to +50 mV. After the second upward step, the current was seen to increase monotonically with time, as if the additional depolarization caused the activation of an outward current. The presence of this time-dependent increase in outward current makes it difficult to rule out the possibility that a small additional amount of charge moved in response to the second step. As a means of determining how much additional charge could have moved without being detected, we used the computer to add or subtract a scaled version of the ON transient at +10 mV from the current elicited by the second upward step. If >10% of the ON transient at +10 mV was added to, or subtracted from, the current elicited by the second step, it was clearly detected as an early "hump" or "notch" on the rising current.

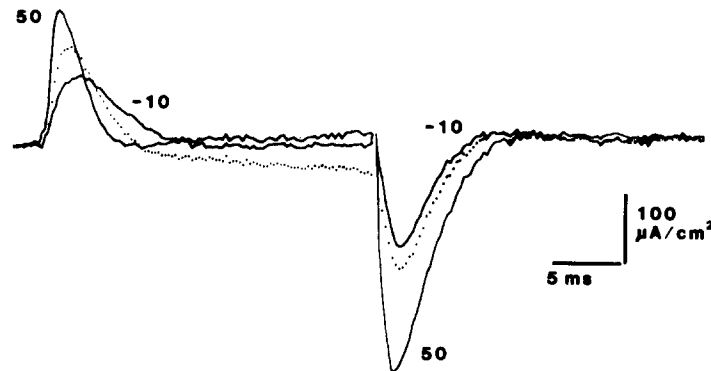


FIGURE 3. Saturation of Q_{ON} . Uncorrected charge movements are shown for test pulses of -10, +10 (dots), and +50 mV. After correction for sloping baselines, the areas under the ON transients were computed to be 26.3, 26.8, and 25.9 nC/ μ F, and under the OFF transients -25.6, -35.6, and -66.5 nC/ μ F, at -10, +10 and +50 mV, respectively. Note that although the ON charge movement at +50 mV decays to a nearly flat baseline, the subsequent OFF charge movement appears to be contaminated by an ionic tail current since $Q_{OFF} > Q_{ON}$. Muscle 99-1. Temperature, 14.4°C.

The smooth increase in current, without any obvious humps or notches, taken together with the data of the previous figure, is consistent with the conclusion that ON charge movement saturates at ~ 0 mV.

The Q_γ Component of Charge Movement

Two components of slow charge movement have been identified in frog twitch muscle (Adrian and Peres, 1979; Huang, 1982; Horowicz and Schneider, 1981; Hui, 1982*b*, 1983*a, b*). In addition to the major component, which has been termed Q_β , a component termed Q_γ is seen at some potentials as a secondary bump on the falling phase of ON charge movements. Based on its voltage dependence and its sensitivity to agents known to interfere with E-C coupling, it has been suggested that Q_γ is more directly associated with calcium release from

the SR than is Q_β (Huang, 1982; Hui, 1982*b*, 1983*a*, *b*; Vergara and Caputo, 1983). Because it has been hypothesized to be causally related to calcium release in frog muscle, we attempted to determine whether a component of charge similar to Q_γ is also present in rat muscle.

In fibers studied at 7°C, no obvious bump was present, and the decay phase of ON charge movements at all test potentials could be well fitted with a single exponential. Similarly, no bump was present in most fibers studied at 15 or 25°C, although in a few fibers a small bump could be seen at potentials in the range -40 to -20 mV. Fig. 5 illustrates an example of a fiber in which a bump that may correspond to the Q_γ component of frog muscle is present in the decay phase at -30 mV. In order to obtain an estimate of the bump's magnitude, the following procedure was adopted. First, single exponentials were fitted, as

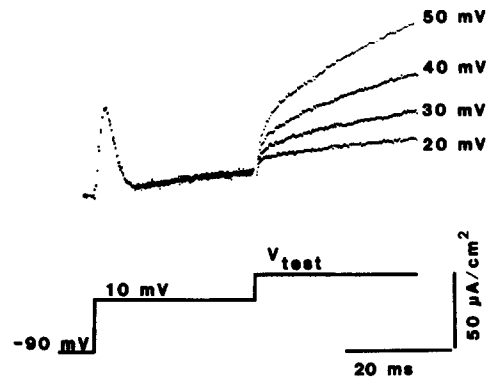


FIGURE 4. Absence of additional charge movement for steps from +10 mV to more depolarized potentials. Potential was stepped from -90 to +10 mV for 31 ms, and then further depolarized to the indicated potentials. Traces are shown uncorrected. The step to +10 mV appears to have been sufficient to have moved nearly all the mobile charge, since only a monotonic increase in outward current is seen after the second upward step. As described in the text, if more than ~10% additional charge had moved between +10 and +50 mV, it would have been visible as a "hump" or "notch" superimposed on the monotonic increase. Muscle 101-5. Temperature, 16.6°C.

described in Methods, to the decay phase of ON and stepped OFF (see below) charge movements at all test potentials except -30 mV. The exponentials (solid lines) superimposed on the charge movements at -40 and -20 mV were obtained in this fashion. Second, the values of τ_{decay} obtained from the best-fitting exponentials were plotted against potential. Third, because the constant field expression (Chandler et al., 1976) gives a good description of the decay rates of charge movement at 7 and 15°C in the rat omohyoid muscle (Simon and Beam, 1985), it was fitted to the τ_{decay} vs. potential data and interpolated to obtain τ_{decay} at -30 mV. An exponential with this value of τ_{decay} and amplitude visually adjusted to match the initial portion of decay is shown superimposed on the charge movement at -30 mV. The area between the current trace and the exponential is ~2.5 nC/ μ F, or ~8% of Q_{max} in this fiber. Of the fibers examined at 15°C, this

represents the largest bump observed. The value might be somewhat different if we had used a different estimating procedure, but it seems clear that the bump in rat muscle is smaller than in frog muscle, where the total movable charge associated with Q_γ has been estimated to be 20–40% of Q_{\max} (Huang, 1982; Hui, 1983b).

In frog muscle, Q_γ is observed as a kinetically distinct component over only a limited range of potentials, outside of which it melds with Q_β . To eliminate the possibility that a larger Q_γ component was going undetected because we failed to examine the appropriate potentials in rat muscle, we explored the potential range between -40 and -20 mV using test pulses at 2.5-mV intervals. This protocol did not reveal any indication of a bump larger than that revealed by

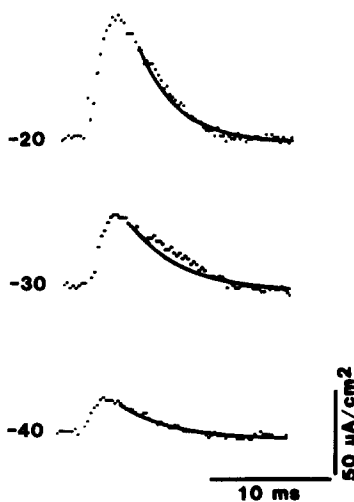


FIGURE 5. A “bump” component of charge movement. ON charge movements are shown for depolarizations to -40 , -30 , and -20 mV. The solid lines are single exponentials, obtained as described in the text, with time constants of 3.4, 3.7, and 3.4 ms, respectively, at -40 , -30 , and -20 mV. The area between the exponential and the data points at -30 mV is 2.5 nC/ μ F. A small bump also appears to be present at -20 mV. Muscle 99-1. Temperature, 14.4°C .

the 10-mV increments of the standard protocol. Because Hui (1982a) had reported that elevating sucrose from 350 to 467 mM abolishes Q_γ in frog muscle, we also measured charge movements using an extracellular solution in which the sucrose concentration had been reduced from 400 to 320 mM. The reduced sucrose did not significantly increase the magnitude of the bump, and muscle movement prevented the testing of still lower sucrose concentrations.

Stepped OFF Charge Movement

The standard ON-OFF pulse protocol permits an analysis of charge movement only for depolarizations to potentials more positive than about -50 mV, because for depolarizations to more negative potentials virtually no charge moves. As a

means of investigating charge movements over the potential range from -90 to -50 mV, a stepped OFF protocol was used. In this protocol, the fiber was first depolarized for 31 ms from -90 to 0 mV in order to move most of the charge, and then repolarized back to an intermediate test potential to study the return movement of charge. The fiber was depolarized to 0 mV, rather than $+10$ or $+20$ mV, where saturation of charge would be more complete, to minimize contamination of the stepped OFF charge movements by ionic currents. Fig. 6 shows a series of charge movements for stepped OFF test potentials ranging from -10 to -70 mV. The amount of recovered charge increases, and, below \bar{V} , the falling phase becomes faster as the repolarizing test potential becomes more

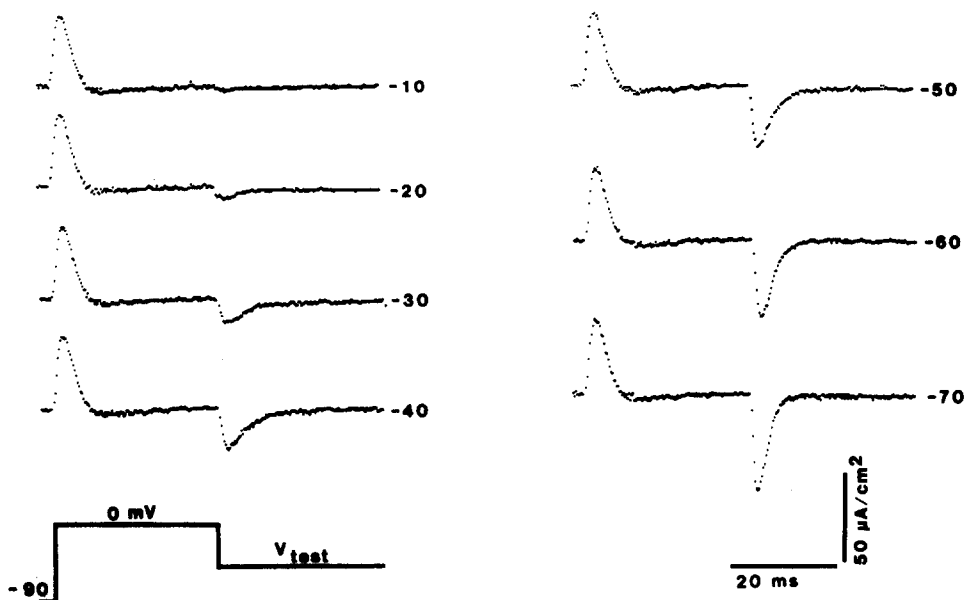


FIGURE 6. Stepped OFF charge movements. The pulse protocol is illustrated on the lower left. The test potential (V_{test}) is indicated to the right of each current trace. Muscle 101-5. Temperature, 16.6°C .

negative. The stepped OFF charge movements have a slow rising phase similar to that of ON charge movements.

The stepped OFF protocol provides an additional means of testing the hypothesis that the measured asymmetry currents result from the rearrangement of membrane-bound charge. Specifically, the amount of charge that moves during any voltage step must depend only on the initial and final potentials, which is not the case for the asymmetry currents predicted by "transient ionic models" (e.g., Mathias et al., 1980). Thus, the sum of the charge moved for an ON transient (Q_{ON}) from -90 mV to a given potential and a stepped OFF transient ($Q_{stepped\ OFF}$) from 0 mV to the same potential should equal the amount of charge that moves for a depolarization from -90 directly to 0 mV, independent of the

intermediate test potential. Fig. 7 is a plot of Q_{total} , the sum of $Q_{\text{ON}} + Q_{\text{stepped OFF}}$, as a function of potential from four fibers at 15°C. The sum is nearly constant over the whole potential range, and roughly equal to Q_{max} , the charge moved for a depolarization directly to 0 mV. This result supports the hypothesis that charge movement is due to the rearrangement of charge confined within the membrane and cannot be easily reconciled with ionic models for charge movement.

Effects of Temperature on the Measured Kinetics of Charge Movement

Fig. 8 illustrates the effect of temperature on the rate of charge movement decay. Plotted as a function of potential are the time constants (τ_{decay}) of exponentials fitted to the falling phases of ON and stepped OFF charge movements at 7, 15, and 25°C. Time constants of decay were obtained for ON charge movements (τ_{ON}) at potentials ranging from -50 to +10 mV, and for stepped

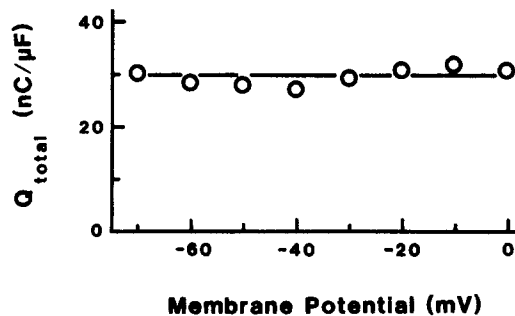


FIGURE 7. The sum (Q_{total}) of Q_{ON} and $Q_{\text{stepped OFF}}$ is independent of potential. The values of Q_{ON} and $Q_{\text{stepped OFF}}$ from four fibers were summed at each potential, averaged, and plotted as a function of voltage. The solid horizontal line was drawn with an ordinate value equal to the average of Q_{total} at all the potentials. Standard errors of the means all fell within the symbols. Temperature, 15°C. Muscles 99-1, 99-2, 100-3, and 101-5.

OFF charge movements ($\tau_{\text{stepped OFF}}$) at potentials ranging from -30 to -90 mV. Values were not obtained for $\tau_{\text{stepped OFF}}$ at 25°C because the stepped OFF charge movements at this temperature were contaminated by ionic currents. The potential dependence of τ_{decay} in the rat omohyoid muscle is similar to that described for the rat EDL muscle (Hollingworth and Marshall, 1981) and frog sartorius muscle (Chandler et al., 1976; Hui, 1983b). Decay rates are slowest at a potential near the midpoint of the Q vs. V relationship and become symmetrically faster on either side of this potential. Thus, in Fig. 8, τ_{decay} at both 7 and 15°C is greatest at -30 mV, which is close to the average values of \bar{V} at those temperatures (-34 mV at 15°C, -32 mV at 7°C). Additionally, as is shown in the next paper (Simon and Beam, 1985), the constant field expression used by others to describe τ_{decay} vs. V in frog muscle (Chandler et al., 1976; Hollingworth and Marshall, 1981; Hui, 1983b) also gives a good description of τ_{decay} vs. V in the omohyoid at both 7 and 15°C.

When the temperature was raised from 7 to 15°C, τ_{decay} decreased by roughly a factor of 2 at all potentials. The Q_{10} calculated from the ratio of τ_{decay} at 7 and 15°C, and averaged over all potentials, is 2.16 ± 0.32 ($n = 10$, range 1.8–2.4). Raising the temperature from 15 to 25°C caused a smaller decrease in τ_{decay} ($Q_{10} = 1.25 \pm 0.3$, $n = 6$), and the magnitude of this decrease varied considerably with potential. The Q_{10} varied between ~ 1.6 at -30 mV, where charge movements are slowest, to ~ 1.0 at $+10$ and -90 mV, where charge movements are fastest. Thus, in contrast to its behavior over the lower temperature range, the Q_{10} between 15 and 25°C is no longer voltage independent.

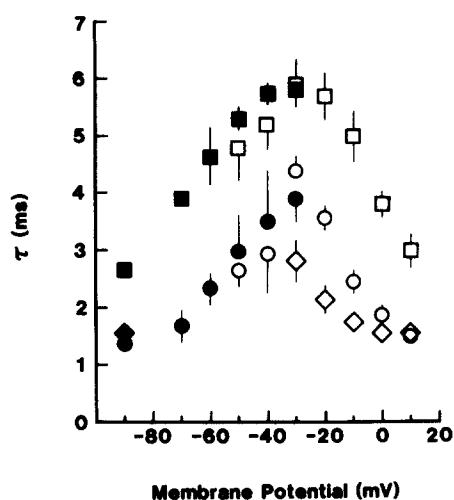


FIGURE 8. The time constant of decay of charge movement (τ_{decay}) as a function of potential at temperatures ($\pm 1^\circ\text{C}$) of 7 (squares), 15 (circles), and 25°C (diamonds). Open symbols were obtained from ON charge movements at test potentials ranging from -50 to $+10$ mV. Filled symbols were obtained from stepped OFF charge movements at test potentials ranging from -30 to -90 mV. Values for $\tau_{\text{stepped OFF}}$ were not plotted at 25°C because the stepped OFF charge movements at this temperature were contaminated by ionic currents. At 25°C, the values of τ_{decay} at -90 mV were obtained from a repolarization to -90 mV from a test step to -10 mV. $\tau_{\text{decay}}(-90$ mV) was found to be independent of the prior test potential; the step to -10 mV was chosen to avoid activation of calcium currents at 25°C. Error bars denote ± 1 SD.

Fig. 9 gives a dramatic illustration of the potential-dependent variation of the effect of temperature on charge movement kinetics. Fig. 9A compares charge movements measured in a fiber at 8 and 15°C for a test pulse to -10 mV, and Fig. 9B illustrates charge movements measured with the same test pulse in another fiber at 15 and 25°C. Raising the temperature from 8 to 15°C caused an increase in the decay rate of both the ON and OFF transients, whereas raising the temperature from 15 to 25°C increased the decay rate of the ON transient but not that of the OFF. A similar comparison shows that increasing the temperature from 15 to 25°C only slightly increases the decay rate of ON charge movements at potentials of 0 and $+10$ mV. In addition to affecting the decay of

charge movement, changing the temperature also affected the rising phase. The duration of the rising phase of both the ON and OFF transients was shorter at 15 than at 8°C. By contrast, the rising phases of the OFF transients were nearly identical at 15 and at 25°C, and the rising phases of the ON transients also matched over most of their time course, despite the fact that the decay at 25°C was faster. Thus, raising the temperature failed to affect either the rising or falling phase of charge movement when these were relatively rapid.

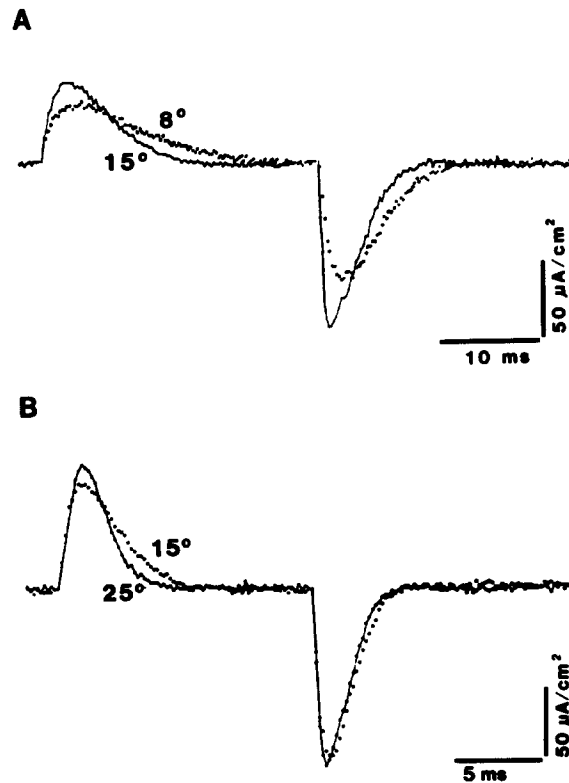


FIGURE 9. The effect of temperature on charge movement kinetics. The test pulse was a 31-ms-long depolarization to -10 mV, superimposed on the holding potential of -90 mV. (A) Muscle 91-4; $\tau_{ON} = 7.12$ (8°C), 4.33 (15°C) ms; $\tau_{OFF} = 3.30$ (8°C), 2.55 (15°C) ms; time constant of the linear capacity transient (τ_{lct}) = 0.95 ms. (B) Muscle 99-2. $\tau_{ON} = 2.54$ (15°C), 1.66 (25°C) ms; $\tau_{OFF} = 1.56$ (15°C), 1.42 (25°C) ms; $\tau_{lct} = 0.8$ ms.

The effect of temperature on the linear capacity transient was also investigated. The linear capacity transient was measured for a step in potential from -135 to -90 mV, and the slow phase of the transient was fitted with a single exponential. The time constant, τ_{lct} , had values of 1.25 ($n = 1$), 0.98 ± 0.09 ($n = 4$), 0.77 ± 0.12 ($n = 8$), and 0.69 ± 0.10 ms ($n = 4$), respectively, at 3.5, 7, 15, and 25°C.

An hypothesis that may account for the effects of temperature on the time course of charge movement is that t-tubular delays alter the measured kinetics.

If charge movement is involved in E-C coupling, then the mobile charge would be expected to reside in the t-system. Thus, the measured kinetics of charge movements recorded on the surface of the fiber would be complicated by the inability of the voltage clamp to rapidly control voltage across the t-tubule membrane. An indication of the time during which voltage across the t-tubule membrane is changing is given by the time course of the slow phase of the linear capacity transient, which, as just described, decays with a time constant of ~ 1 ms and is not strongly affected by temperature. Thus, in the omohyoid, t-tubular delays might be expected to significantly slow measured charge movements whenever the underlying charge redistribution decayed with a time constant on the order of a millisecond, i.e., at high temperatures and at potentials far from \bar{V} . The next paper (Simon and Beam, 1985) presents a quantitative analysis of the effects of tubular delays on the kinetics of charge movement.

DISCUSSION

Charge movement in the rat omohyoid muscle is similar in many respects to charge movement in frog muscle. Based on fits of the Boltzmann expression, the steady state potential dependence of charge moved in the omohyoid agrees closely with that in frog muscle reported by Chandler et al. (1976). Our values of $Q_{\max} = 28.3$ nC/ μ F, $k = 8.7$ mV, and $\bar{V} = -34$ mV are similar to their values of 25 nC/ μ F, 8 mV, and -44 mV, respectively. Strictly speaking, our value for \bar{V} should be adjusted to -41 mV to account for the effect of the higher calcium concentration (10 vs. 2 mM) used in our experiments (Costantin, 1968). If a Q_{10} of 2 is assumed, the time constants of charge movement decay at 7°C in the omohyoid agree with the values determined in frog at 1.4°C by Chandler et al. (1976).

In rat EDL, values of $Q_{\max} = 23.4$ nC/ μ F, $\bar{V} = -19$ mV, and $k = 13.3$ mV have been reported by Dulhunty and Gage (1983), and corresponding values of 48.9 nC/ μ F, -25 mV, and 14 mV have been reported by Hollingworth and Marshall (1981). To compare these values with ours, it is necessary to adjust for differences in the bathing media used in the three sets of experiments. Our medium contained hypertonic sucrose and 10 mM Ca, Hollingworth and Marshall's contained hypertonic sucrose and 2 mM Ca, and Dulhunty and Gage's contained no sucrose, 2 mM Ca, and 2 mM tetracaine. Compared with an isotonic medium containing 2 mM Ca, hypertonic sucrose should have caused a shift in the Q vs. V relationship of about -20 mV (Table II, Almers and Best, 1976), 10 mM Ca a shift of about $+7$ mV (Costantin, 1968), and tetracaine relatively little shift (Almers, 1976). Thus, our value of \bar{V} should be adjusted to -21 mV for comparison with Dulhunty and Gage's value of -19 mV, and our value of \bar{V} adjusted to -41 mV for comparison with the -25 mV reported by Hollingworth and Marshall (1981). Thus, our values for Q_{\max} and \bar{V} agree well with those of Dulhunty and Gage, but less well with those of Hollingworth and Marshall. Our value of k is smaller than reported for EDL.

The Q_γ Component of Charge Movement

One significant difference between charge movements in rat and frog muscle is the absence of a prominent Q_γ component in rat. Thus, Hollingworth and

Marshall (1981) found that a single exponential provided a good fit to the decay phase of the charge movements they measured in the rat EDL, and we found this to be true for the majority of the charge movements we measured in the omohyoid. We occasionally observed charge movements for test potentials in the vicinity of \bar{V} that decayed anomalously slowly or even showed a small secondary rise on the falling phase, which is suggestive of the presence of a small Q_γ component of charge movement.

An estimate of the magnitude of the small rise observed in the ON charge movement to -30 mV in Fig. 5 was obtained by assuming that Q_β decays exponentially, and that the time constant of decay varies with potential according to the constant field expression (Simon and Beam, 1985). With these assumptions, the bump at -30 mV in Fig. 5 represents 2.5 nC/ μ F, considerably less charge than reported for Q_γ in frog (Huang, 1982; Hui, 1983a). One cannot entirely rule out the possibility that a Q_γ component is present in rat with a magnitude comparable to that in frog. For instance, if Q_γ in rat has kinetics and a steady state distribution similar to those of Q_β , then the procedure described above is inappropriate for determining the total magnitude associated with Q_γ . If this were the case, a comparison of charge movements before and after the addition of the pharmacological agents that have been used to suppress Q_γ in frog (Huang, 1982; Hui, 1983a) would be required to separate Q_γ from Q_β in rat.

The Temperature Dependence of Charge Movement Kinetics

The kinetic behavior of the charge movements we have measured in the rat omohyoid muscle at 7 and 15°C is similar to that of charge movements previously measured in frog (Chandler et al., 1976) and rat muscle (Hollingworth and Marshall, 1981), but the charge movements we measured at 25°C reveal important differences. Specifically, at 7 and 15°C , the decay rate of ON and stepped OFF charge movements depends on potential in a fashion well described by the constant field expression (see Simon and Beam, 1985). The decay rate is minimal near \bar{V} and increases symmetrically on either side. Raising the temperature from 7 to 15°C shortens the time to peak and speeds the falling phase of charge movements. For this temperature increase, the Q_{10} is essentially independent of voltage and has a value, averaged over all potentials, of 2.16 ± 0.32 . This Q_{10} is similar to the values reported for frog sartorius muscle by Dr. C. S. Hui (personal communication) and for rat EDL muscle by Hollingworth and Marshall (1981). At both 7 and 15°C , the ON and stepped OFF charge movements decay much more slowly than the linear capacity transient, and thus the effects of t-tubular delays would be expected to be minimal. At 25°C , however, the decay rate of charge movements approaches that of the linear capacity transient, which suggests that at this temperature, delays associated with charging of the t-system might be much more important. We observed that the decay rate at 25°C increased much less as potential was moved away from \bar{V} than at lower temperatures. As a result, the Q_{10} for the temperature increase from 15 to 25°C is much smaller at potentials far from \bar{V} than at potentials near \bar{V} . These results suggest that the mobile charge resides in the t-system and that tubular delays must be incorporated into any kinetic model of charge movement. The following

paper (Simon and Beam, 1985) presents a quantitative analysis of the effect of tubular delays on charge movement kinetics.

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