Comparison of Charge Movement Components in Intact and Cut Twitch Fibers of the Frog

Effects of Stretch and Temperature

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ABSTRACT Charge movements were measured in frog intact fibers with the three-microelectrode technique and in cut fibers with the double Vaseline gap technique. At 13-14°C, the ON segments of charge movement records from both preparations showed an early I₀ component and a late Iᵥ hump component. When an intact fiber was cooled to 4-7°C, the time-to-peak of Iᵥ (tᵥ) was prolonged, but Iᵥ still appeared as a hump. Q-V plots from intact fibers at 4-7°C were fitted with a sum of two Boltzmann distribution functions (method 1). The more steeply voltage-dependent component, identified with Qᵥ, accounted for 32.1% (SEM 2.2%) of the total charge. This fraction was larger than the 22.6% (SEM 1.5%) obtained by separating the ON currents with a sum of two kinetic functions (method 2). The total charge in cut fibers stretched to a sarcomere length of 3.5 μm at 13-14°C was separated into Q₀ and Qᵥ by methods 1 and 2. The fraction of Qᵥ in the total charge was 51.3% (SEM 1.7%) and 53.7% (SEM 1.8%), respectively, suggesting that cut fibers have a larger proportion of Qᵥ:Q₀ than intact fibers. When cut fibers were stretched to a sarcomere length of 4 μm, the proportion of Qᵥ:Q₀ was unchanged. Between 4 and 13°C, the Qᵥ of 1/tᵥ in intact fibers was 2.33 (SEM 0.33) and that of 1/τᵥ was <1.44 (SEM 0.04), implying that the kinetics of Iᵥ has a steeper temperature dependence than the kinetics of I₀. When cut fibers were cooled from 14 to 6°C, I₀ in the ON segment generally became too broad to be manifested as a hump. In a cut fiber in which Iᵥ was manifested as a hump, the Qᵥ of 1/tᵥ was 2.08 and that of 1/τᵥ was <1.47. Separating the Q-V plots from cut fibers at different temperatures by method 1 showed that the proportion of Qᵥ:Q₀ was unaffected by temperature change. The appearance of Iᵥ humps at low temperatures in intact fibers but generally not in cut fibers suggests an intrinsic difference between the two fiber preparations.

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INTRODUCTION

In normally polarized intact frog skeletal muscle fibers, charge movement during the “ON” of a depolarizing pulse can be visually separated into two components. Adrian and Peres (1979) defined the charge associated with the early current component as $Q_a$ and the charge associated with the delayed current component as $Q_v$. To differentiate between current and charge, $I_a$ and $I_v$ will be used in this and the following paper to represent, respectively, the early and delayed charge movement components in current traces, and $Q_a$ and $Q_v$ to represent the time integrals of $I_a$ and $I_v$, respectively. In intact fibers, Hui (1983b) found that the $Q_v$ component so separated is more steeply voltage dependent than the $Q_a$ component. In a recent paper, Hui and Chandler (1990) separated the steady-state $Q-V$ plot from cut fibers into two components and used $Q_a$ and $Q_v$ to denote, respectively, the less steeply and more steeply voltage-dependent components. The two sets of definitions of $Q_a$ and $Q_v$ will be used interchangeably. However, it should be noted that the equivalence of the two sets of definitions has not been established.

The $I_v$ humps recorded in cut fibers by Hui and Chandler (1990) were more prominent than those in intact fibers (Adrian and Peres, 1977, 1979; Huang, 1982; Hui, 1983a, b). The first goal of this paper was to investigate whether cut fibers have a larger fraction of $Q_v$ in the total charge than intact fibers. For both preparations, $Q_a$ and $Q_v$ were separated by fitting a sum of two kinetic expressions to the ON segments of charge movement records (Hui, 1983b) and by fitting a sum of two Boltzmann distribution functions to steady-state $Q-V$ plots (Hui and Chandler, 1990). Although the two methods gave slightly different results, there was definitely a larger fraction of $Q_v$ in the total charge in cut fibers than in intact fibers.

While $I_v$ humps have been routinely observed in intact fibers, it was sometimes absent in charge movement traces recorded from cut fibers. Melzer et al. (1986) suggested that, in highly stretched fibers, $I_v$ might still be present but does not appear as a hump. The second goal of this paper was to study the effect of stretch on the appearance of $I_v$. Results from both intact and cut fibers showed that $I_v$ humps were resolvable in the ON segments of charge movement traces, when the fibers were stretched to a sarcomere length of 3.8-4.0 µm (although the $I_v$ hump in intact fibers might be slightly less obvious due to the inherent difficulty in measuring charge movement in highly stretched intact fibers with the three-microelectrode voltage clamp technique).

Thus, the disappearance of $I_v$ humps in cut fibers is probably not caused by stretch but by other factors. Hui and Chandler (1990) found that $SO_4^{2-}$ replacement of $Cl^-$ in the external solution increased the amount of $Q_a$ in cut fibers two- to threefold, making $I_v$ much less resolvable in the ON segments of TEST minus CONTROL current traces. This paper offers another possible explanation related to the effect of temperature. At 4-7°C, a temperature at which most intact fiber charge movement experiments were performed, $I_v$ humps were always observable in intact fibers but not in most cut fibers, although a steeply voltage-dependent component could still be separated in the $Q-V$ plots of cut fibers. This reveals another intrinsic difference between charge movement in intact and cut fibers. Other factors that might affect the appearance of $I_v$ will be studied in the following paper (Hui, 1991).
METHODS

Muscle and Fiber Preparation

Experiments were performed on muscles from English frogs, *Rana temporaria*, cold-adapted in a refrigerator at ~4°C. Animals were killed by decapitation and pithing. Either whole sartorius or halved semitendinosus muscles were used to study the effect of temperature in intact fibers. The muscle was stretched to ~1.5 times its slack length and pinned on the bottom of the experimental chamber. Sarcomere lengths of the fibers in these experiments were not measured but were probably 3.0–3.2 μm. Fiber contraction was blocked with a hypertonic bathing solution containing 350 mM sucrose.

As the ends of the fibers in a whole muscle cannot be stretched effectively, singly isolated fibers were used to study the effect of stretch in intact fibers. These fibers were dissected from semitendinosus muscles and stored for at least a few hours or overnight in a refrigerator. A fiber was discarded if it showed any sign of damage or did not respond to extracellular stimulation by giving all-or-none twitches; otherwise, it was mounted horizontally in the experimental chamber with two platinum wire loops attached to the tendons at the fiber ends. One loop was secured to a stationary hook and the other to a hook on a sensitive tension transducer (AE802; Akers Electronics, Horten, Norway). Fiber contraction was blocked by stretching the fiber until no twitch tension could be detected by the tension transducer. The end of the fiber used for charge movement measurement was placed on a pedestal to aid microelectrode impalements. The procedure for dissecting and mounting cut fibers has been described in a preceding paper (Chandler and Hui, 1990; see also Kovacs et al., 1983).

Solutions

For intact fiber experiments:

- Solution A (isotonic solution): 115 mM TEACl, 5 mM RbCl, 11.8 mM CaCl₂, 1 mM Na₂-PIPES, 10 μg/ml tetrodotoxin, pH 7.1.
- Solution B (hypertonic solution): 115 mM TEACl, 5 mM RbCl, 1.8 mM CaCl₂, 1 mM Na₂-PIPES, 10 μg/ml tetrodotoxin, 350 mM sucrose, pH 7.1.
- Solution C (hypertonic solution): solution A plus 350 mM sucrose, pH 7.1.

For cut fiber experiments:

- Solution D (relaxing solution): 120 mM K-glutamate, 1 mM MgSO₄, 0.1 mM K₂-EGTA, 5 mM K₂-ATP, pH 7.0.
- Solution E (internal solution): 45.5 mM Cs-glutamate, 20 mM Cs₂-creatine phosphate, 20 mM Cs₂-EGTA, 6.8 mM MgSO₄, 5.5 mM Cs₂-ATP, 5 mM glucose, 5 mM Cs₂-PIPES, 60 μM total Ca²⁺, pH 7.0.
- Solution F (external solution): 120 mM TEA-Cl, 2.5 mM RbCl, 1.8 mM CaCl₂, 2.15 mM Na₂HPO₄, 0.85 mM NaH₂PO₄, 1 μM tetrodotoxin, pH 7.1.

Tea⁺ and Rb⁺ in solutions A, B, C, and F and Cs⁺ in solution E were used to minimize K currents. The elevated [Ca²⁺] in solutions A and C was used to improve the seal around the microelectrodes. TTX in solutions A, B, C, and F was used to block Na current.

Charge Movement Measurements

Intact and cut fiber experiments were carried out with different experimental setups and at different times. The instrumentation, experimental protocol, and method of data analysis were similar to those used by Gilly and Hui (1980) and Hui (1983a, b) for intact fiber experiments, and to those used by Chandler and Hui (1990) and Hui and Chandler (1990) for cut fiber experiments. Both intact and cut fiber experiments were performed on the same species of frog.
and using Cl⁻ as the major anion in the external solution. Charge movement transient was obtained by subtracting a scaled CONTROL current trace from the paired TEST current trace and removing the sloping baseline in the ON or OFF segment in a TEST minus CONTROL current trace.

**Analysis of Steady-State Voltage Distribution of Charge**

Steady-state Q-V plots were analyzed with the correction procedure developed by Hui and Chandler (1990). For one charge species, the Q-V plot was fitted by a single Boltzmann

**Figure 1.** Theoretical Q-V curves describing charge movement in a cut fiber mounted in a double Vaseline gap chamber. (A) Curve 1 was generated according to a single Boltzmann distribution function, Eq. 1, with \( \bar{V} = -31.6 \) mV, \( k = 10.7 \) mV, and \( q_{\text{max}}/C_m = 10.0 \) nC/μF. Curve 2 was generated according to the same equation but with CONTROL charge correction. Curve 3 was generated with gap correction and CONTROL charge correction. (B) Curves 1–3 were generated in the same way as the corresponding curves in A, but with \( \bar{V} = -55.9 \) mV, \( k = 2.7 \) mV, and \( q_{\text{max}}/C_m = 12.6 \) nC/μF. (C) Curves 1–3 were obtained by adding the corresponding curves in A and B. Curve 1 is equivalent to a curve generated by Eq. 2 with the Boltzmann parameters given above.
distribution function, first used by Schneider and Chandler (1973):

\[ Q(V) = Q_{\text{max}} \left[ 1 + \exp \left( -\frac{V - \bar{V}}{k} \right) \right]^{-1} \]  

(1)

where \( Q_{\text{max}} \) represents the maximum moveable charge, \( \bar{V} \) the equidistribution potential, and \( k \) the voltage dependence (or inverse steepness) factor. For two charge species assumed to move in parallel, the \( Q-V \) plot was fitted by a sum of two Boltzmann distribution functions:

\[ Q(V) = Q_{\text{max}} \left[ 1 + \exp \left( -\frac{V - \bar{V}_\beta}{k_\beta} \right) \right]^{-1} + Q_{\text{max}} \left[ 1 + \exp \left( -\frac{V - \bar{V}_\gamma}{k_\gamma} \right) \right]^{-1} \]  

(2)

in which the subscripts \( \beta \) and \( \gamma \) are assigned to the components with a larger and a smaller value of \( k \), respectively.

Eq. 1 describes the distribution of \( Q_o \) or \( Q_s \) if the CONTROL traces are taken at large negative potentials. The distributions of the two components were calculated with the mean values of parameters listed in Table II and are shown as curves 1 in Fig. 1, A and B. In our cut fiber experiments, CONTROL pulses are elicited from \(-110 \) to \(-90 \) mV. The net charge measured at any potential \( V \) is therefore given by the difference between the value on curve 1 and the value on the straight line intersecting curve 1 at \(-110 \) and \(-90 \) mV. The resulting distributions of \( Q_o \) and \( Q_s \) after such correction are shown as curves 2 in Fig. 1, A and B. Apparently, the error introduced by omitting the correction is small for \( Q_o \) and almost zero for \( Q_s \).

The actual situation is different as the resistance of the Vaseline seals in the cut fiber chamber is not infinite. In this case, charge movements in the membranes underneath the two Vaseline seals contribute to the overall charge measured. If these contributions are included, the \( Q-V \) curve has an enhanced foot which rises more substantially than without the contributions (see Fig. 2 B in Hui and Chandler, 1990) and the curve is referred to as one with gap correction. Curve 3 in Fig. 1, A or B, was generated from such a curve by subtracting from it the straight line that intersects it at \(-110 \) and \(-90 \) mV. The two curves 3 are referred to as \( Q-V \) curves with
gap correction and CONTROL charge correction. Interestingly, both curves 3 have a negative slope at positive potentials and the slope is steeper in the $Q_\alpha-V$ curve than in the $Q_\nu-V$ curve.

Curve 1 in Fig. 1C was plotted according to Eq. 2 with the same parameters used for generating the curves in Fig. 1, A and B. Curve 2 in Fig. 1C was generated with CONTROL charge correction and curve 3 with both gap correction and CONTROL charge correction. Each of the three curves is equivalent to the sum of the pair of correspondingly labeled curves in Fig. 1, A and B. It should be noted that the maximum value of $Q$ on curve 3, at around 0 mV, is $\sim 19 \text{nC}/\mu\text{F}$, which is less than the maximum amount of moveable charge, $23.6 \text{nC}/\mu\text{F}$, calculated from $q_{\text{move}}/C_m + q_{\text{move}}/C_m$. An awareness of this difference and its origin would be helpful in understanding some of the results, which might otherwise be puzzling, in this and the following paper.

**Temperature Control**

The bottom of the experimental chamber was cooled by a Peltier device (Midland Ross, Cambridge, MA). Bath temperature was sensed by a thermistor (YSI 44202; Yellow Springs Instrument Co., Yellow Springs, OH) and used for feedback control. Since the thermistor was not located at the same position as the fiber under study, the thermistor reading deviated from the exact temperature of the fiber. After compensating for the deviation as described in Hui (1989), the steady-state temperature at the location of the fiber differed from the thermistor reading by $< 1^\circ\text{C}$. All the temperatures quoted in this paper are thermistor readings.

When a whole muscle was used in an intact fiber experiment, the muscle was pinned on the bottom of the experimental chamber which was in direct contact with the Peltier temperature device. When the chamber was cooled or warmed, the position of the muscle relative to the electrodes was sometimes shifted, dislodging some or all of the electrodes that had been inserted into a fiber. If the fiber became too leaky following reimpalement of the electrodes, the experiment was terminated.

**RESULTS**

**Comparison of Q-V Distributions in Intact and Cut Fibers**

Charge movement components in intact fibers. In a previous paper on charge movement in intact fibers (Hui, 1983b), $Q_\alpha$ and $Q_\nu$ were separated by a mathematical method that was based on the difference in the ON kinetics of $I_\alpha$ and $I_\nu$ (see Eq. 3 below) and was supported by independent pharmacological separations with tetracaine or dantrolene sodium. The mathematical separation technique appeared to be useful, but is definitely not a unique approach for separating $Q_\alpha$ and $Q_\nu$. Recently, Hui and Chandler (1990) introduced another method for separating $Q_\alpha$ and $Q_\nu$ in cut fibers. The method was based on the asymmetry in the $Q-V$ plots, which was first observed in intact fibers by Adrian and Almers (1976; see also Fig. 2 of Hui, 1983a). Specifically, the points at small depolarizations rise more steeply than the symmetric Boltzmann distribution function and the points at large depolarizations rise with a more shallow slope. Hui and Chandler (1990) fitted the $Q-V$ plots by a sum of two Boltzmann distribution functions and found that, in all cut fibers, the quality of fit was improved significantly ($P < 0.05$ based on the likelihood ratio test) from that when a single Boltzmann distribution function was used. It is of interest to apply this method to separate $Q_\alpha$ and $Q_\nu$ in intact fibers and compare the results with those obtained by kinetic separation.
Fig. 2 shows the $Q-V$ plot from Fig. 2 of Hui (1983a). The points in the figure of the previous paper were obtained from time integrals of current transients that had been signal-averaged with 8 or 16 sweeps. The points in Fig. 2 here were averaged further, by using the mean value of charge at each potential, to reduce the scatter of data. Even with these averaging procedures, the points in Fig. 2 still show more scatter than those from single-sweep traces obtained from cut fibers (see Figs. 5 and 7).

The thin curve in Fig. 2 was obtained by fitting Eq. 1 to the points. Following the notation in an earlier paper (Hui and Chandler, 1990), $Q_{\text{max}}$ will be normalized by membrane capacitance and expressed as $q_{\text{max}}/C_m$. The best-fit values of the parameters are $V = -52.2$ mV, $k = 7.3$ mV, and $q_{\text{max}}/C_m = 20.8$ nC/µF. The thick curve was obtained by fitting Eq. 2. The best-fit values of the parameters for $Q_{\beta}$ and $Q_{\gamma}$ are listed in the first row of columns 2–7 of Table I. In this fiber, $V_{\gamma}$ was 15 mV more negative than $V_{\beta}$ and $k_{\gamma}$ was about one-third of $k_{\beta}$. $Q_{\gamma}$ accounted for 40.0% of the total charge, as listed in column 8.

Not all of the $Q-V$ plots from intact fiber experiments could be separated into two components by Eq. 2, either because the number of points was insufficient to define the steep part of the curve or because the range of potential was not wide enough to define the foot and the shallow rising upper part of the curve. The experiment shown in Fig. 2 is the best for demonstrating the separation of $Q_{\beta}$ and $Q_{\gamma}$ by a sum of two Boltzmann distribution functions in an intact fiber; a large number of data points was collected at fine voltage increments because the fiber was stable over several hours.
and the experiment was solely devoted to the study of charge movement in the control state.

Despite the scatter of the data, the thick curve (two Boltzmann distribution functions) in Fig. 2 appears to provide a better fit than the thin curve (single Boltzmann distribution function). To compare the quality of fit statistically, the residual sums of squares from the fits with a single Boltzmann distribution function and with two Boltzmann distribution functions were calculated. Although the sum was reduced from one Boltzmann distribution function to two Boltzmann distribution functions, it does not necessarily imply that the quality of fit was improved because the number of parameters was increased from three to six. One objective way to compare the quality of fit is to apply the likelihood ratio test as described in Hui and Chandler (1990). The likelihood ratio statistic (LRS), defined by Eq. 10 in that paper, is shown in column 9 of Table I.

The $Q-V$ plots of eight other intact fibers were fitted with Eq. 2 and the results are also listed in Table I. On the average, $Q_r$ accounted for 32.1% (range 20.4–41.3%) of

### Table I

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From Hui (1983b):

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Column 1 gives the fiber identifications. Fiber 94181 was bathed in solution B, all others in solution C. *Indicates the fiber was included in Table 3 of Hui (1983b). †Indicates the fiber is the same as in Fig. 1 of Hui et al. (1984). The fibers that are marked by * were from sartorius muscles, whereas the other three fibers were from semitendinosus muscles. Columns 2–7 give best-fit values of the parameters in Eq. 2. Column 8 gives the fraction of $Q_r$ in the total charge. Column 9 gives the likelihood ratio statistics (LRS) for comparing the residual sums of squares from the fits with one Boltzmann distribution function and with two Boltzmann distribution functions (see text for detail). †Values that are significant at $P < 0.05$ with the likelihood ratio test (Bickel and Doksum, 1977). Temperature, 4–7°C. The bottom two rows give the mean values and SEM listed in Table 3 of Hui (1983b).
the total charge. $V_r$ was 13 mV more negative than $V_p$, and $k_r$ was about one-fourth of $k_p$.

The quality of fits with a single Boltzmann distribution function and with two Boltzmann distribution functions were also compared in the other eight fibers, and the values of LRS are also shown in Table I. For all cut fibers in Table IV of Hui and Chandler (1990), the improvement in the quality of fit from one Boltzmann distribution function to two Boltzmann distribution functions was significant at $P < 0.05$, as the values of LRS exceeded 7.8 (the upper 95th percentile of a $\chi^2$ distribution with three degrees of freedom; Bickel and Doksum, 1977). The values of LRS shown in column 9 of Table I in this paper, for intact fibers, are smaller than the values in that paper. Thus, it appears that the improvement in the quality of fit from one Boltzmann distribution function to two Boltzmann distribution functions in intact fibers is not as significant as in cut fibers.

There are two possible explanations for the difference. First, the Q-V plots from intact fibers have larger scatter than those from cut fibers, resulting in a larger contribution of irreducible variance to the total variance in intact fibers. Second, intact fibers have a smaller fraction of $Q_v$ than cut fibers (see comparison in the next section). When the $Q_v$ component is small, the deviation of the overall Q-V plot from a single Boltzmann distribution should be relatively insignificant. The second explanation is supported by the numbers in columns 8 and 9 of Table I. The fiber of Fig. 2 had $Q_v$ accounting for 40.0% of the total charge, the second largest among the intact fibers, and the LRS was the largest. The second fiber in the table had the second smallest fractional amount of $Q_v$, and the LRS was the smallest.

The other method, used by Hui (1983b) to separate $Q_a$ and $Q_v$, was based on the difference in ON kinetics between $I_a$ and $I_v$, which were approximated by a single exponential decay and the time derivative of the logistic curve (Murray, 1979), respectively, in the following equation:

$$I_{ON}(t) = C_\beta \exp\left(-t/\tau_\beta\right) + C_\gamma \frac{d}{dt}\left[1 + \exp\left(-\frac{t - t_{p,\gamma}}{\tau_\gamma}\right)\right]^{-1}$$

(3)

in which $C_\beta$, $C_\gamma$, and $\tau_i$ are the amplitude and time constant of $I_i$ for $i = \beta$ and $\gamma$, and $t_{p,\gamma}$ is the time-to-peak of $I_\gamma$. The second term on the right-hand side, although not unique, provides a good fit to the bell shape of $I_v$ separated by pharmacological agents (Hui, 1983b). The equation was fitted to the ON transients of charge movement traces having an observable hump component and the amounts of $Q_a$ (or $Q_v$) calculated from the time integrals of $I_a$ (or $I_v$) were fitted by Eq. 1. The results in Table 3 of Hui (1983b) are included in Table I for comparison. Apparently the two methods of separation give somewhat different results. The separation by two Boltzmann distribution functions (Eq. 2) gives a larger fraction of $Q_v$ in the total charge, a less negative $V_{p,v}$, a more negative $V_{p,a}$, and a smaller $k_v$ than the separation by two kinetic functions (Eq. 3). The differences are all statistically significant ($P < 0.01$ with the two-tailed $t$ test). On the contrary, the difference in $k_a$ is insignificant ($P > 0.3$ with the two-tailed $t$ test). Nonetheless, the results from both separation methods share some common features: $Q_v$ accounts for one-fourth to one-third of the total charge and $Q_v$ rises more steeply with increasing depolarizations than $Q_a$. 
Charge movement components in cut fibers. Hui and Chandler (1990) reported very prominent \(I_h\) humps in the ON segments of TEST minus CONTROL current traces recorded from cut fibers. One of the objectives of this paper is to investigate whether there is more \(Q_v\) in cut fibers than in intact fibers. Since the separations of \(Q_h\) and \(Q_v\) by Eqs. 2 and 3 gave somewhat different results in intact fibers, a similar comparison was carried out in cut fibers. TEST minus CONTROL current traces from a cut fiber are shown in Fig. 3A. At a small depolarization to \(-55\) mV, \(I_h\) is manifested in the ON segments as a very broad hump with a small amplitude in the decay phase of \(I_h\). As the level of depolarization is increased, the hump becomes larger in magnitude and faster in kinetics.

In cut fibers, there are indications that the \(I_h\) humps are bell shaped, as in intact fibers (see Figs. 4B, 5B, and 9 in the following paper). The ON transients of the traces in Fig. 3A are displayed in expanded scales in Fig. 3B and were separated into \(I_h\) and \(I_v\) components according to Eq. 3. The theoretical curves fitted the data quite well. The residual sums of squares per point for the fits, listed in the figure legend, were comparable to the noise level in the baselines preceding the TEST pulses, confirming the goodness of fit. The amount of \(Q_v\) at each depolarization was estimated from the time integral of \(I_v\) and plotted as a function of membrane potential in Fig. 4 as open squares. To gain some information about the voltage dependence of \(Q_v\), the \(Q_v-V\) plot was assumed to follow Eq. 1. This assumption is an approximation because \(Q_v\) might occupy more than two states. Curve 1 was obtained by fitting Eq. 1 to the open squares, with gap correction and CONTROL charge correction, as described in Methods. The best-fit parameters for \(Q_v\) so obtained are listed in columns 6–8 of Table II under fiber 83242.

The amounts of ON or OFF charge in the traces of Fig. 3A (and other traces not shown) were estimated from the time integrals of the ON or OFF transients and are shown in Fig. 4 as filled and open diamonds, respectively. Below the threshold of \(Q_v\), about \(-65\) mV for this fiber, the ON and OFF charge are almost identical. For the next 10–20 mV, \(I_v\) in the ON segment has a broad time course, making the fitting of baseline unreliable (see Hui and Chandler, 1990, and the text associated with Fig. 6A in the following paper). Consequently, the amount of ON charge in this potential range was not estimated. For further depolarizations up to \(-35\) mV, ON and OFF charge equality is well preserved and the open and filled diamonds at each potential actually overlap with each other. Thus, for all potentials \(<-35\) mV, OFF charge is used to represent the total charge. Beyond \(-35\) mV, the OFF charge diverges from the ON charge. As discussed in Hui and Chandler (1990), the increase in OFF charge could be due to contamination by inward ionic current. When this happens, ON charge is used to represent the total charge (see Fig. 7 in that paper). Since the potential at which the OFF charge rises above the ON charge varies from fiber to fiber and also depends on the ionic conditions, it is impossible to fix a transitional potential for switching the choice from OFF charge to ON charge. Hence, an objective criterion was introduced to make the transition when the OFF charge begins to rise 1–2 nC/\(\mu\)F above the ON charge. In Fig. 4, the transition is set at \(-30\) mV. From \(-30\) to \(+10\) mV the open diamonds are used, whereas for more negative potentials the filled diamonds are used to represent the total charge.

The amount of \(Q_h\) at each depolarization was estimated by subtracting the value of \(Q_v\) given by curve 1 from the corresponding amount of total charge (filled or open
FIGURE 3. Kinetic separation of $I_a$ and $I_v$ in ON segments of TEST minus CONTROL traces from a cut fiber. Fiber identification: 85242. Diameter, 118 μm; sarcomere spacing, 3.5 μm; temperature, 13–14°C. Sapopin treatment was applied to membrane segments in both end pools at time zero. After rinsing, the solutions in the end pools were replaced by solution E. Then, the solution in the center pool was changed to a TEACl solution (solution F). At the 23rd minute, the voltage clamp was turned on and the holding potential was set at $-90$ mV. (A) TEST minus CONTROL current traces elicited from the 124th to the 141st minutes by TEST pulses to the potentials as indicated. During that period, the holding current changed from $-43$ to $-45$ nA and $\tau_v/(\tau_v + \tau_i)$ remained constant at 0.986. All traces are single sweeps. Only representative traces are shown. (B) ON transients of the traces in A are shown in expanded scale. Each transient was fitted by Eq. 3. The residual sums of squares per point for the fits are (in increasing order of depolarization) 0.00635, 0.00781, 0.00698, 0.00822, 0.00651, and 0.00726 (nC/μF)^2. The corresponding mean squared deviations per point in the baselines of the traces preceding depolarization are 0.00287, 0.00728, 0.00484, 0.00538, 0.00727, and 0.00549 (nC/μF)^2, respectively.
Open squares represent the amounts of $Q_b$ obtained by fitting Eq. 3 to the ON segments shown in Fig. 3 (and others not shown) and integrating the second term on the right-hand side of the equation. Curve 1 was obtained by fitting Eq. 1, with gap correction and CONTROL charge correction, to the open squares. Open and filled diamonds represent the total amounts of ON and OFF charge, respectively. Open triangles were obtained by subtracting corresponding values of $Q_b$ on curve 1 from the open diamonds (four largest depolarizations) or filled diamonds (other depolarizations). Curve 2 was obtained by fitting Eq. 1, with the same corrections, to the open triangles.

To compare the above separation of $Q_b$ and $Q_s$ with the separation by a sum of two Boltzmann distribution functions, the filled and open diamonds in Fig. 4 are replotted in Fig. 5. The filled diamonds at potentials $<-30$ mV and the open diamonds between $-30$ and $+10$ mV were fitted by Eq. 2, with corrections. The best-fit parameters for $Q_b$ are listed in columns 3–5 in Table II (under fiber 83242). In this fiber, $Q_s$ accounted for 68.9% of the total charge, as shown in column 9.
best-fit parameters for the two components are: $V_{\beta} = -30.7 \text{ mV}$, $k_{\beta} = 11.6 \text{ mV}$, $q_{\beta,\text{max}}/e_m = 6.4 \text{ nC}/\mu\text{F}$, $V_{\gamma} = -53.7 \text{ mV}$, $k_{\gamma} = 3.1 \text{ mV}$, and $q_{\gamma,\text{max}}/e_m = 10.2 \text{ nC}/\mu\text{F}$. Here the subscripts $\beta$ and $\gamma$ refer to the less steeply and more steeply voltage-dependent component. The individual $Q_{\beta-V}$ and $Q_{\gamma-V}$ curves so obtained (labeled 1 and 2) are also displayed in Fig. 5. These two curves are similar to the correspondingly labeled curves in Fig. 4. By this second method of separation, $Q_{\beta}$ accounted for 61.4% of the total charge in this fiber. This value, listed in column 10 of Table II, is somewhat smaller than the value in column 9 obtained by the first method of separation.

Attempts were made to estimate the error involved in fitting Eq. 2 if the values of OFF charge were used at large depolarizations instead of the values of ON charge. If all the filled diamonds in Fig. 5 up to $+10 \text{ mV}$ were included in the fit, the fitting routine did not converge. If the point at $+10 \text{ mV}$ was omitted, the best-fit parameters for the two Boltzmann components are: $V_{\beta} = -27.3 \text{ mV}$, $k_{\beta} = 11.7 \text{ mV}$, $q_{\beta,\text{max}}/e_m = 9.5 \text{ nC}/\mu\text{F}$, $V_{\gamma} = -54.0 \text{ mV}$, $k_{\gamma} = 2.9 \text{ mV}$, and $q_{\gamma,\text{max}}/e_m = 9.6 \text{ nC}/\mu\text{F}$. This shows that any ionic contamination of OFF charge by inward ionic current would lead to an overestimation of $q_{\beta,\text{max}}/e_m$, as expected, because the distribution of $Q_{\beta}$ is determined by the shallow rise in the upper part of the $Q-V$ curve where ionic contamination is most serious. On the other hand, the distribution of $Q_{\gamma}$, as determined by the steep rise in the lower part of the $Q-V$ curve, is affected minimally.

Many charge movement experiments were performed on cut fibers in this study. Only experiments on cut fibers stretched to a sarcomere length of 3.5 $\mu\text{m}$ and bathed in TEA.Cl Ringer from the start of the experiment are included in the analysis. In each experiment, $Q_{\beta}$ and $Q_{\gamma}$ were separated by both methods, but only the best-fit parameters obtained by fitting Eq. 3 are listed in Table II. The fractions of $Q_{\gamma}$ in the total charge obtained by both methods for each experiment are listed in columns 9 and 10 of the same table. The mean values of the best-fit parameters obtained by fitting Eq. 2 (see legend of Table II) are not significantly different from the mean values of the best-fit parameters obtained by fitting Eq. 3, shown in the bottom two rows of Table II ($P > 0.3$ for each pair with the two-tailed $t$ test). A comparison of the mean values in columns 9 and 10 shows that the separation of the charge components by two kinetic functions gives a slightly larger fraction of $Q_{\gamma}$ in the total charge than the separation by two Boltzmann distribution functions, but the difference is not statistically significant ($P > 0.3$ with the two-tailed $t$ test). This is in contrast to the situation in intact fibers, in which the two methods gave significantly different results. However, the separation of charge components by two Boltzmann distribution functions is considered to be less reliable in intact fibers than in cut fibers (see above).

Results in Table II show that the charge associated with the $I_h$ hump in the ON segments of TEST minus CONTROL current traces from cut fibers has a steeper voltage dependence than that associated with $I_{\beta}$, which agrees qualitatively with the results from intact fibers (Hui, 1983b). More importantly, the Boltzmann parameters associated with the voltage distribution of the time integral of $I_h$ have values similar to those associated with the more steeply voltage-dependent component in the $Q-V$ plot of the total charge. Thus, it seems justified to identify the charge component separated by the first method with the charge component separated by the second method and use the same notation, $Q_{\gamma}$, to represent the charge component. The same argument applies to $Q_{\beta}$. Any difference between the Boltzmann parameters for
## Table II

Q-V Distributions of $Q_\alpha$ and $Q_\gamma$, Obtained by Kinetic Separation of ON Currents, in Cut Fibers with Sarcomere Length 3.5 μm

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Column 1 gives the fiber identifications. All fibers were from semitendinosus muscles. Column 2 gives the values of $r_i/(r_i + r)$. ON currents were separated into $I_\alpha$ and $I_\gamma$ by Eq. 3, as shown in Fig. 3B. For each fiber, Eq. 1 was fitted to a $Q_\alpha$-V plot or a $Q_\gamma$-V plot, with gap correction and CONTROL charge correction, as shown in Fig. 4. The best-fit parameters are listed in columns 3–8. Column 9 gives the fraction of $Q_\alpha$ in the total charge estimated from the values in columns 5 and 8. The total charge in each fiber was also separated into two components by fitting Eq. 2, with corrections, to each $Q$-V plot similar to the one shown in Fig. 5. The mean values of the best-fit parameters in Eq. 2 are: $V_b = -33.0$ (SEM 1.3) mV, $k_b = 10.9$ (SEM 0.4) mV, $q_{\alpha \max \over C_a} = 11.2$ (SEM 0.5) nC/μF, $V_\gamma = -56.1$ (SEM 0.6) mV, $k_\gamma = 2.9$ (SEM 0.2) mV, and $q_{\gamma \max \over C_a} = 12.0$ (SEM 0.7) nC/μF. Column 10 gives the fraction of $Q_\gamma$ in the total charge estimated by the latter method. Temperature, 13–14°C for all fibers.
Charge Movement in Intact and Cut Fibers

$Q_b$ or $Q_v$ obtained by the two methods of separation could be attributed to scatter of the data or to the errors involved in approximating the true voltage distributions of $Q_b$ and $Q_v$ by Eq. 2 or in approximating the true kinetics of $I_b$ and $I_v$ by Eq. 3.

Interestingly, separating charge movement by a sum of two Boltzmann distribution functions shows different characteristics of $Q_b$ and $Q_v$ in the two fiber preparations (Table I and legend of Table II). The values of $k_b$ and $k_v$ for cut fibers are larger than those for intact fibers and the differences are statistically significant ($P < 0.05$ with the two-tailed $t$ test). The values of $V_b$ and $V_v$ in cut fibers are more negative than those in intact fibers, which can be attributed to the elevated $[Ca^{2+}]$ in the external solution in intact fiber experiments, but this alone cannot explain why the difference in $V_b$ is smaller and insignificant ($P > 0.3$ with the two-tailed $t$ test), whereas the difference in $V_v$ is larger and highly significant ($P < 0.001$ with the two-tailed $t$ test). $Q_b$ in cut fibers accounts for 51.3% (range 30.1–65.2%) of the total charge, which is substantially larger than the 32.1% (range 20.4–41.3%) in intact fibers separated by the same method. The difference is highly significant ($P < 0.001$ with the two-tailed $t$ test). This difference is not due to the method of separation chosen, as the separation by Eq. 3 also resulted in a similar difference. In fact, the latter method yielded an even smaller fraction of $Q_v$ in the total charge in intact fibers (bottom two rows in Table I), making the difference between the two preparations even more significant. Furthermore, it is unlikely that the difference arises from the difference in temperature, 4–7°C in intact fiber experiments versus 13–14°C in cut fiber experiments (see below).

**Effect of Stretch on Charge Movement**

Effect of stretch on charge movement in cut fibers. Charge movement experiments were performed on cut fibers stretched to a sarcomere length of 4.0 μm. Except for the sarcomere length, the conditions for these experiments were identical to those in the experiments listed in Table II. Fig. 6 shows TEST minus CONTROL current traces from one of the experiments. The ON segments of these traces are similar to those in Fig. 3 with an $I_v$ hump clearly visible. Similar $I_v$ humps were also observed in other fibers stretched to the same sarcomere length.

The areas of the OFF transients from the traces shown in Fig. 6 (and others not shown) are plotted as a function of potential in Fig. 7. Only values of OFF charge are shown because there was no sign of ionic contamination up to −20 mV. Since the goal of this experiment was to explore the characteristics of $Q_v$, traces were taken at 1-mV increments in the potential range in which $I_v$ humps were observable, resulting in more points in the $Q$-$V$ plot than usual. The smooth curve was obtained by fitting Eq. 2 to the points, with corrections. The quality of fit was much better than that with one Boltzmann distribution function and the improvement is statistically significant ($P < 0.05$ based on the likelihood ratio test). The best-fit values for the parameters are listed in the figure legend. From the values of $q_m/e_m$ obtained for the two components, $Q_v$ in this highly stretched fiber accounted for 61.2% of the total charge.

Five other experiments were performed in which cut fibers were stretched to a sarcomere length of 4 μm and the $Q$-$V$ plots were fitted by a sum of two Boltzmann distribution functions, with corrections. The mean values of the best-fit parameters are: $\bar{V}_b = -38.9$ (SEM 1.1) mV, $k_b = 8.5$ (SEM 0.6) mV, $q_{b,m}/e_m = 9.4$ (SEM 0.8)
This experiment was carried out in collaboration with Dr. Wei Chen.

nC/μF, \( V_0 = -58.0 \) (SEM 1.8) mV, \( k_0 = 2.2 \) (SEM 0.3) mV, and \( q_{\text{max}}/\kappa_m = 9.6 \) (SEM 1.0) nC/μF. These values are not statistically different from the corresponding values obtained from cut fibers stretched to a sarcomere length of 3.5 μm (\( P > 0.05 \) for each pair with the two-tailed \( t \) test) except for the mean values of \( k_0 \) (\( P < 0.01 \) with the two-tailed \( t \) test). The mean value of the fraction of \( Q_s \) in the total charge at a sarcomere length of 4 μm is 50.0% (SEM 2.3), which is almost identical to that at a sarcomere length of 3.5 μm.

\[ q_{\text{max}}/\kappa_m = 9.6 \text{ nC/\mu F} \]

\[ V_0 = -58.0 \text{ mV} \]

\[ k_0 = 2.2 \text{ mV} \]

\[ q_{\text{max}}/\kappa_m = 9.6 \text{ nC/\mu F} \]
sarcomere length of 3.5 μm (Table II). The difference between the two mean values is insignificant \((P > 0.8\) with the two-tailed \(t\) test). A general conclusion of these results is that the steady-state voltage distributions of \(Q_a\) and \(Q_v\) appear to be similar in cut fibers stretched to a sarcomere length of either 3.5 or 4 μm.

Effect of stretch on charge movement in intact fibers. Attempts were made to study the effect of stretch on charge movement in intact fibers. As it is difficult to highly stretch a whole muscle or a muscle bundle without damaging its fibers, these experiments were performed on singly isolated intact fibers. Since the fibers were highly stretched, there was no need to block contraction with a hypertonic bathing solution. The fibers were thus bathed in an isotonic TEA solution, similar to the hypertonic solution in the experiment of Fig. 2 without the added sucrose. These single fiber experiments were extremely difficult. Of twelve experiments in which the fiber did not break after impalement with microelectrodes, only three gave charge movement records completely free of movement artifact and only one lasted long enough to give a complete \(Q-V\) curve.

In the most successful experiment, the sarcomere length of the fiber in the region between the two voltage electrodes was 3.8 μm, and probably well over 4.0 μm at the center (Huxley and Peachey, 1961; Hui and Gilly, 1979). Since such a highly stretched intact fiber usually does not survive very long, the charge movement traces were not heavily signal averaged. Although the traces were more noisy, they resembled those recorded from less stretched intact fibers bathed in a hypertonic solution. \(I_v\) hump began to appear at about −35 mV and was most noticeable at −25 mV, the usual potential range in intact fibers. It is possible that both \(I_p\) and \(I_v\) in this fiber were smaller in amplitude than those in less stretched intact fibers, because \(q_{max}/C_m\) of the total charge was only 17.3 nC/μF, smaller than the mean value in Table II. However, \(I_v\) hump was definitely not completely abolished. Unfortunately, because of the scatter of data and the insufficient number of points taken, the \(Q-V\) plot was not fitted by a sum of two Boltzmann distribution functions.

Another interesting observation was made on a separate fiber which was stretched slightly less than the one described above. The sarcomere length in the region where charge movement was measured was 3.6 μm. In the early phase of the experiment, an \(I_v\) hump was clearly visible in the ON segments of the charge movement traces. Concomitantly, the traces showed movement artifacts. As the experiment progressed, \(I_v\) became smaller and slower, probably due to rundown of the fiber. Finally, \(I_v\) completely disappeared and, at the same time, the traces were free of movement artifact. This experiment showed that there is a correlation between the appearance of the \(I_v\) hump and the generation of tension.

Effects of Temperature on Charge Movement

Effect of temperature on charge movement components in intact fibers. Fig. 8 shows TEST minus CONTROL current traces recorded from an intact fiber at both 4 and 13°C. The traces in Fig. 8A are typical of those recorded at the lower temperature. The \(I_v\) humps in this figure are not as prominent as those in some other published records (for example, Fig. 1C of Hui, 1983a, which shows the most prominent humps the author has observed so far). The fiber was then warmed to 13°C and the traces in Fig. 8B were recorded. Warming obviously speeded the kinetics of \(I_v\), as reflected by a
reduction in $t_{p\gamma}$. The change in the kinetics of $I_p$ in the ON segment was not apparent as the decay phase of $I_p$ was obscured by the presence of $I_v$. An attempt was made to expand the time scale of a trace in B and superimpose the expanded trace on the corresponding trace in A. No scaling was able to match both $I_p$ and $I_v$, implying that the kinetics of $I_p$ and $I_v$ might have been affected differently.

**FIGURE 8.** Effect of temperature on charge movement in an intact fiber. TEST minus CONTROL current traces recorded from a fiber in a sartorius muscle bathed in solution C at 4°C (A) and 13°C (B). Fiber identification: 2NI11. Resting potential after impalement of the first voltage electrode was −70 mV. $l$, 200 μm; $l'$, 40 μm; holding potential, −80 mV. 15 min after the voltage clamp was turned on, charge movement traces in A were recorded at 4°C. The temperature was then raised to 13°C. After 12 min of equilibration, more traces were recorded, some of which are shown in B. At the end of the experiment, resting potential was −65 mV. The numbers shown between the two columns indicate the potentials during the TEST pulses. In some traces, one or two "erratic" points at the "make" or "break" of the pulses were omitted (see Gilly and Hui, 1980). To convert $\Delta V$ signals to membrane current density as shown in the vertical scale bar, $R$, was assumed to be 375.6 Ω cm (A) and 282.9 Ω cm (B). A sloping baseline was fitted to the ON or OFF segment of each trace from the late phase of the charge movement transient either to the end of the segment or to the point marked by a vertical bar. These baselines are represented by the thin straight lines. In A, the top trace is an average of eight sweeps and all others four sweeps. In B, the top three traces are averages of eight sweeps and all others four sweeps. The arrowheads indicate the peaks of $I_v$ in the traces obtained by fitting Eq. 3.

To quantitate the effect of temperature on the kinetics of $I_v$, $t_{p\gamma}$ in each ON segment of a TEST minus CONTROL current trace was determined by three separate methods. The first method was to fit Eq. 3 to the ON transient, as in Fig. 3 B. The second method was to fit, wherever possible, a parabolic curve to the points around the peak of the hump and approximate the peak of the hump by the peak of
Hui Charge Movement in Intact and Cut Fibers

the parabola. The third method was to locate \( t_{p,v} \) by visual inspection of the ON transient displayed at a highly expanded scale. Results of the three methods agreed fairly well with each other. Thus, although Eq. 3 might not be the exact representation of the waveforms of \( I_a \) and \( I_v \), the values of \( t_{p,v} \) obtained from it were probably not in serious error.

Columns 4 and 5 of Table III list the values of \( t_{p,v} \) at 4 and 13°C for all the traces in Fig. 8. The ratio of the value at 13°C to that at 4°C at each potential, listed in column 6, appears to be independent of the potential and has a mean value of 0.63 (range 0.54–0.78), which can be converted to a \( Q_{10} \) of 1.70 for \( 1/t_{p,v} \) in this fiber.

The fits of Eq. 3 to the ON transients also provided values of \( \tau_p \). For each of the two temperatures, the reciprocals of these values were plotted as a function of membrane potential (not shown). Each set of data was fitted by a U-shaped curve as in Fig. 6 of Hui (1983b). The amplitudes at the troughs of the curves were 0.0454 ms\(^{-1}\) at 4°C and 0.0620 ms\(^{-1}\) at 13°C. The ratio of the two amplitudes is 1.37, which can be converted to a \( Q_{10} \) of 1.41 for \( 1/\tau_p \) in this fiber. Another estimate of this \( Q_{10} \) can be obtained from the decay of OFF currents when the membrane potential was repolarized back to −80 mV. Since no hump component can be visualized in the OFF currents in intact fibers, it has always been assumed that the restorations of \( Q_p \) and \( Q_v \) back to the resting positions share similar kinetics (see also Huang, 1984). This assumption provides an approximation for \( \tau_p \) at −80 mV. The approximation is reasonable because in intact fibers \( Q_v \) accounts for one-fourth to one-third of the total charge, implying that the OFF kinetics of charge movement should be dominated by that of \( I_p \).

<table>
<thead>
<tr>
<th>( V_v )</th>
<th>( 4^\circ C )</th>
<th>( 13^\circ C )</th>
<th>( 4^\circ C )</th>
<th>( 13^\circ C )</th>
<th>Ratio</th>
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<tr>
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<tr>
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<td>15.4</td>
<td>0.54</td>
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</tr>
<tr>
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<tr>
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<td>2.99</td>
<td>15.2</td>
<td>9.3</td>
<td>0.61</td>
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</table>

Mean | 4.15   | 2.96   | 0.63 |
SEM  | 0.09   | 0.06   | 0.02 |

Same fiber as in Fig. 8. Column 1 gives the membrane potentials during the TEST pulses. Columns 2 and 3 give the values of \( \tau_p \) at −80 mV, obtained by fitting a single exponential decay to the OFF transients. Columns 4 and 5 give the best-fit values of \( t_{p,v} \), obtained by fitting Eq. 3 to the ON segments of the traces shown in Fig. 8, A and B, respectively. Column 6 is obtained by dividing the value in column 5 by the value in column 4.
The values of $\tau_{\text{off}}$ for the total current at 4 and 13°C in the traces of Fig. 8 are listed in columns 2 and 3 in Table III. The mean values are 4.15 ms at 4°C and 2.96 ms at 13°C, giving a $Q_{10}$ of 1.45 for $1/\tau_{\text{off}}$ in this fiber, which is very close to the value for $1/\tau_0$ obtained at the trough of the ON rate constant versus $V$ curve described above. Thus, the ON kinetics of $I_v$ has a larger $Q_{10}$ than either the ON kinetics of $I_0$ or the OFF kinetics of the total current. Since the $Q_{10}$ of $1/\tau_{\text{off}}$ is a weighted average of those of $1/\tau_0$ and $1/\tau$, at $-80$ mV, and if the OFF kinetics of $I_v$ has a larger $Q_{10}$ than the OFF kinetics of $I_0$ (as is true for the ON kinetics), that will make the $Q_{10}$ of $1/\tau_{\text{off}}$ even smaller than that of $1/\tau_{\text{off}}$ at $-80$ mV.

Similar values of $Q_{10}$ were obtained from another fiber. The value for $1/\tau_{\text{off}}$ was 1.35 and that for $1/\tau_0$ was 2.21. In a third experiment, the order of temperature change was reversed; i.e., charge movement traces were recorded at 13°C first before temperature was lowered to 4°C. The values of $Q_{10}$ in that experiment were 1.53 for $1/\tau_{\text{off}}$ and 3.09 for $1/\tau_0$. The values of $Q_{10}$ averaged over the three fibers were 1.44 ($\pm 0.04$) for $1/\tau_{\text{off}}$ and 2.33 ($\pm 0.33$) for $1/\tau_0$. Thus, the $Q_{10}$ of $1/\tau_0$ is clearly larger than the $Q_{10}$ of $1/\tau_{\text{off}}$ and the difference between the $Q_{10}$’s of $1/\tau_0$ and $1/\tau_0$ could be even larger.

The three values of $Q_{10}$, 1.70, 2.21, and 3.09, for $1/\tau_0$ from the three intact fibers have a large scatter. Other than fiber-to-fiber variation, part of the scatter can be explained by the order in which the traces were recorded and the rundown of the fibers. It was observed previously that as an experiment progressed, $\tau_0$ at the same potential and temperature increased monotonically (see Fig. 5 of Hui, 1983b). In the two experiments in which the $Q_{10}$ of $1/\tau_0$ was estimated to be 1.70 and 2.21, charge movement traces were recorded at the lower temperature before the higher temperature. Part of the accelerating effect of warming on $\tau_0$ was probably canceled by this rundown effect, and so the value of $Q_{10}$ could be underestimated. In the third experiment, the order of temperature change was reversed, and the value of $Q_{10}$ could be overestimated. In carrying out the experiments, the order of temperature change was intentionally alternated to minimize systematic error. Attempts to bracket the results by returning to the initial temperature were unsuccessful because the fibers could not last that long.

The estimations of $Q_{10}$’s are not meant to be highly quantitative for the following reasons. First, $\tau_0$ was used to characterize the kinetics of $I_v$ without any reference to the width of the hump. Second, all three methods for estimating $\tau_0$ are approximate. Nonetheless, values obtained by using the different methods seemed to agree with each other. Third, the uncertainty in estimating $\tau_0$ at various potentials from the ON segments of charge movement traces was coupled to the uncertainty in estimating $\tau_0$, as both were fitted simultaneously with Eq. 3. On the other hand, the estimation of $\tau_0$ from OFF currents is not without drawback, because it is based on the assumptions that (a) $I_0$ decays with a single time constant, and (b) $I_0$ and $I_v$ decay with the same time constant, both of which may not be exactly correct. Further complication will arise if the OFF currents are contaminated by ionic currents, but the contamination should be minimal after small or intermediate depolarizations. When the contamination became noticeable after large TEST pulses, the $\tau_{\text{off}}$’s were excluded from the average.
Effect of temperature on charge movement components in cut fibers. Fig. 9 shows TEST minus CONTROL current traces elicited by a TEST pulse to $-40 \text{ mV}$ in a cut fiber at different temperatures. When resolvable, $I_v$ in the ON segment is marked by an arrowhead. Since the average value of $\bar{V}_v$ in cut fibers (Table II) is 11 mV more negative than that in intact fibers (Table I), the first trace in Fig. 9, taken at $-40 \text{ mV}$ and at $14^\circ\text{C}$, should be compared with the fifth or sixth trace in Fig. 8B, taken at $-30$ or $-28 \text{ mV}$ and at about the same temperature. Although the traces look similar, a close comparison reveals that the kinetics of charge movement appears to be slower in the cut fiber than in the intact fiber. $t_{p,v}$ is 13.4 ms in the fifth or sixth trace of Fig. 8B and 17.0 ms in the first trace of Fig. 9. Also, the value of $\tau_{\text{eff}}$ has an average of 2.96 ms in the traces of Fig. 8B (at $-80 \text{ mV}$) and is 6.40 ms in the first trace of Fig. 9 (at $-90 \text{ mV}$). One possible cause for the faster kinetics in intact fibers is that they were bathed in a hypertonic solution, which increased the speed of charge movement in rabbit skeletal muscle fibers (Lamb, 1986).

When the temperature was lowered, the kinetics of both ON and OFF currents in Fig. 9 became slower and the hump in the ON segment became progressively less
pronounced. At 6°C the hump could hardly be visualized. When the temperature was increased from 6 to 20°C, the hump in the ON segment was restored and $t_{p,y}$ was monotonically decreased, indicating that the retarding effect of low temperature on $I_v$ was reversible. The magnitude of $I_v$ in the ON segment of the seventh trace at 14°C appears to be smaller than that in the first trace at the same temperature. This may be due to an extremely slow recovery of $I_v$ after cooling. The values of $\tau_{\text{off}}$ at various temperatures were estimated from the OFF currents of the traces. The $Q_{\text{off}}$ of $1/\tau_{\text{off}}$ obtained by linear regression is 1.52, which is close to the mean value of 1.44 in intact fibers.

The amounts of ON/OFF charge in the first five traces are: 13.4/13.3, 14.5/13.9, 14.2/14.5, 14.6/14.5, and 14.4/14.8 nC/µF. They show that, when $I_v$ became obscure during cooling, the equality of ON and OFF charge was preserved, within experimental error, and the amount of charge per unit capacitance was increased rather than decreased. Thus, cooling did not suppress $I_v$ but probably broadened its waveform substantially instead. This may partially explain the absence of a hump component in the cut fiber charge movement traces from other laboratories, since their experiments were generally performed at temperatures below 10°C.

The change in the shape of $I_v$ in the ON segment as a result of cooling in Fig. 9 was typical of experiments performed on cut fibers. In one of seven cut fibers in which the effect of temperature on charge movement was studied, $I_v$ appeared as a hump at low temperatures, as shown in Fig. 10. Although this fiber was atypical, it provided information about the $Q_{\text{on}}$ of $1/t_{p,y}$ in cut fibers. When the fiber was cooled from 14 to 5°C, $t_{p,y}$ was lengthened and the effect was reversible. Eq. 3 was fitted to the ON transients to obtain the values of $t_{p,y}$ at various temperatures. The $Q_{\text{on}}$ of $1/t_{p,y}$ obtained by linear regression is 2.08, which is similar to the mean value of 2.33 in intact fibers.

The decay of the OFF currents in the traces of Fig. 10 appears to be slower than that in Fig. 9. From the values of $\tau_{\text{off}}$ at various temperatures from Fig. 10, the $Q_{\text{off}}$ of $1/\tau_{\text{off}}$ obtained by linear regression was 1.47. When averaged over the experiments of Figs. 9 and 10 and five other experiments, the value was 1.45 (±0.08), which is almost the same as the mean value in intact fibers.

A possible explanation for the difference between the shapes of $I_v$ at low temperatures in Figs. 9 and 10 is that the proportion of $Q_v:Q_b$ at -40 mV was larger in the fiber of Fig. 10 than that of Fig. 9. In the ON segments of the first five traces in Fig. 10, the peak of $I_v$ actually rises above the peak of $I_b$, very different from the situation in Fig. 9. Moreover, if the $\tau_{\text{off}}$ of $I_v$ is larger than that of $I_b$ in cut fibers at -90 mV, then the slower decay of OFF currents in Fig. 10 is consistent with a larger proportion of $Q_v$ in that fiber.

In the experiment of Fig. 10, in addition to the traces shown, charge movement was measured at various potentials at both 14 and 6°C. The average values of $c_a$ from CONTROL traces were 0.229 and 0.220 µF/cm at 14 and 6°C, respectively. The $Q-V$ plots at the two temperatures (not shown) were separately fitted by a sum of two Boltzmann distribution functions. The best-fit values of $q_{\text{off}}/c_a$, $\bar{V}_b$, $k_b$, $q_{\text{on}}/c_a$, $\bar{V}_v$, and $k_v$ at 14°C were 6.4 nC/µF, -30.7 mV, 11.6 mV, 10.2 nC/µF, -53.7 mV, and 3.1 mV, respectively, whereas at 6°C they were 9.7 nC/µF, -22.9 mV, 12.1 mV, 13.6 nC/µF, -51.1 mV, and 3.8 mV, respectively. The fraction of $Q_v$ in the total charge therefore changed minimally from 61% at 14°C to 58% at 6°C. Similar constancy in
the fraction of $Q_v$ was observed in two other cut fiber experiments in which complete sequences of runs were taken at both temperatures, but the $I_v$ hump disappeared at the lower temperature.

Results in this section show that when cut fibers were cooled from 14 to 6°C, the waveform of $I_v$ in the ON segments of TEST minus CONTROL current traces was generally not manifested as a hump. This is markedly different from the properties of $I_v$ in intact fibers. However, when the $I_v$ hump was resolvable in cut fibers by Eq. 3, its $t_p, v$ might share a similar $Q_{10}$ with that in intact fibers. In addition, the proportion of $Q_v:Q_i$ in cut fibers might not be changed when the temperature was altered between 6 and 14°C.

**DISCUSSION**

$I_v$, Hump in Intact and Cut Fibers

Hui (1983b) separated charge movement in intact fibers by a sum of two kinetics functions and found that, in each fiber, the $Q_r-V$ plot associated with the delayed hump component has a steeper voltage dependence than the $Q_i-V$ plot associated with the early current component. Recently, Hui and Chandler (1990) separated the...
Q-V plots of the total charge in cut fibers into two components by a sum of two Boltzmann distribution functions, one having a steeper voltage dependence than the other. Based on these two separation methods, the fraction of the steeply voltage-dependent component in the total charge estimated by Hui and Chandler in cut fibers was larger than the fraction estimated by Hui in intact fibers. In this paper, the fractions estimated with both methods of separation were compared in intact as well as cut fibers. The first accomplishment was the identification of the time integral of $I_+$ with the more steeply voltage-dependent component in the two Boltzmann fit, thereby justifying the usage of $Q_s$ to refer to both quantities (likewise for $Q_a$).

The results also showed that the fraction of $Q_s$ in the total charge was larger in cut fibers than in intact fibers, independent of the method of separation used. Some of the differences in protocol of the two types of experiments could contribute to the difference in the fraction. For example, the external solution in intact fiber experiments is usually hypertonic and the internal solution in cut fiber experiments is nonphysiological. Either of these could possibly affect the proportion of $Q_s:Q_a$. In addition, if the modification of the Boltzmann distribution function to correct for contributions due to charge movement from underneath the Vaseline seals is not exactly accurate, it could contribute to the difference. The 11.8 mM external $[Ca^{2+}]$ in intact fiber experiments (versus 1.8 mM in cut fiber experiments) could increase the amount of $Q_a$ in intact fibers (Brum et al., 1988). However, Table I shows that the fraction of $Q_s$ in the total charge for intact fiber 94181, which was bathed in a solution containing 1.8 mM $Ca^{2+}$, was similar to that for intact fiber 08152, which was bathed in a solution containing 11.8 mM $Ca^{2+}$, and both fractions were smaller than the average fraction for cut fibers (Table II). Thus, the difference in $[Ca^{2+}]$ alone cannot completely account for the difference in the fraction between the two fiber preparations.

Another difference between charge movement in the two fiber preparations is that although the $I_+$ hump has been observed routinely in intact fibers (Adrian and Peres, 1977, 1979; Huang, 1982; Hui, 1982, 1983a, b), it was sometimes absent in cut fiber charge movement records (for example, Melzer et al., 1986). Melzer et al. (1986) suggested that the absence of $I_+$ humps in cut fibers could be because the fibers were highly stretched. Results in this paper show that $I_+$ humps definitely existed in cut fibers stretched to a sarcomere length of 4.0 μm and perhaps also in intact fibers stretched to 3.8 μm. The fraction of $Q_s$ in the total charge in cut fibers was the same for a sarcomere length of 3.5 or 4.0 μm. Thus, stretch is not the cause for the absence of $I_+$ humps in cut fibers. Instead, the substitution of $Cl^-$ in the external solution with $SO_4^{2-}$ is a possible explanation (Hui and Chandler, 1990). The effect of temperature studied in this paper is another possibility. Other possible factors affecting the appearance of $I_+$ will be described in the following paper (Hui, 1991).

This paper shows that, under the conditions of the present experiments, $I_+$ in cut fibers appeared as a prominent hump at temperatures above 10°C. When the temperature was lowered to a few degrees Celsius, the hump usually disappeared within minutes but a $Q_s$ component could still be separated from the Q-V plot, implying that $I_+$ might still be present but was not manifested as a hump. Since $I_+$ is routinely observed in intact fibers as a hump at a few degrees Celsius, there appears to be a genuine difference between cut and intact fibers. The reason for this
difference is unknown, but could be related to a difference in the physiological states of the two preparations.

$Q_{10}$ of Charge Movement Kinetics

The kinetics of $I_b$ and $I_s$ in cut fibers appeared to be slower than those in intact fibers. Part of the difference could be caused by extrinsic factors such as the composition of solutions, the speed of the voltage clamp circuitry, and the distortion by the Vaseline gap, etc. The slight difference in holding potential should also lead to some difference in $\tau_{off}$, but the difference is in the wrong direction. Also, $I_s$ could decay more slowly than $I_b$ on repolarization. Since $I_s$ has a larger amplitude in cut fibers than in intact fibers, any difference between the OFF decay time constants of $I_b$ and $I_s$ could be more noticeable in cut fibers.

Although the kinetics of $I_b$ and $I_s$ were different in the two preparations, their $Q_{10}$'s did not seem to differ much. For either preparation, $1/\rho_{\gamma}$ has a larger $Q_{10}$ than $1/\tau_{\gamma}$. It can be argued that the $Q_{10}$ of $1/\tau_{off}$ is not equivalent to the $Q_{10}$ of $1/\tau_{\gamma}$ but to a weighted average of the $Q_{10}$'s of $1/\tau_{b}$ and $1/\tau_{s}$. However, the error in the approximation should be small in intact fibers in which the fraction of $Q_b$ in the total charge is small. Moreover, since the $Q_{10}$ of $1/\tau_{off}$ is smaller than the $Q_{10}$ of $1/\tau_{s}$, the $Q_{10}$ of $1/\tau_{b}$ should be even smaller.

The difference in $Q_{10}$ between the kinetics of $I_b$ and $I_s$ is consistent with the idea that $Q_b$ and $Q_s$ are two distinct pools of charge arising from different origins. Counter-examples could be constructed: for example, $Q_b$ could be the trigger for Ca release from the sarcoplasmic reticulum, whereas $Q_s$ could arise as a result of the release (Cserrnoch et al., 1989; Pizarro et al., 1990), in which case both $Q_b$ and $Q_s$ belong to the same pool of charge in the tubular membrane and the $Q_{10}$ for the kinetics of $I_s$ would then be governed by the overall $Q_{10}$ of all the steps leading to, and including, Ca release.

Comparison with Existing Results

Very little information is available concerning the $Q_{10}$ of charge movement in frog skeletal muscle. This is the first time the $Q_{10}$ of $I_s$ kinetics is reported. In a review paper, Adrian (1978) gave a value of 2.6 for the $Q_{10}$ of charge movement rate constant in intact fibers bathed in a TEA$^+$SO$_4$ solution with 350 mM sucrose. As $I_s$ is expected to exist in the moderately hypertonic solution, their rate constants could very well be lumped values for both charge components, but it is difficult to compare the values of $Q_{10}$ in this paper with their lumped value.

Subsequently, Hollingworth and Marshall (1981) repeated the experiments and obtained a value of 1.5–2.0 for the $Q_{10}$ of charge movement rate constant between 2 and 15°C. They also discussed that the ON segments of their traces might not decay with a single exponential. However, on inspecting the traces in their Fig. 7, it is not obvious that an $I_s$ hump could be resolved. Their solution contained 450 mM sucrose, which generally suppressed the appearance of the $I_s$ hump (Chandler et al., 1976; Adrian and Almers, 1976). The value for $I_b$ reported here would agree with the lower limit of their value.

Hollingworth and Marshall (1981) also studied the $Q_{10}$ of charge movement kinetics in rat skeletal muscle. They gave a value of 1.2–1.8 for the $Q_{10}$ of charge
movement rate constant in the same temperature range as their experiments on frogs. Later, Simon and Beam (1985) reported a value of 2.16 for the \( Q_{10} \) of charge movement rate constant in rat skeletal muscle between 7 and 15°C. The reason for the slight difference between the values obtained by the two groups of investigators is unknown, but could be due to the difference in the muscles used or the difference in the configurations of the three-microelectrode voltage clamps. Both values were probably pertinent to the temperature dependence of \( I_v \) kinetics, as \( I_v \) hump was absent in their charge movement records. In comparing results from frog and rat muscles, it is not clear whether the temperature range of 4–13°C in frogs is equivalent to the same or a higher temperature range in rats. Simon and Beam (1985) gave a value of 1.25 for the \( Q_{10} \) of \( I_v \) in rat muscle between 15 and 25°C, similar to the value for \( I_v \) in this paper. However, they mentioned that the value of \( Q_{10} \) at the higher temperature range was voltage dependent, which complicates the comparison with results from frogs.

Possible Molecular Model for \( Q_v \)

At present, the role of \( Q_v \) in excitation–contraction coupling is still controversial. \( Q_v \) could be the trigger for Ca release which further mobilizes more \( Q_v \) (Huang, 1982; Hui, 1983b; Vergara and Caputo, 1983). Alternatively, \( Q_v \) could be mobilized by the release and could then trigger further Ca release (Csernoch et al., 1989; Pizarro et al., 1990). The main difference between the two hypotheses is that, in the former “trigger hypothesis,” \( Q_v \) is not assigned any role in Ca release, whereas in the latter “feedback hypothesis,” \( Q_v \) and \( Q_v \) belong to the same set of voltage sensors for the release. On the other hand, the ability of a fiber to release Ca in the absence of \( I_v \) hump (Melzer et al., 1986) could rule out the association of \( Q_v \) with Ca release, whether it is the cause or consequence. Results in this and the following paper (Hui, 1991) broaden our perception of \( Q_v \). \( I_v \) is generally manifested as a hump in the ON current in intact fibers, but under a variety of conditions (e.g., low temperature) \( I_v \) might have a very broad waveform in cut fibers such that it cannot be visualized as a hump but still contributes to the current transient. Whether it is manifested as a hump or not, its charge constitutes the steeply voltage-dependent component in the \( Q-V \) plot. The steep voltage dependence of this component maintains its close association with Ca release, but provides no clue about whether it is the cause or consequence of the release.

Eq. 3 was introduced as a phenomenological model to separate \( I_v \) and \( I_v \), and was supported by results from pharmacological separation (1983b). If \( Q_v \) is the trigger for Ca release, which is our working hypothesis, Eq. 3 could have a mechanistic basis. The logistic curve has been used successfully to describe population growth (Murray, 1979). The second term on the right-hand side of Eq. 3, being the time derivative of the logistic curve, can be used to represent the change in population over time. The population under investigation is, presumably, the functional dihydropyridine receptors in the transverse tubules. These dihydropyridine binding proteins were found to have four subunits and to be arranged in register with the ryanodine binding proteins in the junctional SR (Block et al., 1988). From the small value of \( k_v \), it appears that \( I_v \) can only flow when the subunits are clustered.
It is not known with certainty whether, when the fiber is at rest, the subunits of a dihydropyridine binding protein are assembled into a functional unit or whether the dihydropyridine binding proteins are in electrical continuity with the projections or feet of the ryanodine binding proteins. If they are not, it is possible that, when a fiber is depolarized, before a dihydropyridine binding protein can trigger Ca release, it has to go through conformational change that leads to tetramerization and/or making contact with a foot. The growth of $Q_v$ units can be described, as an approximation, by the logistic curve that rises steeply at the foot because of some cooperative interaction between adjacent units and flattens at the upper part because of the saturation in the growth of the units. The kinetics of $I_v$, not rate-limited by the relaxation of $Q_v$ but by the growth of the population of tetramers or contacts, can therefore be described by the time derivative of the logistic curve. Although somewhat speculative, this proposed mechanism explains the peculiar kinetics of $I_v$.

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