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CHARGE MOVEMENT AND MECHANICAL REPRIMING IN SKELETAL MUSCLE

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SUMMARY

1. Muscles were placed in a solution which depolarized the membrane to −30 to −20 mV so that mechanical activation was made refractory. Mechanical repriming and the recovery of voltage dependent charge movement were studied using a voltage clamp technique.

2. Mechanical repriming was investigated by determining the duration of a hyperpolarizing pulse required to elicit a just-visible contraction for various post-pulse potentials. As the post-pulse potential was made more positive shorter repriming times were required to produce a threshold contraction. The relationship approached a minimum repriming time for very positive post-pulse potentials.

3. These results suggest that hyperpolarization gradually removes some component of the activation mechanism from a refractory state and that the effectiveness of the amount which has recovered depends on the post-pulse potential. A quantitative explanation is given using a simple model in which the essential component is assumed to be the charge movement process.

4. The rate of repriming contraction is voltage dependent; at −160 mV the rate is about twice that at −120 mV. Between 4 and 10° C the rate has a Q_{10} of about 9.

5. Recovery of charge movement was studied using a repriming duration less than that required to produce a threshold contraction. The observed charge movement increased linearly with repriming time, consistent with the approximately linear initial segment of a slow exponential recovery process. Extrapolation of the recovery curve indicated that 2–5 nC/μF of charge is reprimed in the time necessary to reprime a threshold contraction.

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6. The charge which recovers during a subthreshold repriming pulse is distributed according to membrane potential in the same way as a fully reprimed charge.

7. These results are consistent with the hypothesis that voltage dependent charge movement is an intermediate step in excitation-contraction coupling.

8. The characteristics of a second type of charge movement are also described.

INTRODUCTION

In other papers a general description was given of a voltage dependent charge movement in skeletal muscle (Schneider & Chandler, 1973; Adrian & Almers, 1976*a*, *b*; Chandler, Rakowski & Schneider, 1976*a*, *b*). Although the physiological function is not known with certainty, the results to date seem most consistent with the idea that the charge process plays a role in excitation-contraction coupling.

Unfortunately, in the previous experiments it was not possible to make a direct comparison between the properties of mechanical activation and those of the charge movement. Hypertonic solutions were used to block mechanical movement so that the rather large contractions which would otherwise occur would not dislodge the internal micro-electrodes. The finding that the charge movement is reversibly inactivated by prolonged depolarization (Adrian & Almers, 1976*b*; Chandler *et al.* 1976*b*) in the same way as contraction is inactivated (Hodgkin & Horowicz, 1960), suggested that it might be possible to compare directly some of the properties of contraction and of charge movement by studying the recovery of these processes in mechanically refractory fibres.

In the present experiments muscles were placed in a solution which depolarized the membrane to -30 to -20 mV. Repriming was accomplished by imposing relatively brief hyperpolarizations with a micro-electrode voltage clamp. If care is taken to avoid producing more than small contractions, there is no obvious damage to the fibre and experiments lasting 1–2 hr can be carried out. The general conclusion from the study is that the recovery of contraction is similar in several ways to the recovery of charge, lending support to the idea that charge movement is an intermediate step in excitation-contraction coupling. A preliminary account of some of the results has been published (Chandler, Schneider, Rakowski & Adrian, 1976*c*).

METHODS

Sartorius muscles were dissected from English frogs (*Rana temporaria*) and stored in Ringer solution (solution E, Chandler *et al.* 1976*b*). 40–60 min before beginning the experiment the solution was changed to one of the same tonicity containing 40 mM-Rb_2SO_4, 55 mM $(TEA)_2SO_4$, 8 mM $CaSO_4$, 10^{-6} g tetrodotoxin/ml and 1 mM

Tris-maleate buffer (pH 7). Muscle fibres placed in this solution depolarize to around -20 mV, contract, then relax as they become mechanically refractory.

For the electrical measurements the three micro-electrode voltage-clamp technique developed by Adrian, Chandler & Hodgkin (1970) was used with the modifications and digital data collection techniques described by Chandler *et al.* (1976*a*). Fig. 1 indicates the positions of the internal micro-electrodes. According to linear cable theory (Adrian *et al.* 1970) the membrane current density at electrode 1 is proportional to the potential difference $V_2 - V_1$, denoted as ΔV. The switch (SW) is shown in the position used for measuring charge movement. Measurements of linear cable properties (usually carried out at the beginning of an experiment) and of mechanical threshold were made with the switch in the other position, using voltage V_2 for feed-back control.

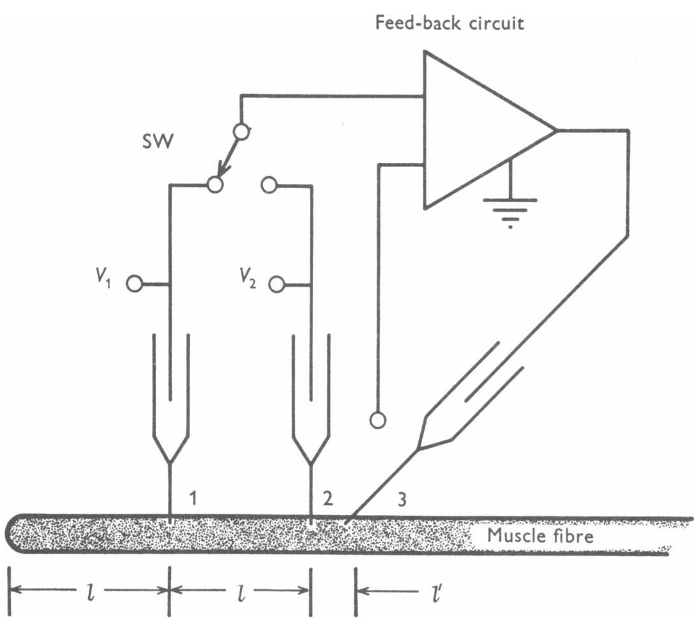


Fig. 1. Diagram indicating the feed-back amplifier and the positions of the micro-electrodes. Electrodes 1 and 2 were used to measure potentials V_1 and V_2; electrode 3 was used to pass current. According to Adrian *et al.* (1970) the current density i_m at electrode 1 is given by

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

where $\Delta V = V_2 - V_1$ and r_i is the internal longitudinal resistance. The switch (SW) was set on V_1 for measurements of charge movement and on V_2 for measurements of cable properties or of contraction threshold.

Charge movement measurements

The procedure for measuring charge movement was the same as described earlier (Chandler *et al.* 1976*a*) except for two modifications. Firstly, the voltages used in the control and the test runs were exactly the same, only the repriming duration was varied. A short duration, usually 0·25 sec, was used for the control whereas longer times were used for the test. Secondly, the command pulse was exponentially rounded with a time constant 0·6–2·0 msec. The amount of rounding was adjusted so that the ΔV trace was always in the range of the amplifiers. Therefore, even the first ΔV points of a transient were reliable and could be used without change.

The charge movement transients in partially reprimed fibres are small so that it is important to obtain an estimate of the resolution of the method. An indication of the error which would arise because of noise was determined in the following way. Standard deviations were measured on difference traces, base line minus base line, signal averaged the same number of times as $\Delta V_{test} - \Delta V_{control}$ traces. The upper trace in Fig. 8*A* is an example of one such base-line difference. Three separate traces from this fibre gave values 0·0946, 0·1006 and 0·1034 mV for the standard deviation of the base-line noise, with an average of 0·0995 mV. Standard deviations for the first fifty base-line points of the nine traces in Fig. 8*A* gave a similar average value, 0·0980 mV (range 0·0832–0·1187 mV). The average value of the two groups of measurements, 0·099 mV, when converted to the charge which moves during the 1 msec sampling interval and when normalized for capacitance corresponds to 0·055 nC/μF.

The time integrals of charge movement transients, plotted in Fig. 9, were determined from the first twenty-five points, following the voltage step, of the ΔV traces in Fig. 8*A*. The base line for these twenty-five points was extrapolated from the best fit straight line through points 26 to 100. Taking 0·055 nC/μF for the value of the standard deviation of the measurement of a single point, the standard deviation of the sum of the first twenty-five points of the transient should be $\sqrt{25} = 5$ times 0·055 or 0·275 nC/μF. If we assume for convenience that the sum of the twenty-five extrapolated base-line points has the same standard deviation, the standard deviation of the difference, which is the integrated charge movement, is $\sqrt{2}$ times 0·275 or 0·389 nC/μF. The vertical bar in the lower right part of Fig. 9 extends ± 1 S.D., from $-0·389$ to $+0·389$ nC/μF, and gives an indication of the error in the measurement of charge movement. The same procedure was used to estimate the standard deviations shown in Figs. 11, 13 and 14.

Mechanical measurements

For experiments on mechanical repriming, contraction was observed under a dissecting microscope. V_2 was used for feed-back control and the command pulse was rounded 1–2 msec. This delay reduced the spatial non-uniformity of voltage around the current passing electrode which occurs transiently during a step change in potential. For experiments on mechanical properties alone, part *A* of the Results, only the electrodes numbered 2 and 3 in Fig. 1 were inserted.

RESULTS

The results are presented in three separate sections. Part A deals with experiments on contractile repriming, using two micro-electrodes for the voltage clamp. Part B is concerned with experiments in which repriming of both contraction and charge were observed. In part C the characteristics of a second type of charge movement are described.

A. Contractile repriming

Effect of post-pulse potential on threshold repriming time

Early in the investigation it was observed that the duration of the repriming pulse necessary to elicit a just-visible contraction could be altered by varying the post-pulse potential. As the potential of the second pulse was varied from +40 to +100 mV, the threshold repriming time was nearly constant and approached a minimum value. For less positive potentials the threshold repriming time was increased. Fig. 2 shows a plot

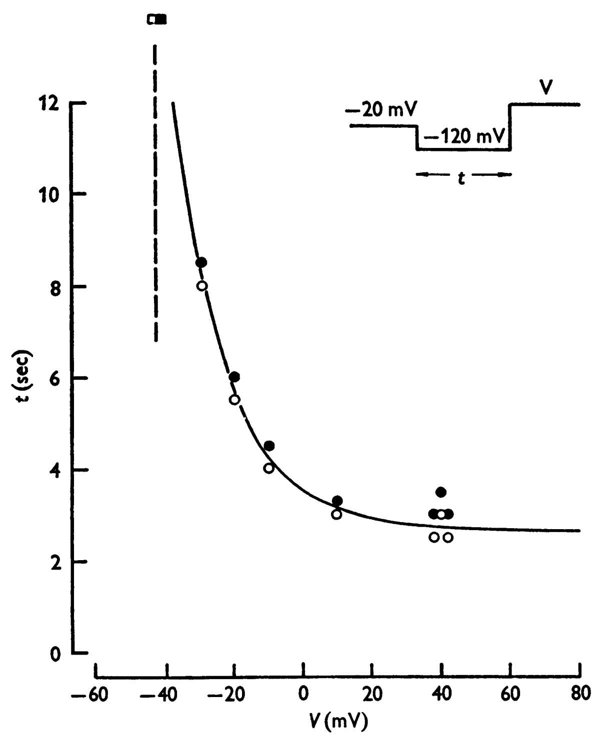


Fig. 2. Repriming time as a function of post-pulse potential. ●, shortest repriming time which gave a contraction; ○, longest repriming time without contraction. Three separate determinations were made at $V = 40$ mV, one at the beginning of the run, one midway and one at the end. The pattern of voltage pulses is indicated at upper right. The duration of the post-pulse was 10 sec for $V = -30$ mV and 2 sec for other voltages. The continuous curve is drawn according to eqns. (5) and (9) with best fit parameters from Fig. 4. The vertical dashed line corresponds to the mechanical threshold measured after the fibre was fully reprimed, as described in the text in connexion with symbols □ and ■. Electrode 2 was 186 μm from the end of the fibre, $l' = 47$ μm. Fibre 29·3, temperature 4·1–4·4° C. Initial resting potential −27 mV, holding potential −20 mV.

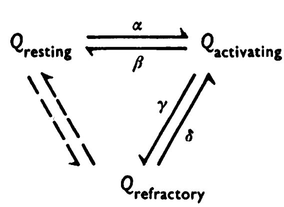
of repriming duration *vs.* post-pulse potential, V, in a fibre in which V was varied from -30 to $+40$ mV. The open circles correspond to the longest repriming times for which no contraction was observed, the filled circles indicate the shortest times which resulted in a just visible contraction. Threshold durations were estimated to be halfway between the open and filled circles. The minimum repriming time for large values of V was about 2·7 sec.

To obtain reproducible results in this kind of experiment and at this temperature it was necessary to use long intervals of time between successive pulse sequences to allow complete inactivation. In the fibre in Fig. 2 a 3 min interval was used after short repriming pulses. As V was decreased to -30 mV and the repriming duration increased, the interval between pulse sequences was increased to 5 min.

Expected behaviour of the charge movement process during repriming

Experiments of the type illustrated in Fig. 2 consistently showed that longer repriming times were required using a negative post-pulse than using a strongly positive post-pulse. The simplest interpretation is to suppose that hyperpolarization gradually removes some component of the activation mechanism from an inactivated or refractory state, and that the effectiveness of the amount which has recovered depends on the potential of the post-pulse. Since this type of behaviour is also shown by the charge movement process (Adrian & Almers 1976*b*; Chandler *et al.* 1976*a*, *b*) it is of interest to analyse the results on the assumption that the component of the activation mechanism which is reprimed is charge movement.

The charge movement experiments indicate that there are at least three configurations for the charge, called *resting*, *activating* and *refractory* in Chandler *et al.* (1976*b*). Assuming that the rate constants are instantaneous



functions of membrane potential, transitions between *resting* and *activating* and between *activating* and *refractory* configurations follow exponential time courses for a step change in potential. Direct transitions between *resting* and *refractory* configurations may occur, as indicated by the dashed arrows, although at the present time there is no experimental requirement for them. Values of the rate constant $(\alpha+\beta)$ measured in the cold are several orders of magnitude greater than those of $(\gamma+\delta)$. At $2°$ C $(\alpha+\beta)$ is order of magnitude 0·1 msec^{-1} and $(\gamma+\delta)$ is order of magnitude 0·1 sec^{-1}

An additional requirement is that there is a total amount of charge Q_{max},

$$Q_{max} = Q_{resting} + Q_{activating} + Q_{refractory}. \quad (1)$$

The average value of Q_{max}, divided by capacitance, in six fibres reported by Chandler *et al.* (1975*a*) was 25 nC/μF.

At a holding potential of -20 mV the steady-state distribution would be

$$Q_{resting} = 0, \quad (2{\cdot}1)$$

$$Q_{activating} = 0, \quad (2{\cdot}2)$$

$$Q_{refractory} = Q_{max}. \quad (2{\cdot}3)$$

On hyperpolarization to -120 mV, $\delta \gg \gamma$ so that the amount in the *refractory* configuration tends to decline exponentially to zero with time constant $\tau = \delta^{-1}$. Since $\beta \gg \alpha$ and $\beta \gg \delta$ the amount in the *activating* configuration remains near zero, giving

$$Q_{resting} = Q_{max}[1-\exp(-t/\tau)], \quad (3{\cdot}1)$$

$$Q_{activating} = 0, \quad (3{\cdot}2)$$

$$Q_{refractory} = Q_{max} \exp(-t/\tau). \quad (3{\cdot}3)$$

When the period of hyperpolarization is terminated and the potential is switched to the post-pulse level V, the charge in the *resting* configuration rapidly distributes between *resting* and *activating* according to the distribution function $F(V)$,

$$Q_{activating}/(Q_{resting}+Q_{activating}) = F(V). \quad (4)$$

In the rather simple model used by Chandler *et al.* (1976*a*) the functional form of $F(V)$ is given by

$$F(V) = \frac{1}{1+\exp[-(V-\bar{V})/k]}. \quad (5)$$

After a few tens of msec

$$Q_{resting} = Q_{max}[1-\exp(-t/\tau)][1-F(V)], \quad (6{\cdot}1)$$

$$Q_{activating} = Q_{max}[1-\exp(-t/\tau)]F(V), \quad (6{\cdot}2)$$

$$Q_{refractory} = Q_{max} \exp(-t/\tau), \quad (6{\cdot}3)$$

where t is the duration of the hyperpolarization. In the following seconds the charge in the *resting* and *activating* configurations decreases along an exponential time course and becomes *refractory*.

If charge in the *activating* configuration were involved in the activation of contraction, for example by regulating calcium release from the sarcoplasmic reticulum, one might expect contraction to occur if the amount exceeded a *threshold* value. At threshold itself

$$Q_{\text{activating}} = Q_{\text{threshold}}. \tag{7}$$

The expected changes in Q_{resting}, $Q_{\text{activating}}$ and $Q_{\text{refractory}}$ which have just been described are shown in Fig. 3. In the diagram $Q_{\text{activating}}$ exceeds $Q_{\text{threshold}}$ during the initial part of the post-pulse so that contraction would be activated. If V were less positive the peak value of $Q_{\text{activating}}$ would be reduced, eventually reaching a level which does not exceed the contraction threshold.

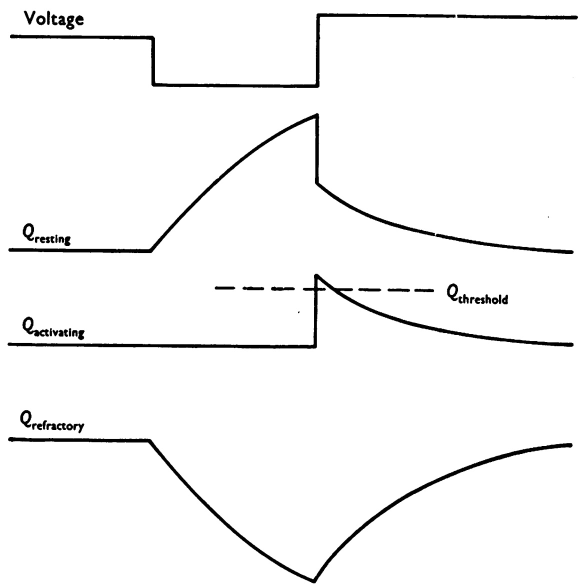


Fig. 3. Time course of Q_{resting}, $Q_{\text{activating}}$ and $Q_{\text{refractory}}$ during a repriming experiment. The upper diagram shows membrane voltage and the other diagrams, charge. At the holding potential the voltage is sufficiently positive that $Q_{\text{resting}} = Q_{\text{activating}} = 0$ and $Q_{\text{refractory}} = Q_{\text{max}}$, eqn. (2). During the period of hyperpolarization Q_{resting} increases exponentially, $Q_{\text{activating}} = 0$ and $Q_{\text{refractory}}$ decreases exponentially according to eqn. (3). Within less than 0·1 sec after the onset of the post-pulse Q_{resting} decreases and $Q_{\text{activating}}$ increases according to the distribution function $F(V)$, eqns. (4)–(6). Thereafter the amounts decrease exponentially towards zero while $Q_{\text{refractory}}$ increases to Q_{max}. If $Q_{\text{activating}}$ exceeds $Q_{\text{threshold}}$ during the initial part of the post-pulse a contraction is elicited.

For a pulse sequence that results in a threshold contraction eqns. (6·2) and (7) can be combined to give

$$\frac{1}{\tau[1-\exp(-t/\tau)]} = F(V)\left[\frac{Q_{\text{max}}}{\tau Q_{\text{threshold}}}\right]. \tag{8}$$

The term in square brackets on the right side of the equation is constant. In the limiting case in which only a small fraction of Q_{max} is reprimed, $t/\tau \ll 1$ and eqn. (8) becomes

$$\frac{1}{t} \doteqdot F(V)\left[\frac{Q_{max}}{\tau Q_{threshold}}\right]. \tag{9}$$

Comparison of the voltage dependence of mechanical repriming and of charge movement

The filled circles in Fig. 4 represent a plot of $(1/t)$ *vs.* V from the experiment in Fig. 2. According to eqn. (9), valid for $t/\tau \ll 1$, the points give the distribution function $F(V)$ scaled by the constant factor $(Q_{max}/\tau Q_{threshold})$. The smooth curve represents a best least-squares fit of eqns. (5) and (9) which gave $\bar{V} = -18{\cdot}2$ mV, $k = 16$ mV and $Q_{max}/\tau Q_{threshold} = 0{\cdot}373$ sec^{-1}. Values from this and five other experiments are given in Table 1.

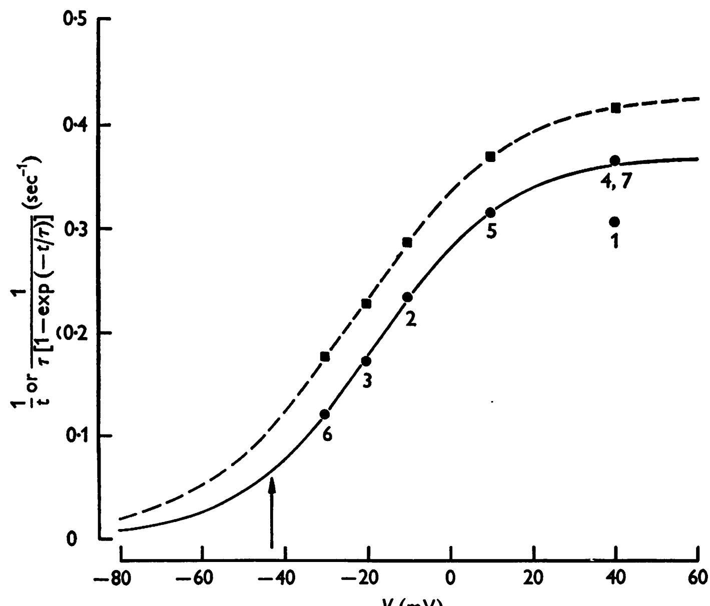


Fig. 4. Sigmoid relationship between the reciprocal of the repriming duration and the post-pulse potential. ●, $(1/t)$ *vs.* V from the experiment in Fig. 2. The sequence in which the measurements were made is indicated by small numerals. The continuous curve is drawn according to eqns. (9) and (5) using best fit parameters $\bar{V} = -18{\cdot}2$ mV, $k = 16$ mV and $Q_{max}/\tau Q_{threshold} = 0{\cdot}373$ sec^{-1}. The first observation was omitted from the fit. ■, $1/\tau[1-\exp(-t/\tau)]$ *vs.* V calculated from the same experiment using $\tau = 10$ sec and omitting data point 1. The interrupted curve was calculated from eqns. (8) and (5) and best fit parameters $\bar{V} = -23$ mV, $k = 19$ mV, $Q_{max}/\tau Q_{threshold} = 0{\cdot}431$ sec^{-1}. The arrow drawn at -43 mV indicates the contraction threshold which was measured when the fibre was fully reprimed, as described in the text.

At the end of the repriming runs in the experiment in Fig. 2 the holding potential was changed to -100 mV. After 5 min the mechanical threshold for the fully reprimed fibre was determined using 200 msec depolarizing pulses. A pulse to -44 mV gave no contraction (open square, Fig. 2) whereas one to -42 mV gave visible movement (filled square, Fig. 2). The midpoint, -43 mV, was taken as threshold. This voltage is indicated by the vertical dashed line in Fig. 2 and by the vertical line with arrow in Fig. 4.

TABLE 1. Best fit parameters for contraction repriming experiments

(1) Fibre	(2) Temperature (° C)	(3) $\bar{V}$ (mV)	(4) k (mV)	(5) $\frac{Q_{max}}{\tau Q_{threshold}}$ (sec^{-1})	(6) $t_{V=\infty}$ (sec)
23·3	5·4	−30·8	10·5	0·252	3·97
28·5	9·8	−18·7	8·2	0·409	2·44
29·1	4·2	−21·8	10·3	0·223	4·48
29·3	4·2	−18·2	16·0	0·373	2·68
36·2	5·2	−17·9	9·6	0·206	4·85
37·4	5·5	− 5·8*	17·1*	0·164*	6·10*
Average ± S.E. of mean		−21·5 ± 2·4	10·9 ± 1·3	0·293 ± 0·041	3·68 ± 0·48

The repriming voltage was -120 mV except for fibre 37·4 in which -80 mV was used. Columns (3) to (5) give best fit parameters using eqns. (5) and (9). Column (6) gives reciprocals of values in (5); these represent the minimum repriming times required for large positive post-pulses.

* Values for -80 mV excluded from average.

For the model to be completely consistent with the results, the amount of $Q_{activating}$ required to give a threshold contraction in the fully reprimed condition should be the same as that required in a partially reprimed condition. At $V = -43$ mV the continuous curve in Fig. 4 is 0·17 times its maximum value so that $Q_{threshold}/Q_{max} = 0{\cdot}17$. This value and the value 0·373 sec^{-1} for $Q_{max}/\tau Q_{threshold}$ gives $\tau = 15{\cdot}8$ sec. Quite clearly the inequality $t/\tau \ll 1$ does not hold for the less positive post-pulses which required repriming durations up to 8 sec.

The filled squares in Fig. 4 represent the same data plotted as $1/\tau[1-\exp(-t/\tau)]$, as suggested by eqn. (8), assuming somewhat arbitrarily that $\tau = 10$ sec. The sigmoid relationship is similar to that shown by the circles but with a larger plateau value and a slight shift to more negative potentials. The best fit parameters for the dashed curve drawn using eqns. (8) and (5) are $\bar{V} = -23$ mV, $k = 19$ mV and $Q_{max}/\tau Q_{threshold} =$ 0·431 sec^{-1}. The value of 0·431 sec^{-1} for the last parameter and $\tau = 10$ sec give $Q_{threshold}/Q_{max} = 0{\cdot}23$. This number is in close agreement with the number 0·25 obtained as the ratio of the value of the dashed curve at

-43 mV to the maximum value and indicates a satisfactory agreement between the data determined both in the fully reprimed and partially reprimed conditions.

The general conclusion from this kind of experiment is that the distribution function $F(V)$ which is obtained from the mechanical repriming curve is very similar to the distribution function measured directly for charge. The average value of k determined by mechanical measurements was $10{\cdot}9 \pm 1{\cdot}3$ mV (five observations, column 4 Table 1) and from charge measurements was $7{\cdot}8 \pm 0{\cdot}7$ mV (six observations, Chandler *et al.* 1976*a*). These two values are not significantly different ($P > 0{\cdot}05$). The two values of $\bar{V}$, taken at face value, are different by about 20 mV. This difference, however, is most likely attributed to the fact that different solutions were used for the two types of experiments.

The repriming measurements gave values of $-21{\cdot}5 \pm 2{\cdot}4$ mV for $\bar{V}$ (five observations, column 3, Table 1) whereas the charge measurements, in hypertonic solution, gave $-44{\cdot}1 \pm 3{\cdot}4$ mV (six observations, Chandler *et al.* 1976*a*). The difference corresponds to a shift of 22·6 mV, a value which may be 2–5 mV too large since the estimates of $\bar{V}$ in Table 1 were calculated assuming $t \ll \tau$. A rough indication of the shift expected from the difference in solutions is given by the shift in the threshold for mechanical activation. In the solution used for the mechanical measurements the contraction threshold for the fully reprimed fibre was -43 mV (Fig. 2). The threshold in the solution used for charge movement is estimated to be -58 mV, the essential assumption being that a threefold increase in osmolality in the external solution produces a -10 mV shift in the activation threshold (Chandler *et al.* 1976*b*). The difference between the thresholds, 15 mV, is similar in magnitude and direction to the difference between the determinations of $\bar{V}$. When this shift is taken into account the two values of $\bar{V}$ do not appear to be significantly different.

Effect of repriming potential on threshold repriming time

The general shape of the curve relating repriming time *vs.* post-pulse potential is the same at different repriming potentials, as indicated by the experiment in Fig. 5. In this fibre repriming was studied at two voltages, -120 mV (open circles) and -160 mV (filled circles). The curve for -120 mV represents the best fit using the same procedure as described for the experiment in Figs. 2 and 4. The curve for -160 mV, also a good fit, was obtained by scaling the first curve by a constant factor, 0·48. In two other experiments using the same two repriming potentials (fibres 30·1 and 36·1) values of 0·55 and 0·51 were obtained for the scaling factor.

The experiment in Fig. 6 shows that the threshold repriming time for a constant post-pulse voltage decreased monotonically as the repriming potential was varied from -80 to -230 mV. The data are adequately described by assuming that the repriming rate constant is proportional to $(V - V^*)$ with $V^* = -66{\cdot}2$ mV.

Effect of temperature on repriming

Fig. 7 shows an experiment in which the repriming curve was measured at 4·1° C (circles), at 10·7° C (triangles), then finally at 4·1° C (squares). The upper curve was drawn according to a best fit of eqns. (5) and (9).

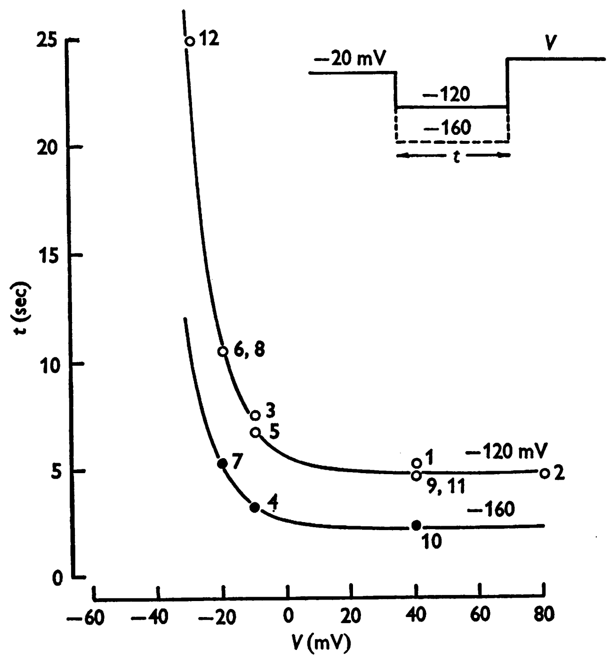


Fig. 5. Repriming curve determined at two different repriming potentials. The sequence of the determinations is indicated by small numerals. The threshold repriming time, t, is plotted as a function of the post-pulse potential V as indicated in the inset in the upper right. ○, measurements at a repriming potential of −120 mV; ●, at −160 mV. The continuous curve through the open symbols is based on eqns. (5) and (9) with best fit parameters $\bar{V} = -17{\cdot}9$ mV, $k = 9{\cdot}6$ mV and $Q_{\max}/\tau Q_{\text{threshold}} = 0{\cdot}206\ \text{sec}^{-1}$. The lower curve is 0·48 times the upper curve. Fibre 36·2, temperature 5° C. Two micro-electrode clamp, electrode spacing not recorded. Initial resting potential −22 mV, holding potential −20 mV.

The lower curve was then obtained by multiplying by a constant factor which was adjusted to give a best fit. The value of the scaling factor, 0·238, implies that the Q_{10} of the repriming process is extremely high, 8·9. Another fibre studied at 3·5 and 7·2° C gave $Q_{10} = 8{\cdot}7$.

B. Repriming charge movement and contraction

The shape of the repriming curve is consistent with the known behaviour of the charge movement process and the assumption that contraction occurs if $Q_{\text{activating}}$ exceeds $Q_{\text{threshold}}$ at the beginning of the post-pulse. It is clearly important to find out whether charge movement is actually reprimed under the conditions of these experiments and, if so, to study the characteristics of partially reprimed charge.

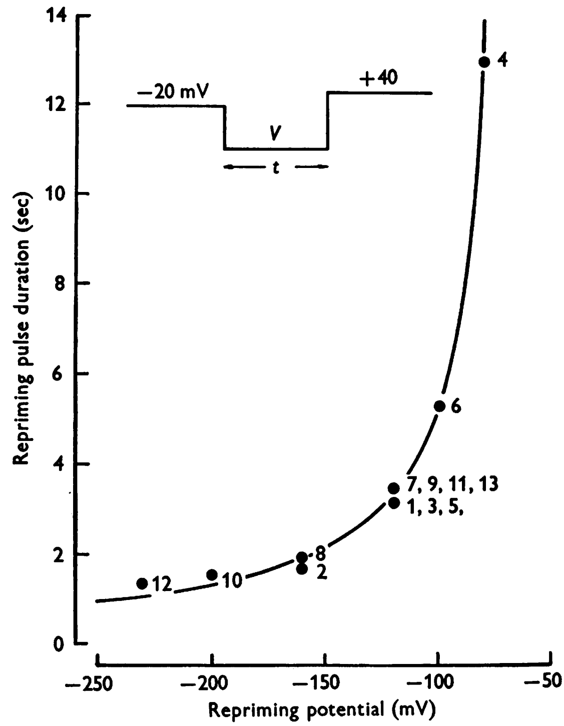


Fig. 6. The effect of repriming potential on the time necessary to reprime contraction, constant post-pulse. The continuous line is drawn according to the equation

$$t = b/(V - V^*)$$

with best fit parameters $b = -179\cdot3$ mVsec and $V^* = -66\cdot2$ mV. The numbers beside the data points indicate the sequence in which the determinations were made. Electrode 2 was 372 μm from the end of the fibre, $l' = 65$ μm. Fibre 30·1, 5° C. Initial resting potential -26 mV, holding potential -20 mV.

All experiments described in the remainder of this paper were carried out using the three micro-electrode voltage-clamp technique. The usual protocol was to first clamp using electrode 2 for feed-back control (Fig. 1). Measurements of linear cable properties were made using 100 msec pulses, $\pm$ 10 mV exponentially rounded with a time constant of 40 μsec. Then the exponential delay was increased to 1–2 msec and threshold durations for mechanical repriming were determined, taking care not to allow a vigorous contraction. Finally, the clamp control was switched to electrode 1 for measurements of charge movement associated with subthreshold amounts of repriming. An interval of at least 2 min was used to separate successive determinations of either mechanical threshold or charge movement.

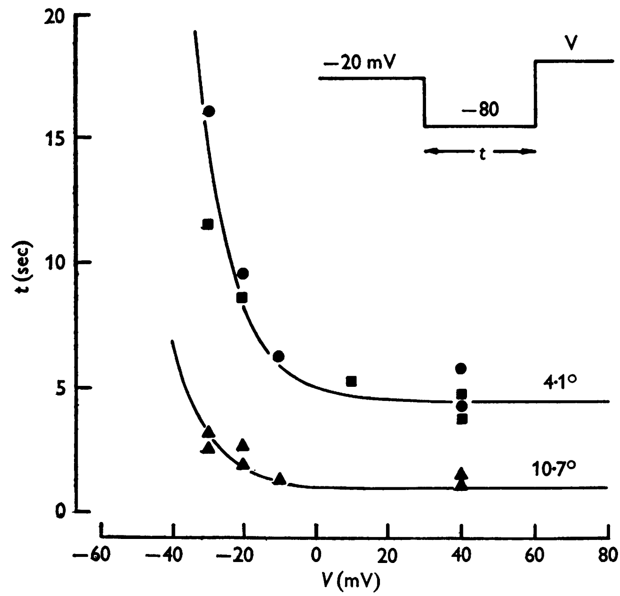


Fig. 7. Effect of temperature on the repriming curve. ●, determinations of mechanical threshold for repriming at -80 mV at 4·1° C; ▲, 10·7° C, ■, return to 4·1° C. The pulse sequence is indicated at the upper right. The curve for 4·1° C is drawn according to eqns. (5) and (9) with best fit parameters $\bar{V} = -21{\cdot}8$ mV, $k = 10{\cdot}3$ mV and $Q_{max}/\tau Q_{threshold} = 0{\cdot}223$ sec^{-1}. The lower curve is 0·238 times the upper curve, implying $Q_{10} = 8{\cdot}9$. Electrode 2 was 372 μm from the end of the fibre; $l' = 47$ μm. Fibre 29·2. Initial resting potential -23 mV, holding potential -20 mV.

Recovery of charge movement during sub-threshold repriming pulses

The traces in Fig. 8*A* show charge movement transients after different durations of hyperpolarization to -90 mV and measured following a post-pulse to $+30$ mV. The first two traces in Fig. 8*A* give an indication of the inherent noise in the measurements. The first trace shows the difference between two base-line ΔV records and the second trace shows the difference between two 'control' ΔV records, obtained by repriming for 0·25 sec. The other traces show differences between test and control records, $\Delta V_{test} - \Delta V_{control}$, where repriming times for the 'test' measurements are listed at the left. Control times were always 0·25 sec. It is clear from the Figure that the charge movement transients, although small, became progressively larger as the repriming duration was increased.

The traces in Fig. 8*B* show subtracted voltage records displayed at the same gain. They are flat within the resolution of the measurement, showing that the charge movement transients in Fig. 8*A* were not due to inequalities in the test and control voltage steps.

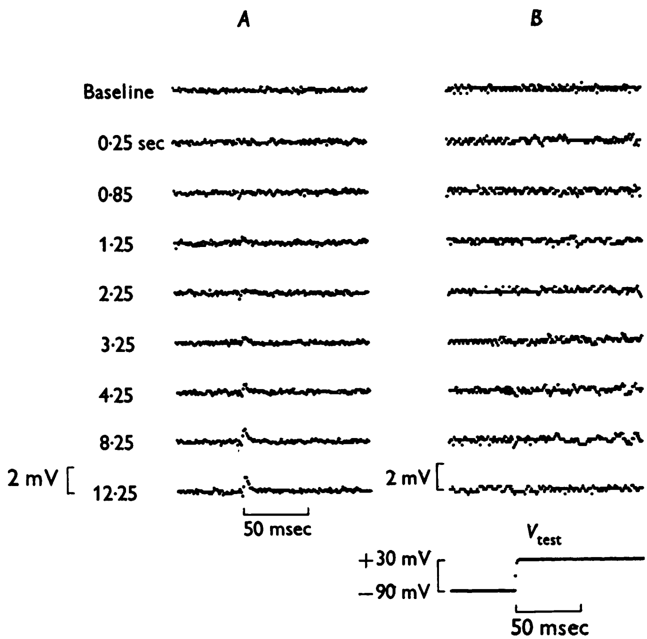


Fig. 8. Recovery of charge movement with increasing durations of repriming. *A*, traces of $\Delta V_{test} - \Delta V_{control}$ for various repriming times as indicated at the left. *B*, traces of $V_{test} - V_{control}$. The base-line traces are the subtraction of two records in which no pulses were applied. The traces for 0·25 sec are the subtraction of two 'control' records in which a repriming prepulse to -90 mV for 0·25 sec was followed by a pulse to $+30$ mV as illustrated in the lower right. In subsequent records a control record was subtracted from a test record in which progressively longer durations of repriming were used. All records are the average of two sweeps. In this and subsequent Figures each point represents the average value of the signal measured during a 1 msec interval. Electrode spacing $l = 368$ μm, $l' = 93$ μm. Cable measurements gave $r_i = 2{\cdot}775$ MΩ/cm, $c_m = 0{\cdot}3126$ μF/cm, radius = 55·3 μm, $\lambda = 0{\cdot}282$ cm. 1 mV on ΔV corresponds to 0·174 μA/cm or 0·555 μA/μF. Fibre 38·2, temperature 5·5° C; command pulse rounded with a 0·6 msec time constant. Initial resting potential -33 mV, holding potential -30 mV.

Fig. 9 shows the recovery of charge movement as a function of repriming time. The amount of charge movement was obtained by integrating the transient component of ΔV as described in the legend and was normalized by dividing by total fibre capacitance. An indication of the error involved in the determinations is given by the ± 1 S.D. bar, estimated according to the procedure described in Methods. The two points plotted at $t = 0{\cdot}25$ sec were calculated from the first two traces in Fig. 8*A* and theoretically should give values of zero. Within experimental error the data points in Fig. 9 increase linearly with time as would be expected for the initial part of an exponential. The line has a slope of 0·291 nC/μFsec. A linear increase with time is also seen in Fig. 10 in which charge and repriming duration are plotted on expanded scales. In Fig. 10 data have been pooled from three fibres.

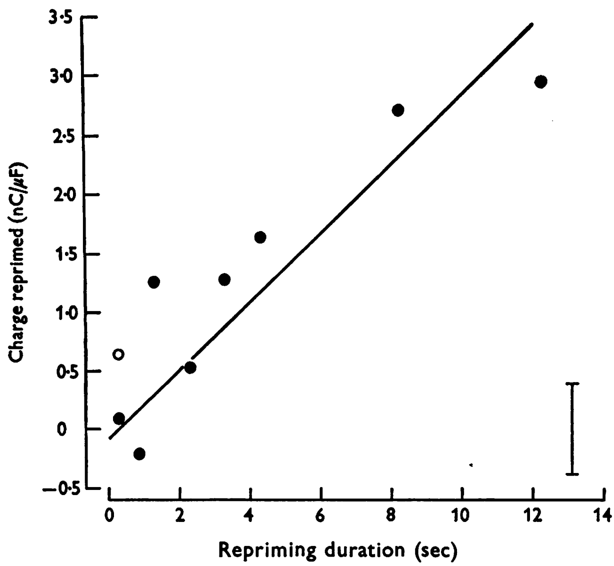


Fig. 9. Initial linear recovery of charge movement. ●, charge movement areas determined from the last eight records in Fig. 8*A*. Each determination was made the same way. A straight line was fitted to the points numbered 26–100 following the voltage pulse. This line was extrapolated to the beginning of the pulse and the difference between points 1–25 and the extrapolated base line was taken as the charge movement transient. The same procedure was used for all other estimates of charge movement described in Results, part B. ○, area determined in a similar manner from the first record in 8*A*. The line drawn is the best least squares fit to the data with the constraint that charge equal zero at 0·25 sec, the control pulse duration. The slope of the line is 0·291 nC/μFsec. The vertical bar in the lower right part of the figure indicates ± 1 S.D. of the measurement of charge movement, $\pm 0{\cdot}389$ nC/μF, estimated as described in Methods.

Estimates of charge movement expected at the mechanical threshold

Before the traces in Fig. 8 were taken, contractile repriming was studied using the same pulse voltages but with the clamp control on electrode 2. A period of 16 sec repriming gave no contraction whereas 18 sec gave a visible response. Threshold was taken as the average, 17 sec. If the amount of reprimed charge increases linearly with time during this period (0·291 nC/μFsec in Fig. 9), threshold repriming would be associated with the recovery of 4·95 nC/μF. On the basis of the model used to explain the results in Part *A*, the value 4·95 nC/μF would correspond to $Q_{\text{threshold}}$ and would indicate that $Q_{\text{threshold}}$ is 0·198 times the average value of $Q_{\max}$ measured by Chandler *et al.* (1976*a*), 25 nC/μF.

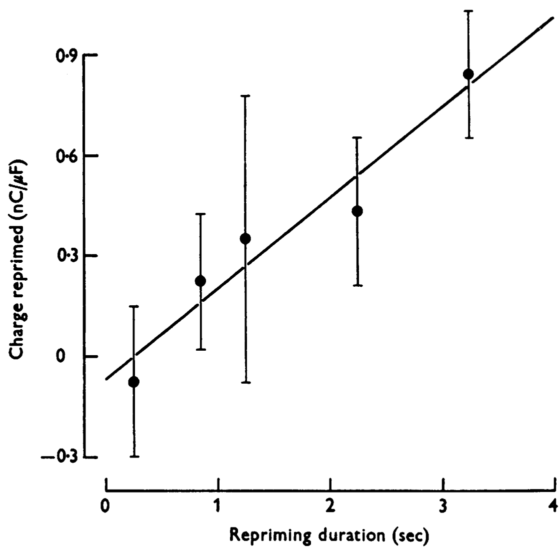


Fig. 10. Initial linear recovery of charge movement for short repriming durations. Results from two runs on each of three fibres, using the same protocol as Figs. 8 and 9, have been averaged and plotted on expanded scales. The vertical lines indicate ± 1 S.E. of mean. The slope of the fitted line is 0·270 nC/μFsec. For fibres 38·1 and 39·1 the holding potential was -20 mV, the repriming potential was -80 mV and the post-pulse potential was $+40$ mV. In fibre 38·2 all these voltages were 10 mV more negative. Temperature 3–5° C.

An initial rate of 0·291 nC/μFsec and $Q_{\max} = 25$ nC/μF implies a time constant of 86 sec for an exponential process, eqn. (6·2) and a value of unity for $F(V)$ during the post-pulse. Using an exponential recovery curve a somewhat smaller amount of charge, 4·5 nC/μF or 0·18 times $Q_{\max}$, should be reprimed in 17 sec. The general conclusion is that 4·5–5·0 nC/μF of charge is reprimed at the repriming duration which results in a threshold contraction.

Table 2 gives results from a total of seven fibres. Column (4) gives the observed rate of charge appearance; (5) gives the time constant for charge appearance on the assumption that $Q_{\max} = 25$ nC/μF and $F(V) = 1$

during the post-pulse; (6) gives the repriming duration for a threshold contraction; and (7) gives $Q_{threshold}$ assuming a linear time course for charge recovery. The values in column (7) range from 1·95 to 4·95 nC/μF with an average value of 3·52 nC/μF. Using Q_{max} = 25 nC/μF this implies that on the average $Q_{threshold}/Q_{max}$ = 0·14 with a range of values 0·08–0·20.

TABLE 2. Repriming contraction and charge movement

(1) Fibre	(2) Temperature (° C)	(3) Post-pulse potential (mV)	(4) Rate of charge appearance (nC/μFsec)	(5) Charge repriming time constant (sec)	(6) Contraction repriming time (sec)	(7) $Q_{threshold}$ (nC/μF)
37·1	5·0	40	0·300	83	6·5	1·95
37·4	5·5	40	0·578	43	6·5	3·76
		40	0·422	59	6·5	2·74
38·1	5·5	40	0·209	120	18	3·76
38·2	5·5	30	0·291	86	17	4·95
38·3	5·5	40	0·190	132	> 12	
39·1	3·0	40	0·146	171	23·5	3·43
39·2	3·0	40	0·149	168	27	4·02
Average ± S.E. of mean				108 ± 17		3·52 ± 0·36

The repriming potential was −80 mV except for fibre 38·2 in which −90 mV was used. Column (4) gives the repriming rate for charge movement and column (5) gives the corresponding time constants, assuming exponential recovery (eqn. 6·2), Q_{max} = 25 nC/μF and $F(V)$ = 1 during the post-pulse. Column (6) gives the minimum time required to reprime contraction. The values of $Q_{threshold}$ in column (7) are the product of the values in (4) and (6); they represent the amount of charge reprimed when contraction is just reprimed, assuming a linear recovery for charge.

Effect of post-pulse potential on the observed rate of charge repriming

In the experiments in Fig. 8–10, the post-pulses were sufficiently positive to ensure that almost all the reprimed charge moved from the resting to the activating configuration during the post-pulse and thus was observed electrically. Fig. 11 shows charge plotted against repriming duration in an experiment in which measurements were made at two different post-pulse voltages, +40 mV (filled circles) and −40 mV (open circles). The repriming potential was −80 mV. The charge measured at +40 mV recovered at a rate of 0·19 nC/μFsec given by the slope of the continuous line. The measurements at −40 mV gave a recovery rate of 0·05 nC/μFsec, dashed line, but this value does not seem to be different from zero.

Q vs. V relationship following subthreshold repriming

It is of some interest to see whether the small amount of charge movement which recovers during a subthreshold repriming pulse depends on post-pulse potential in the same way as fully reprimed charge, eqn. (5). Fig. 12 shows records from an experiment designed to examine this point. Column *A* shows difference records $\Delta V_{\text{test}} - \Delta V_{\text{control}}$ where the test duration was 20 sec, the control duration was 0·25 sec and the repriming potential was -80 mV. It is apparent from the traces that as the post-pulse potential was increased from -60 to $+39$ mV the charge transient became progressively larger. For comparison, column *B* shows that records obtained from the subtraction of two successive control ΔV transients are flat, as they should be.

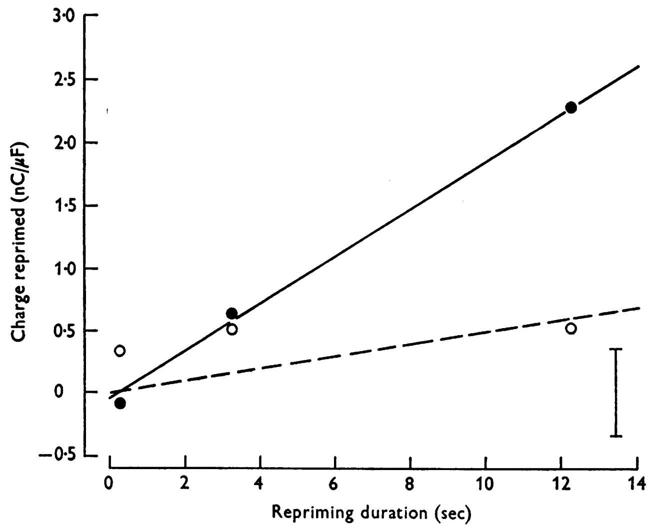


Fig. 11. Effect of post-pulse potential on the apparent rate at which charge movement is reprimed. Measurements of charge movement area were made from difference traces $\Delta V_{\text{test}} - \Delta V_{\text{control}}$ signal averaged twice. The repriming duration of ΔV_{test} is given by the abscissa; control duration was 0·25 sec. Repriming potential, -80 mV. Post-pulse potential: ●, $+40$ mV; ○, -40 mV. The lines are the best least-squares fits constrained to give zero charge at the control pulse duration, 0·25 sec. The slope of the continuous line is 0·19 nC/μFsec and of the dashed line is 0·05 nC/μFsec. Fibre 38·3, temperature 5·6° C. Initial resting potential -29 mV, holding potential -20 mV. The vertical bar in the lower right part of the Figure extends from $-0·350$ to $+0·350$ nC/μF, indicating ± 1 S.D. of the measurements.

The areas of the transients in Fig. 12 are plotted in Fig. 13 as a function of post-pulse potential. The filled circles give the measured charge movement from the traces in Fig. 12*A* whereas the open circles were obtained from the control traces in Fig. 12*B*. The open circles should give zero charge and do so within the reliability of the measurements. The vertical bar at the lower right shows ± 1 S.D. of the charge measurement, estimated as previously described in Methods.

The continuous curve in Fig. 13 represents a least squares fit using eqn. (5) and an adjustable value for the maximum amount, 3·3 nC/μF, indicated by the dashed line; $\bar{V}$ = −10 mV and k = 22 mV. This experiment indicates that partially reprimed charge depends on post-pulse potential in a sigmoid manner similar to that shown for fully reprimed charge.

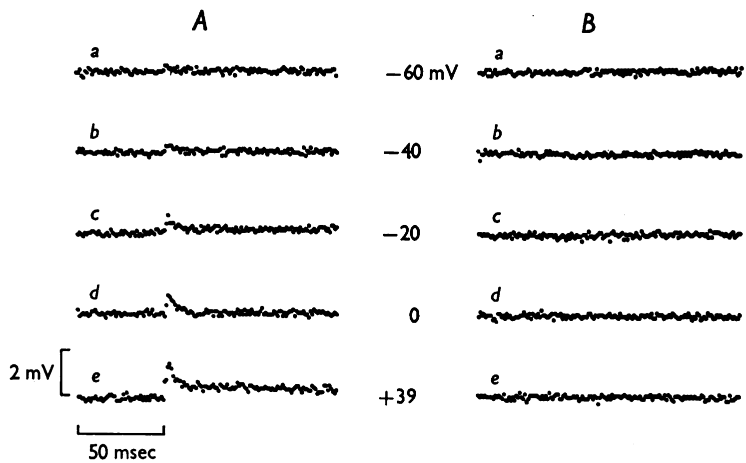


Fig. 12. Effect of post-pulse potential on observed charge movement. A, traces of $\Delta V_{\text{test}} - \Delta V_{\text{control}}$ for various post-pulse potentials as indicated. B, $\Delta V_{\text{control}} - \Delta V_{\text{control}}$ for two successive control records. All records have been signal averaged three times. The test determinations used 20 sec of repriming at −80 mV followed by a post-pulse to the potential indicated. Control determinations were similar except that repriming lasted only 0·25 sec. The command pulse was rounded with a time constant of 0·6 sec. The duration required to reprime a threshold contraction was 27 sec using a post-pulse to +40 mV and clamping on V_2. Electrode spacing l = 377 μm, l' = 93 μm. r = 2·420 MΩ/cm, c_m = 0·3660 μF/cm, radius = 61·5 μm, λ = 0·369 cm. 1 mV on ΔV corresponds to 0·194 μA/cm or 0·530 μA/μF. Fibre 39·2, temperature 3·2° C. Initial resting potential −22 mV, holding potential −20 mV.

Comparison of $F(V)$ from mechanical measurements and $Q(V)$ from sub-threshold charge measurements

In one experiment a fibre with three micro-electrodes inside gave reproducible results for over 2 hr without any visible damage. In this case it was possible to make a full set of both mechanical and charge measurements.

The first measurements, made after the run for cable properties, gave the repriming parameters in Table 2, first line fibre 37·4. The second set of measurements concerned the effect of post-pulse potential on a sub-threshold amount of reprimed charge. The results, normalized to give a maximum value of unity, are shown by the filled circles in Fig. 14. The open circles, which should have a value of zero, were evaluated from control traces in exactly the same manner as the open circles in Fig. 13. The curve in Fig. 14 represents a best least-squares fit of eqn. (5) to the filled circles, $\bar{V} = -5$ mV and $k = 12{\cdot}8$ mV.

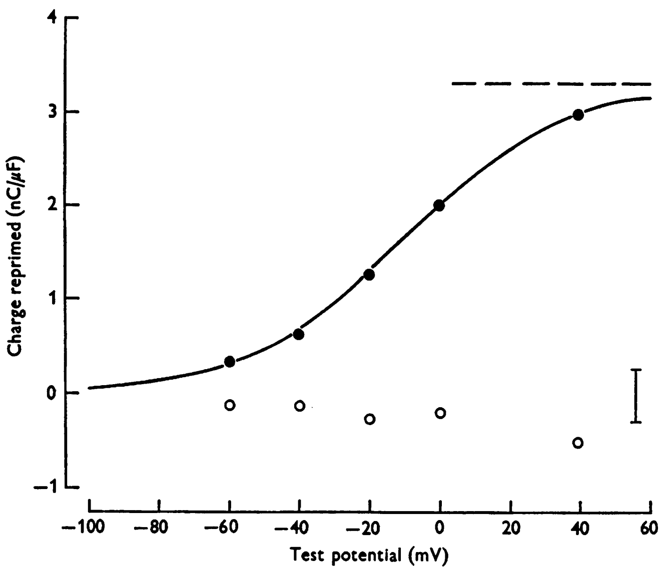


Fig. 13. Voltage dependence of partially reprimed charge. ●, charge movement determined from the records in Fig. 12*A*. ○, areas determined in a similar manner from control records, Fig. 12*B*. The continuous line was determined by a least-squares fit using eqns. (4) and (5) and an adjustable value for the total charge in the resting and activating states, indicated by the dashed line. $\bar{V} = -10$ mV, $k = 22$ mV and total charge $= 3{\cdot}3$ nC/μF. The vertical line in the lower right indicates ± 1 S.D. of the measurements, $\pm 0{\cdot}288$ nC/μF.

The final set of measurements on the fibre gave the repriming curve for contraction and showed that the threshold duration for a $+40$ mV post-pulse had not changed from the initial value of 6·5 sec (Table 2, second line fibre 37·4). Values of $(1/t)$, normalized to a maximum value of unity, are shown by the filled squares in Fig. 14.

The general conclusion from the experiment is that the two methods for measuring charge distribution, indirectly by observing contractile repriming and directly by measuring Q *vs.* V for subthreshold amounts of charge, are in agreement.

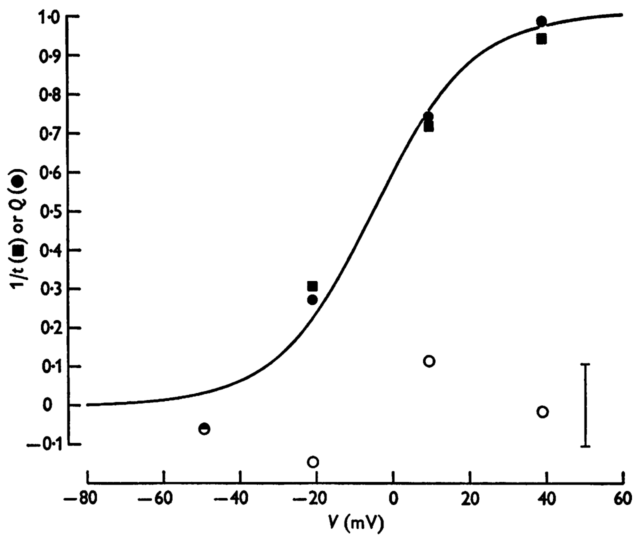


Fig. 14. Comparison of $F(V)$ from mechanical measurements and $Q(V)$ from subthreshold charge measurements. ●, charge movement determined from $\Delta V_{test} - \Delta V_{control}$ records; ○, charge determined from the subtraction of two successive control records. The procedure was exactly the same as used in Figs. 12 and 13 except that the records were signal averaged 4 times and the duration of the repriming pulse was 6 sec. The original data for the filled circles, in units nC/μF, were fitted according to eqn. (5) and a scaling factor. The data for open and filled circles were divided by the scaling factor, 2·59 nC/μF, for plotting. The curve is drawn from eqn. (5) using the best fit values $\bar{V} = -5$ mV and $k = 12{\cdot}8$ mV. ■, $(1/t)$ *vs.* V from contractile repriming measurements. The original data, in units sec^{-1}, were fitted using eqns. (5) and (9). The value for $Q_{max}/\tau Q_{threshold}$, 0·164 sec^{-1}, was used to scale the data for plotting. The best fit values for $\bar{V}$ and k were −5·8 and 17·1 mV, respectively (curve not shown). Fibre 37·4, temperature 5·4–5·8° C. Initial resting potential −20 mV, holding potential −20 mV. The vertical bar in the lower right shows ± 1 S.D. of the normalized charge measurements, ± 0·106.

C. Characteristics of a second type of charge movement

In depolarized fibres, in which the usual charge movement has been inactivated, the electrical capacitance shows a voltage dependence suggesting the presence of a second type of charge movement (Almers, 1975; Schneider & Chandler, 1976). This process, which will be referred to as 'Charge 2', shows a much broader voltage dependence than 'Charge 1'.

Estimates of Charge 2 currents can be obtained in fibres in 80 mM-Rb solution as shown in Fig. 15. The diagram in part *A* shows the pattern of voltage pulses used to obtain the traces in part *B*. For ΔV_{test} the pulse pattern is indicated by the continuous line. A conditioning prepulse to +29 mV was applied 100 msec before superimposing the hyperpolarizing test pulse to voltage V, duration 100 msec. The magnitude of this pulse was $49n$ mV where $n = 1, 2, 3$ or 4. For $\Delta V_{\text{control}}$ the conditioning prepulse was to +78 mV and the superimposed pulse was always 49 mV in magnitude, as shown by the dashed line; n records were taken and summed for each $\Delta V_{\text{control}}$.

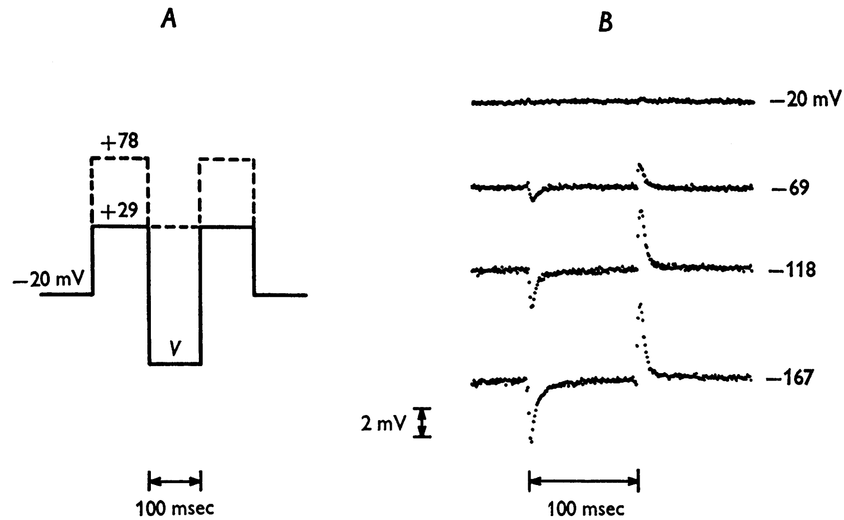


Fig. 15. A second type of charge movement. A, the continuous line shows the test pulse sequence and the dashed line the control pulse sequence used to obtain the charge movement transients shown at the right. Records of from one to four control pulses were summed, as appropriate, and the sum was subtracted from the test pulse record. A correction factor was calculated and applied to the control ΔV transients as described by Chandler *et al.* (1976*a*) to compensate for slight differences in the test and the summed control voltage excursions. B, records of $\Delta V_{\text{test}} - \Delta V_{\text{control}}$ for pulses to the test potential V, indicated besides each record. The command pulse was rounded by a 1 msec time constant. Four runs were signal averaged. Electrode spacing $l = 368$ μm, $l' = 92$ μm. Cable measurements gave $r_i = 4{\cdot}779$ MΩ/cm, $c_m = 0{\cdot}1516$ μF/cm, radius $= 43{\cdot}2$ μm, $\lambda = 0{\cdot}568$ cm; 1 mV on ΔV corresponds to $0{\cdot}1029$ μA/cm or $0{\cdot}679$ μA/μF. Fibre 25·7, temperature 4·0° C. Initial resting potential −35 mV, holding potential −20 mV.

The traces in Fig. 15 B show $\Delta V_{\text{test}} - \Delta V_{\text{control}}$ beginning 50 msec after the start of the conditioning prepulse and ending just before the prepulse was switched off. The voltage during the hyperpolarization for ΔV_{test} is indicated beside each record. The first record shows very little sign of a charge movement transient whereas the others show increasing amounts of charge movement.

Areas of the charge movement transients in Fig. 15*B* were determined as described in Chandler *et al.* (1976*a*). A consistent feature in this and in three other experiments was that the magnitudes of the 'on' areas were less than those of the 'off'. The ratios were usually in the range of 0·5–0·9. The reason for this discrepancy is not known although the presence of small time-varying ionic currents seems to be the most likely explanation.

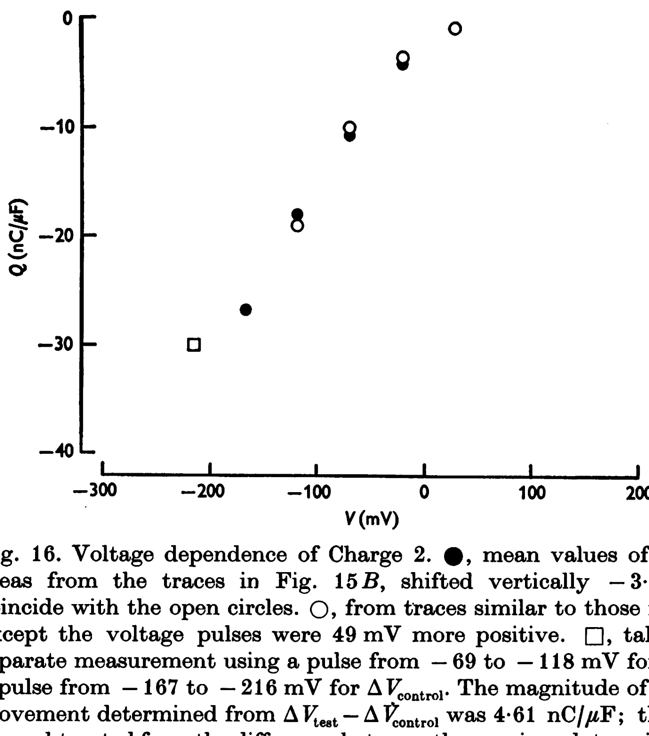


Fig. 16. Voltage dependence of Charge 2. ●, mean values of on and off areas from the traces in Fig. 15*B*, shifted vertically −3·8 nC/μF to coincide with the open circles. ○, from traces similar to those in Fig. 15*B* except the voltage pulses were 49 mV more positive. □, taken from a separate measurement using a pulse from −69 to −118 mV for ΔV_{test} and a pulse from −167 to −216 mV for $\Delta V_{\text{control}}$. The magnitude of the charge movement determined from $\Delta V_{\text{test}} - \Delta V_{\text{control}}$ was 4·61 nC/μF; this amount was subtracted from the difference between the previous data points at −118 and −69 mV to give the charge expected between −216 and −167 mV. This difference was then added to the value at −167 mV to give the value plotted as the open square.

Fig. 16 shows *Q vs. V* for Charge 2 from the experiment in Fig. 15. The values of *Q* are averages of on and off areas and may be somewhat in error because of the inequality in areas mentioned in the previous paragraph. The filled circles are from the traces in Fig. 15*B*, shifted −3·8 nC/μF to coincide with the open circles. The open circles were obtained in the same manner on the same fibre but adding +49 mV to the voltage pulses. The zero reference for *Q* was based on the control for the open circles, a pulse from $+127$ to $+78$ mV. The open square was obtained in a slightly different fashion, as described in the legend.

The data in Fig. 16 show that Q for Charge 2 varies over practically the

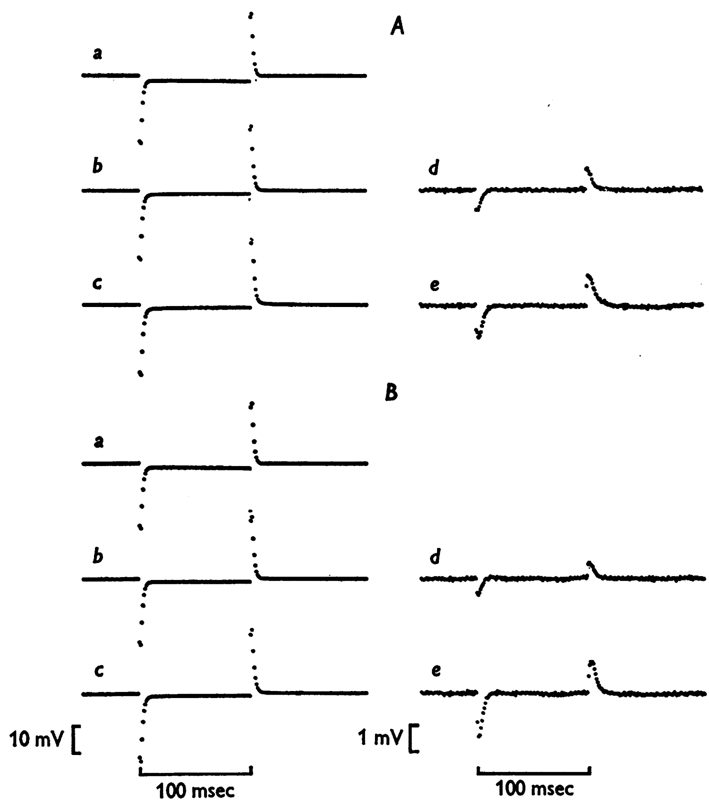


Fig. 17. Effect of holding potential on Charge 2. Run A was made from a holding potential of -20 mV; run B was begun 2 min after changing to -118 mV. The records are signal averaged 8 times. Each run took about 2 min. The records at the left were taken using a -49 mV pulse superimposed on a conditioning prepulse to -167 (a), -118 (b) or -69 mV (c). The records at the right are subtracted records, $d = b-a$ and $e = c-a$. Only the transient components are shown on the right; ionic currents have been subtracted using the procedure described in Chandler *et al.* (1975*a*). The slow ionic currents can be seen as slight slopes in the records on the left by viewing the record along the base line and holding the page at eye level. These residual ionic currents are small and slow and do not appreciably affect the determination of charge movement area. The command pulse was rounded by a 1 msec time constant. Electrode spacing was $l = 368$ μm, $l' = 97$ μm. Cable measurements gave $r_i = 4{\cdot}923$ mΩ/cm, $c_m = 0{\cdot}1804$ μF/cm, radius $= 42{\cdot}8$ μm, $\lambda = 0{\cdot}401$ cm; 1 mV on ΔV corresponds to $0{\cdot}100$ μA/cm or $0{\cdot}554$ μA/μF. Fibre 25·9, temperature 3·6° C. Initial resting potential -26 mV, holding potential -20 mV.

entire measurable range of voltage. Because of this it is difficult to obtain an adequate control record in which it is clear that the charge transient is entirely absent. In spite of the uncertainties, the results indicate that the total amount of Charge 2 is 30–40 nC/μF, $\bar{V}$ is in the neighbourhood of -100 mV and k is probably 40–60 mV. Thus, although the total amount of Charge 2 is similar to the amount of Charge 1 the values for k, which determines the steepness of the voltage dependence curve, are different.

Effect of holding potential on Charge 2

An interesting possibility which was considered at one stage of the investigation was whether the slow inactivation of Charge 1 on depolarization actually represents a conversion of Charge 1 to Charge 2. On this basis recovery on repolarization would involve a return from Charge 2 to 1. To test this idea an experiment was carried out to see whether Charge 2 disappears when fibres in high Rb solution are hyperpolarized for several minutes.

Fig. 17 shows results from one of two experiments. The records in part *A* were taken when the holding potential was -20 mV. Then the holding potential was switched to -118 mV and after 2 min the records in part B were taken. The pulse voltages were the same in *A* and *B* and were negative to the contraction threshold in the fully reprimed fibre.

The left side of Fig. 17 shows ΔV records at low gain for a 49 mV hyperpolarization from different conditioning prepulses, -167 mV for *a*, -118 mV for *b* and -69 mV for *c*. The right side shows difference records at 10 times higher gain, $d = b-a$, $e = c-a$. It is clear that traces *Ad* and *Bd* are similar as are traces *Ae* and *Be*, indicating that Charge 2 was not greatly affected by the change in holding potential.

Two other sets of measurements were made on the same fibre but are not shown. Run *C* was made beginning 7 min after the holding potential was changed to -118 mV. After this was finished the holding potential was slowly returned over a 6 min period to -20 mV, without visible contraction. Three minutes after the potential reached -20 mV run *D* was made.

The *d* traces in runs *A*–*D* were similar in appearance and showed similar amounts of charge movement. The same was true for the *e* traces. For *d* the average on and off areas gave 4·31 (*A*), 2·88 (*B*), 2·54 (*C*) and 2·37 (*D*) nC/μF. For *e* the values were 8·91 (*A*), 8·93 (*B*), 8·80 (*C*) and 9·94 (*D*) nC/μF.

This experiment indicates that Charge 2 currents, measured at negative potentials so that Charge 1 currents are very small, are not appreciably altered by changing the holding potential from -20 to -118 mV. Since this change is sufficient to fully restore Charge 1, the conversion hypothesis is ruled out.

DISCUSSION

The main conclusion from the repriming experiments is that recovery of contraction is affected by voltage in much the same manner as recovery of charge movement. The results can be explained rather simply by assuming that contraction occurs during the relatively long post-pulse if the charge in the activating configuration reaches a threshold level. This explanation can account quantitatively for the shape of the repriming curve, i.e. the relationship of repriming time *vs.* post-pulse potential as seen in Fig. 2. Furthermore, since the shape is determined by the voltage dependence of the distribution function for charge, eqn. (5), factors which change the rate of repriming should alter the repriming curve by only a constant scaling factor. Within the accuracy of our measurements this was the case when the rate was changed either by voltage (Fig. 5) or by temperature (Fig. 7).

Thus far three different methods have been used to estimate the distribution function for charge; (1) direct measurement of charge in fully reprimed fibres in hypertonic solution (Adrian & Almers, 1976*b*; Chandler *et al.* 1976*a*), (2) indirect measurement from experiments on mechanical repriming in isosmotic solution (Figs. 4 and 14, Table 1), and (3) direct measurement of a small amount of reprimed charge in isosmotic solution (Figs. 13 and 14). The results from methods (1) and (2) seem to agree if allowance is made for the effect of the two solutions on $\overline{V}$. In the one experiment in which it was possible to use methods (2) and (3) on the same fibre (Fig. 14) the same distribution curve was obtained with either technique. The agreement between the different methods shows that the charge movement interpretation of the mechanical repriming curve provides a consistent explanation for the experimental results. Although the results are internally consistent there is a slight indication that $\overline{V}$ may be influenced by repriming potential. The values observed on fibres reprimed at -120 mV (method 2, Table 1) are more negative than those found at -80 mV (method 2, fibre 37·4 in Table 1 and Fig. 14; method 3, Fig. 13 and 14), but more experiments are needed to settle this point.

An obvious experiment which was attempted but without success was to measure recovery of charge and contraction at different repriming potentials and to see whether the threshold duration for contraction is associated with a constant amount of charge. At $V = -120$ mV the charge movement currents were partially obscured by currents resembling delayed ionic currents so that accurate measurements of charge could not be made. At $V = -140$ mV the delayed currents were even larger compared with the charge currents. If charge movement activates contraction this result would be expected from previous work in which delayed ionic currents were measured without TEA following repriming at different potentials. In these experiments the amount of delayed rectification was not constant at threshold durations for repriming contraction. Rather, the delayed currents increased as the repriming potential was made more negative (R. H. Adrian and A. L. Hodgkin, personal communication).

Several observations are consistent with the threshold level of charge, $Q_{\text{threshold}}$, being 0·1–0·2 times the value for Q_{max}. Firstly, the threshold for activation in the hypertonic TEA solution used by Chandler *et al.* (1976*a*) was estimated as -58 mV. Using $\bar{V} = -44$ mV and $k = 8$ mV a value of 0·15 is obtained for Q/Q_{max} at this voltage (Chandler *et al.* 1976*b*). Secondly, the threshold for the fibre in Fig. 4 after full repriming was -43 mV. At this voltage the distribution function was 0·17–0·25 times its maximum value, the exact number depending on the value used for the repriming time constant. Thirdly, in the experiments described in Part *B* of the results an average value of 3·5 nC/μF of charge was reprimed at the time that a threshold contraction was reprimed, range 2–5 nC/μF (Table 2). Dividing by 25 nC/μF for Q_{max} (Chandler *et al.* 1976*a*) gives an average value of 0·14, range 0·08–0·20.

In conclusion, the charge movement hypothesis provides a consistent explanation of the threshold mechanical repriming behaviour of skeletal muscle.

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REFERENCES

Adrian, R. H., Chandler, W. K. & Hodgkin, A. L. (1970). Voltage clamp experiments in striated muscle fibres. *J. Physiol.* **208**, 607–644.

Adrian, R. H. & Almers, W. (1976*a*). The voltage dependence of membrane capacity. *J. Physiol.* **254**, 317–338.

Adrian, R. H. & Almers, W. (1976*b*). Charge movement in the membrane of striated muscle. *J. Physiol.* **254**, 339–360.

Almers, W. (1975). Observations on intramembrane charge movements in skeletal muscle. *Phil. Trans. R. Soc.* B **270**, 507–513.

Chandler, W. K., Rakowski, R. F. & Schneider, M. F. (1976*a*). A non-linear voltage dependent charge movement in frog skeletal muscle. *J. Physiol.* **254**, 245–283.

Chandler, W. K., Rakowski, R. F. & Schneider, M. F. (1976*b*). Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle. *J. Physiol.* **254**, 285–316.

Chandler, W. K., Schneider, M. F., Rakowski, R. F. & Adrian, R. H. (1976*c*). Charge movements in skeletal muscle. *Phil. Trans. R. Soc.* B **270**, 501–505.

Hodgkin, A. L. & Horowicz, P. (1960). Potassium contractures in single muscle fibres. *J. Physiol.* **153**, 386–403.

Schneider, M. F. & Chandler, W. K. (1973). Voltage dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature, Lond.* **242**, 244–246.

Schneider, M. F. & Chandler, W. K. (1976). Effects of membrane potential on the capacitance of skeletal muscle fibres. *J. gen. Physiol.* (in the press.)