J. Physiol. (1980), **301**, *pp.* 175–190
With 8 text-figures
Printed in Great Britain

VOLTAGE-DEPENDENT CHARGE MOVEMENT IN FROG SLOW MUSCLE FIBRES

BY W. F. GILLY* AND CHIU SHUEN HUI†

From the Department of Physiology, Yale University Medical School, 333 Cedar Street, New Haven, Connecticut 06510, U.S.A.

(*Received 26 April 1979*)

SUMMARY

1. Voltage-clamp experiments on frog slow and twitch fibres were carried out using the three-micro-electrode technique. Potassium currents were blocked by tetraethylammonium. Contraction was blocked by 2 mM-tetracaine.

2. After subtracting the linear capacitive and leakage currents, the ΔV(test − control) traces from slow fibres show 'on' and 'off' charge movements similar to those observed in twitch fibres.

3. The time integrals of the 'on' and 'off' transients, Q_{on} and Q_{off}, in slow fibres are, as in twitch fibres, almost equal in magnitude but opposite in direction.

4. The charge–voltage distribution is well fitted by a sigmoid curve given by

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

which has been successfully applied to twitch fibres. Data from three fibres gave $\bar{V} = -25$ mV, $k = 13$ mV, and $Q_{max} = 7$ nC/μF. Thus, intramembranous charge in slow fibres has the same steady-state voltage distribution as that in twitch fibres, but the quantity of maximum movable charge is only 1/4 to 1/3 as large.

5. Charge movement in slow fibres does not inactivate completely when the fibres are held at −20 to 0 mV for durations as long as 30 min.

6. These results show that charge movement exists in slow fibres and may serve the same function in regulating contractile activation as that postulated for twitch fibres. The lack of complete inactivation may be consistent with the ability of slow fibres to maintain maximal tension during prolonged depolarizations.

INTRODUCTION

In the preceding papers (Gilly & Hui, 1980*a*, *b*) we compared the contractile and electrical properties of frog slow and twitch muscle fibres. From the fact that slow fibres contract so slowly, one might guess that the mechanism responsible for con-

* Present address: Biology Department/G5, University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A.

† To whom reprint request should be sent. Present address: Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, U.S.A.

0022-3751/80/7180-0169 $07.50

tractile activation in slow fibres is different from that in twitch fibres but, as we have discussed previously, this need not be the case.

Earlier work in electron microscopy showed that frog slow fibres have little sarcoplasmic reticulum and few transverse tubules and triads (Peachey & Huxley, 1962; Page, 1965). As techniques in electron microscopy were improved, more internal membranes were seen in slow fibres (Flitney, 1971), and triads much the same as those in twitch fibres have been found (Franzini-Armstrong, 1973). Moreover, Nasledov, Mandelstam & Radzjukewich (1972) found that the contractility of single slow fibres was vulnerable to glycerol shock as is the case in twitch fibres. They proposed that there might not be any essential difference in the processes leading to contractile activation in the two fibre types.

We have reported that both slow and tetrodotoxin-poisoned twitch fibres follow the same strength–duration relationship for activation of threshold contractions under identical voltage-clamp conditions (Gilly & Hui, 1980*a*). This observation is consistent with the possibility that the mechanisms underlying contractile activation in the two fibre types are similar.

The least understood step in excitation–contraction coupling in skeletal muscle is the signal transmission from the transverse tubule to the sarcoplasmic reticulum, here referred to as tubule:sarcoplasmic reticulum coupling. In twitch fibres, intramembranous charge movements have been observed which may be involved in this coupling (Schneider & Chandler, 1973; Chandler, Rakowski & Schneider, 1976*a*; Adrian & Almers, 1976). In light of the similar morphology of the internal membrane systems in slow and twitch fibres, it is of interest to find out whether charge movements, similar to those in twitch fibres, exist in slow fibres. Preliminary reports of some of the results have appeared (Gilly & Hui, 1978; Chandler, Gilly & Hui, 1978).

METHODS

The experimental techniques were generally the same as those described in the preceding papers (Gilly & Hui, 1980*a*, *b*). Slow fibres from *Rana temporaria* pyriformis muscle and twitch fibres from sartorius muscle were used for the experiments.

Solutions are given in Table 1. Since movement artifacts can contaminate electrical measurements, 2 mM-tetracaine (Lüttgau & Oetliker, 1968) was used to block fibre contraction. In most experiments, 11·8 mM-Ca^{2+} was added to aid sealing the impalement sites (De Mello, 1973; Stefani & Steinbach, 1969). Experiments were performed at 3°–7 °C.

TABLE 1. *Composition of solutions* (mM)

Reference	K^+	Rb^+	Na^+	TEA^+	Ca^{2+}	Cl^-	Tetracaine
A	2·5	—	115	—	1·8	121	2
B	2·5	—	—	115	1·8	121	2
C	2·5	—	—	115	11·8	141	2
D	—	25	—	92·5	11·8	141	2
E	—	65	—	50	11·8	138	2

All solutions were buffered to pH of 7·0–7·2 by either 1·5 mM-Na phosphate (A and B) or 2·0 mM-PIPES buffer (C, D, and E). Twitch fibre experiments were carried out in presence of TTX (10 μg/ml.).

Pulse protocol

In our experiments, test pulses were compared with control pulses of the same polarity and superimposed on a hyperpolarizing conditioning pulse. For test pulses no larger than +80 mV, control pulses of equal magnitude were used. For larger test pulses, control pulses were set at 60 or 80 mV and the ΔV(control) record was scaled up for subtraction (and the number of control pulses used for signal averaging was increased accordingly).

Effect of applying voltage clamp at V_2

Because high resistance (30–50 MΩ) electrodes were used, the feed-back signal for voltage clamp was taken from the V_2 electrode in most of the experiments. Leakage components were removed by a numerical method similar to that described by Chandler *et al.* (1976*a*). When the clamp was on V_2, corrections on ΔV(test) and ΔV(control) were performed separately to allow for any difference in rise times of the test and control pulses.

In a few experiments, in which relatively short electrode spacings were used, we were able to compare charge movements measured by applying the clamp at either V_1 or V_2 in the same slow fibre. Within the resolution of our results, the amount of movable charge was independent of the choice of the feed-back location, as expected. However, the kinetics of charge movements was slower when the clamp was applied at V_2, owing to the additional delay imposed by the longitudinal cable in addition to the unavoidable tubular delay. No attempt was made to correct for such complications.

Estimation of error in the measurements

Since charge movement signals in slow fibres are small, it is important to obtain an estimate of the accuracy of the measurements. The total charge during the pulse, Q_{on}, was obtained by summing the values of the points during the pulse (likewise for the total charge after the pulse, Q_{off}). The summing procedure was usually complicated by one or two erratic points at the 'on' and the 'off' of the pulse. These points were discarded, which led to an underestimation of the values of charge. The error introduced, estimated by fitting a fast exponential rise and a slower exponential decay to the 'on' segment (likewise for the 'off' segment) was $< 3\%$ or $< 10\%$ when one point or two points were dropped, respectively. No attempt was made to correct for this.

The second source of error arises from the noise in the ΔV traces. Electrical noise was inevitable because high-resistance electrodes were used. In addition, an irregular, low frequency noise of unknown (possibly mechanical) origin was observed. The errors arising from these and other sources were estimated from a ΔV(test − control) trace in a manner similar to that used by Chandler *et al.* (1976*a*). For example, in record *a* of Fig. 1*A*, the standard deviation of the sum of the points during the pulse is 0·158 nC/μF. This represents the uncertainty in Q_{on} (likewise for Q_{off}). Other traces in the same experiment were more noisy, and the over-all standard deviation averaged 0·302 nC/μF, as indicated by the vertical bar on the right of Fig. 3.

The third source of error, as discussed below, is the omission of the charge at the holding potential.

RESULTS

Charge movement in slow fibres

Records from one experiment are shown in Fig. 1. Fig. 1*A* shows ΔV(test − control) traces obtained when the fibre, held at −100 mV (*a–c*) or −80 mV (*d–i*), was depolarized to the indicated potentials. With increasing depolarization, the peak current increases and the decay becomes faster. These traces are similar to those obtained in twitch fibres. Fig. 1*B* shows the subtractions of the corresponding feed-back voltage traces, i.e. V_2(test) − V_2(control).

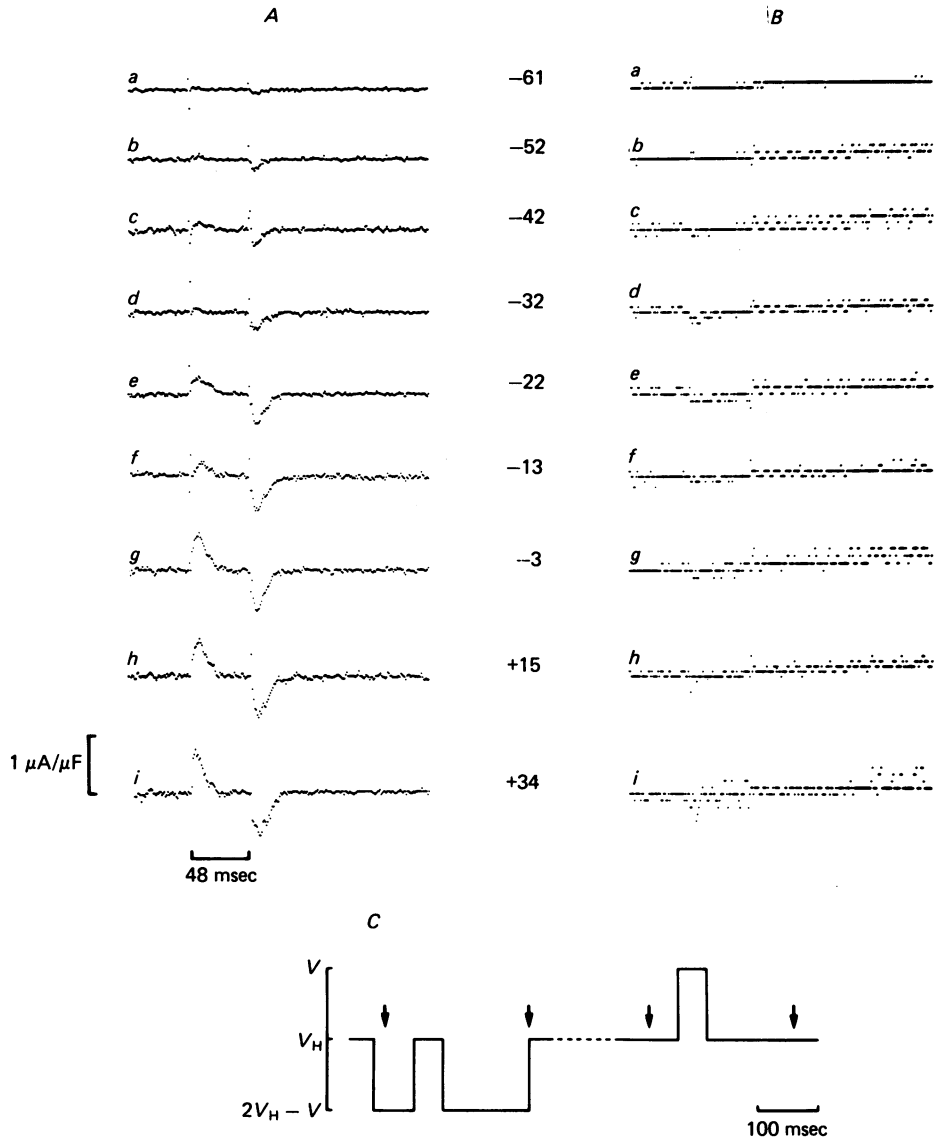

Fig. 1. Charge movement in slow fibre. *A*, ΔV(test − control) traces for depolarizations to voltages, in mV, as indicated at the right. Each point corresponds to a duration of 0·96 msec. Points one to fifty are taken before the depolarizing pulse, points fifty-one to 100 during the pulse, and points 101 to 250 after the pulse. In traces *h* and *i* of both *A* and *B*, the fifty-first and 101st points are erratic and are omitted to avoid overlap with adjacent traces. Fibre diameter was measured optically both in the up–down and left–right directions, and the geometric mean is 72·1 μm. Electrode spacing: l = 273 μm, l' = 20 μm. R_i: assumed to be 271·8 Ω.cm. C_m = 6·557 μF/cm² at the beginning of the experiment. The product $R_i.C_m$ decreased monotonically so that 1 μA/μF corresponds to 1·244 mV of ΔV (at the beginning) and 0·768 mV (at the end). Fibre 4141-77; sarcomere length = 2·9 μm; 5·0 °C; solution C in Table 1; holding potential, V_H, was −100 mV for traces *a*, *b* and *c*, and −80 mV for the rest; feed-back voltage at V_2. Traces *a*, *b*, *c* and *e* are averages of thirty-two sweeps; others are averages of sixteen sweeps. *B*, V_2(test) − V_2(control) traces with the same gain as *A*. The slow creep is due

Comparison of 'on' and 'off' areas

In both nerve (Armstrong & Bezanilla, 1974) and twitch muscle, the equality of the charge carried by the non-linear transient current at the 'on' and the 'off' of a brief test pulse indicates that the current is capacitive in nature. It is thus important to test if this equality of charge is true in slow muscle as well. Q_{on} *vs.* Q_{off} from the experiment of Fig. 1 are plotted as filled circles in Fig. 2. Similar data obtained from

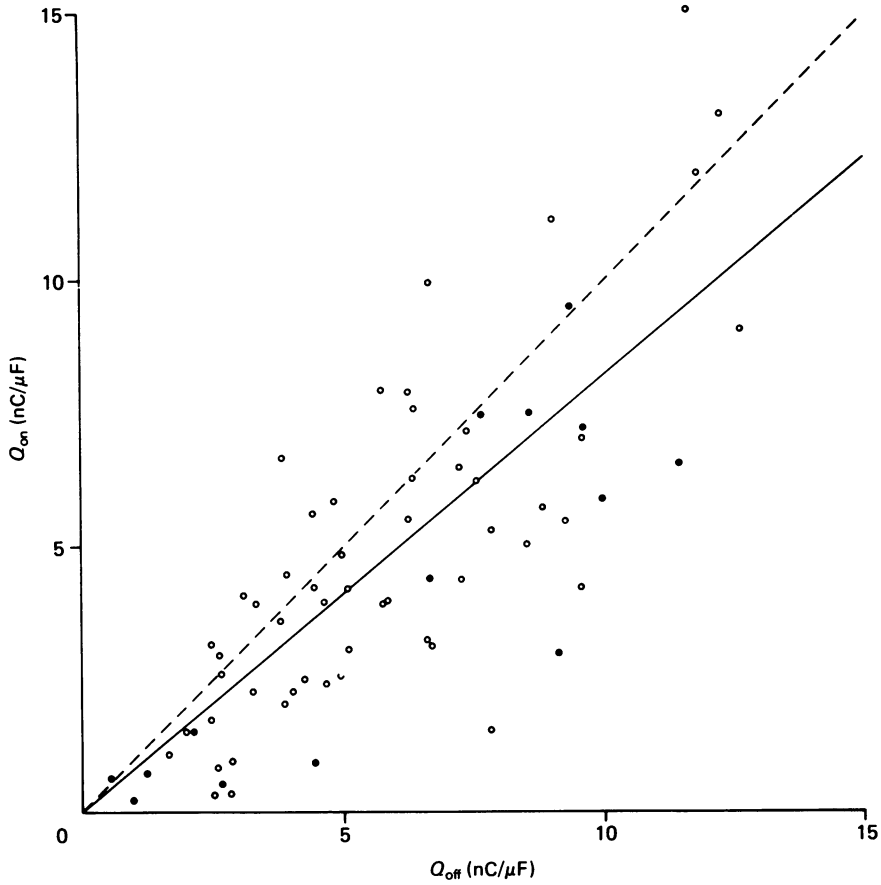

Fig. 2. Q_{on} *vs.* Q_{off} at different test potentials. Q_{on} and Q_{off} are time integrals of charge movements at the 'on' and 'off' of the pulse normalized with respect to the capacitance measured from the respective ΔV(control) trace (Schneider & Chandler, 1976): ●, from fibre of Fig. 1; ○, from fifteen other fibres. Dashed line is the line of unity slope. Continuous line is obtained by least squares fit to the points. It has a slope of 0·821 ± 0·038. Solution C in Table 1; feed-back voltage at V_2; 3°–7 °C.

to a slight decay of the hyperpolarizing conditioning pulse. *C*, pulse protocol: control pulse on the left and test pulse on the right. $V_H = -80$ or -100 mV. Pulse duration was 48 msec which was shorter than the minimum duration (about 70 msec) of a large depolarizing pulse required to make a twitch (Almers & Best, 1976) or slow (authors' unpublished results) fibre contract in tetracaine solution. The arrows mark the beginning and the end of analogue-to-digital data conversion, which lasted for exactly 240 msec, corresponding to 250 points.

fifteen other fibres are also shown as open circles. A straight line through the origin was fitted to all the points by the method of least squares and is shown by the continuous line, which has a slope of 0·821 ± 0·038. For comparison, the dashed line of slope 1 is also shown.

The deviation of the slope from unity can probably be accounted for by uncertain-

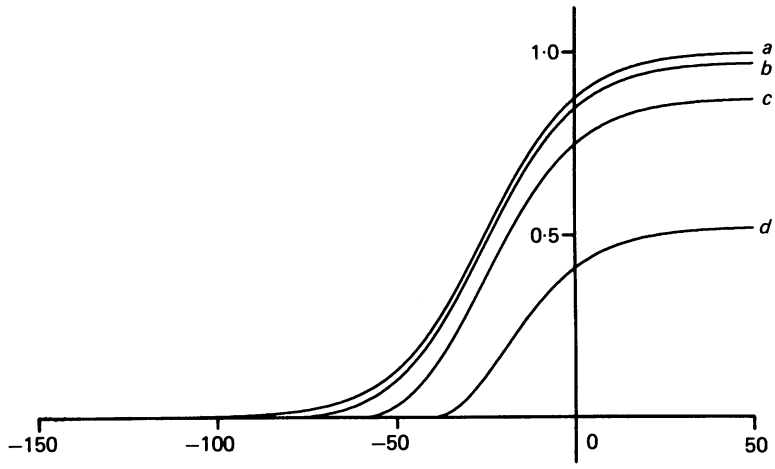

Fig. 3. Effect of V_H on the apparent distribution function. The curves are plotted according to

$$F_{app}(V) = f(V) - 2f(V_H) + f(2V_H - V),$$

where $f(V)$ is given by eqn. (2) and $\bar{V}$ and k are set at −25 mV and 13 mV, respectively. Only the segments of the curves to the right of V_H are shown. *a*, V_H at $-\infty$, and practically superimposable on curves with $V_H \leqslant -100$ mV; *b*, V_H at −80 mV; *c*, V_H at −60 mV; *d*, V_H at −40 mV.

ties in estimating base lines. In the following analysis, the quantity of charge at any potential, $Q(V)$, is generally taken as the arithmetic mean of Q_{on} and Q_{off}.

Characteristics of charge movement

The distribution and movement of intramembranous charges can be described by a two-state model in which charges move with a single time constant from a site on one side of the membrane to a site on the other side during depolarization and move back (also with a single time constant) following repolarization. This is undoubtedly an over-simplification as some of the charge traces show decays more complicated than a single exponential. Because of the high noise level in our records, it was not sensible to construct a more complicated scheme with higher order kinetics.

The ensemble of charges at steady state can be described by Boltzmann's distribution, and the quantity of charge that occupies the second site at any potential is given by (Schneider & Chandler, 1973):

$$Q(V) = f(V)\, Q_{max}, \tag{1}$$

where

$$f(V) = \left[1+\exp\left(-\frac{V-\bar{V}}{k}\right)\right]^{-1}. \quad (2)$$

$\bar{V}$ is the potential at which the charges distribute equally between the two sites and k is a steepness factor. The zero reference of eqn. (2) is implicitly set at $Q(V = -\infty)$. Since the holding potential, V_H, in most of our experiments was at -80 mV, the equation has to be corrected. However, theoretical calculations as illustrated in Fig. 3 show that the correction is negligible for $V_H < -80$ mV.

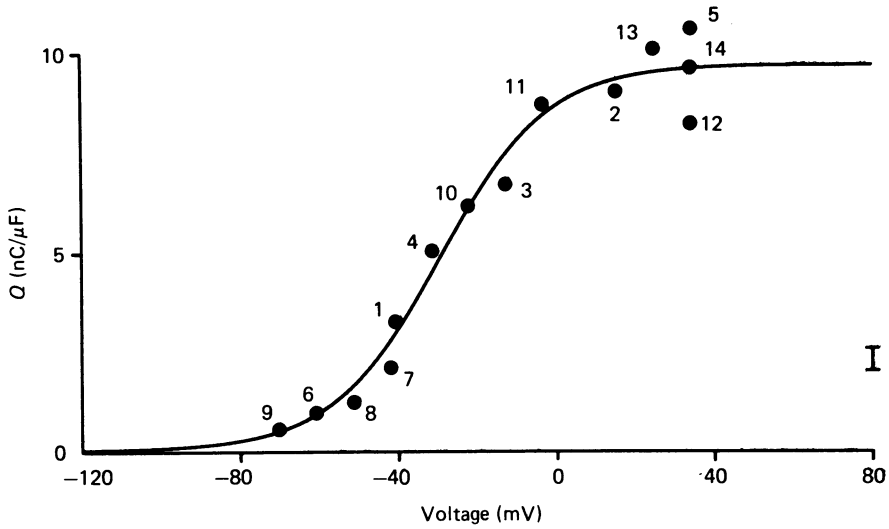

Fig. 4. Voltage dependence of charge movement in slow muscle. In this and the following Figures, V refers to V_1. The numbers next to the points indicate the order of recording. Points 6, 7, 8 and 9 were taken when the fibre was held at -100 mV. The rest of the points were measured directly from -80 mV and had 0·6 nC/μF (obtained from a semi-log plot of the four points at -100 mV) added to their original values. The smooth curve was drawn according to eqns. (1) and (2), and was fitted to the points by least squares. The best fit parameters were $\bar{V} = -29{\cdot}3$ mV, $k = 13{\cdot}9$ mV, $Q_{max} = 9{\cdot}7$ nC/μF. The vertical bar in the lower right indicates ± 1 S.D. of the measurements, equal to $\pm 0{\cdot}302$ nC/μF.

Values of Q_{on} and Q_{off} are obtained from Fig. 1 and the mean values of Q are plotted against V in Fig. 4. A least squares fit of eqns. (1) and (2) to the points, with zero reference at $Q(-100)$, gives the smooth sigmoidal curve, showing that the model is equally applicable to slow fibres. After shifting the zero reference to $Q(-80)$, the best fit values of the parameters $\bar{V}$, k, and Q_{max} became $-27{\cdot}5$ mV, 12·2 mV and 9·0 nC/μF, respectively. The differences between these values and those before the shift (see legend of Fig. 4) are more drastic than the theoretical prediction of Fig. 3 and probably reflect an over-estimate of $Q(-80)$. These values and those from two other experiments (using $Q(-80)$ as zero reference) are listed in Table 2.

We also made a few measurements of charge movement using normal Ringer solution plus 2·0 mM-tetracaine (solution A) instead of tetraethylammonium (TEA^+)

solution. As in twitch fibres (Almers, 1976), the threshold of delayed rectification was shifted to the right (i.e. to a less negative potential) and it was possible to remove the small K^+ current together with the leakage ionic current. Charge movements so obtained in three fibres gave Q–V curves (not shown) almost identical to that of Fig. 4. Average values of the best fit parameters are listed in the first row of Table 3. The similarity of Q–V curves in Na^+ and TEA^+ solutions implies that TEA^+ ions do not perturb the movement of intramembranous charges.

TABLE 2. Parameters for fitting Q–V curve of slow fibres in tetracaine solution

(1) Fibre reference	(2) Voltage range (mV)	(3) $\bar{V}$ (mV)	(4) k (mV)	(5) Q_{max} (nC/μF)
4121-77	$-41 \rightarrow +34$	$-18{\cdot}9$	17·8	7·6
4141-77	$-71 \rightarrow +34$	$-27{\cdot}5$	12·2	9·0
4283-77	$-40 \rightarrow +26$	$-30{\cdot}0$	9·0	4·3
Average ± S.E. of mean		$-25{\cdot}4 \pm 2{\cdot}7$	$13{\cdot}0 \pm 2{\cdot}1$	$6{\cdot}9 \pm 1{\cdot}1$

Column (2) gives the range of potential covered in the measurements. Columns (3), (4) and (5) give best fit parameters for eqns. (1) and (2); solution C, temperature 3–7 °C. The mean values are shown again in the second row of Table 3.

TABLE 3. Properties of charge movement in isosmotic solutions

Fibre type	Solution	$\bar{V}$ (mV)	k (mV)	Q_{max} (nC/μF)	Reference
Slow*	Na, Cl†	-23	14	8 [3]	Gilly & Hui
Slow*	TEA, Cl† 11·8 mM-Ca	-25	13	7 [3]	Gilly & Hui
Twitch	TEA, Cl† 11·8 mM-Ca	-24	8	29‡	Gilly & Hui
Twitch*	TEA, Cl† 11·8 mM-Ca	-25	9	22§	Hui & Gilly (1979)
Twitch	TEA, SO_4†	-27	13	29 [4]	Almers & Best (1976)
Twitch	TEA, Cl	-30	12	25‖ [5]	Schneider & Chandler (1976)

* Stretched to sarcomere lengths about 3·0 μm.
† 2 mM-tetracaine added.
‡ $\bar{V}$ and k from two fibres, Q_{max} from four fibres.
§ $\bar{V}$ and k from three fibres, Q_{max} from eight fibres.
‖ Q_{max} assumed from the average value obtained in hypertonic solution.

Comparison with results in twitch fibres

Charge movements in frog twitch fibres inactivated by tetracaine have been studied in some detail (Almers & Best, 1976; Almers, 1976). We have performed similar experiments on a few twitch fibres held at -80 mV, with voltage clamped at V_1. Within the resolution of our measurements, we did not detect much difference between the kinetics of charge movements in slow and twitch fibres. Best fit values of the parameters from the steady-state Q–V curves of twitch fibres are entered in Table 3, along with published results of others for comparison.

It can be seen that our results agree reasonably well with those of Almers & Best (1976). The small difference in $\bar{V}$ is most likely due to differences in Ca^{2+} concentra-

tions, because 11·8 mM-Ca^{2+} in our solutions should shift the Q–V curve to the right (Schlevin, 1978). The main difference between the values from slow and twitch fibres is the three- to four-fold smaller value of Q_{max} in slow fibres. However, our charge movement experiments on slow fibres were performed at sarcomere lengths of about 3 μm, whereas twitch fibre experiments by us and others were usually done

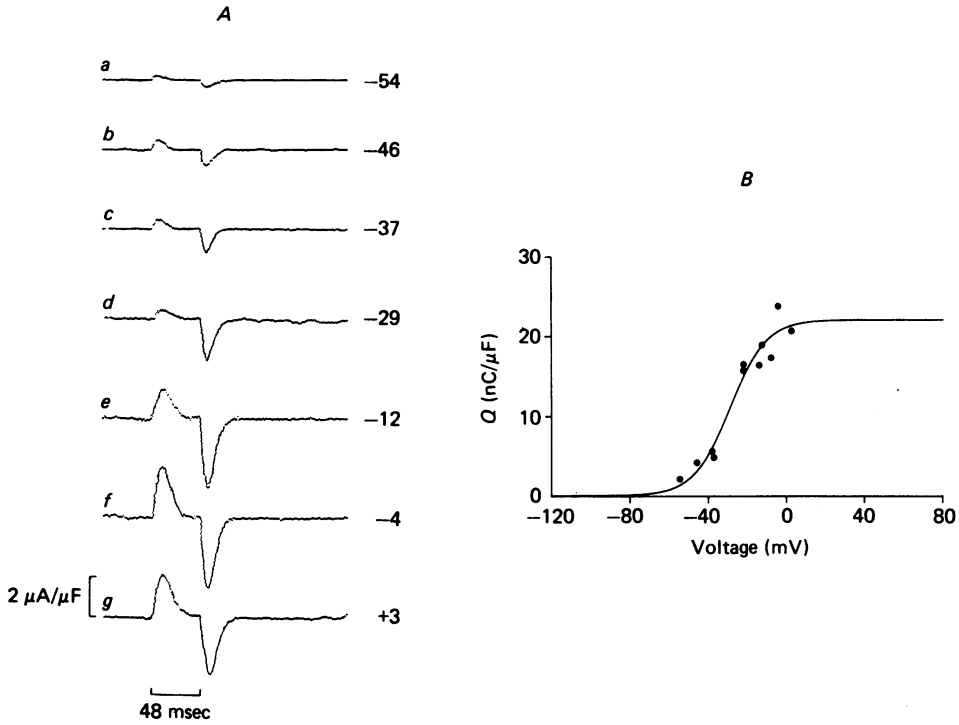

Fig. 5. Charge movement in a stretched semitendinosus twitch fibre. A, ΔV(test − control) traces for depolarizations to voltages as indicated in mV at the right. Pulse protocol and data acquisition same as Fig. 1; electrode spacing: l = 250 μm, l' = 20 μm; R_i assumed to be 255·2 Ω.cm. 1 μA/μF corresponds to 1·136 mV (at the beginning, and 1·478 mV (at the end). Fibre 0234-78; sarcomere length = 3·0 μm; 7 °C; solution C in Table 1; V_H = −80 mV; feed-back voltage at V_2. Trace a is average of eight sweeps; others are averages of four sweeps. B, steady-state Q–V plot. The points were obtained from time intregrals of current traces some of which are shown in A. The smooth curve was drawn according to eqns. (1) and (2), and was fitted to the points by least squares. The best fit parameters were $\bar{V}$ = −28·6 mV, k = 9·3 mV, Q_{max} = 22·1 nC/μF.

on sartorius fibres at sarcomere lengths of about 2·5 μm. Since charge movement may disappear in highly stretched twitch fibres (Adrian, Caputo & Huang, 1978), it seems possible that the relatively small value of Q_{max} in slow fibres might be due to the slight stretch.

To settle this point, we measured charge movement in semitendinosus twitch fibres stretched to sarcomere lengths of 3 μm. Charge traces from one such experiment are shown in Fig. 5A and the corresponding steady-state Q–V plot in Fig. 5B. The average least squares fit values of Q_{max}, k, and $\bar{V}$ from stretched twitch fibres are

shown in the fourth row of Table 3 (see also Hui & Gilly, 1979). It can be seen that Q_{max} in stretched twitch fibres does not diminish appreciably. The general conclusion is that the smaller value of Q_{max} in slow fibres is an intrinsic characteristic of this kind of fibre.

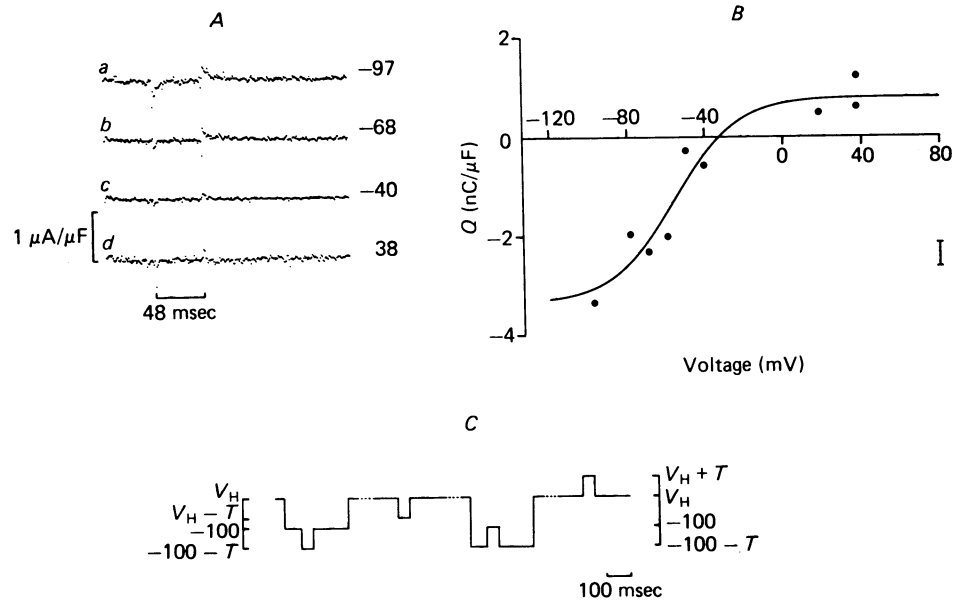

Fig. 6. Charge movement in a slow fibre depolarized to −20 mV. *A*, ΔV(test − control) traces for hyperpolarizations or depolarizations to voltages, in mV, as indicated at the right. Mean fibre diameter is 67·4 μm. Electrode spacing: l = 273 μm, l' = 20 μm. R_i assumed to be 263·4 Ω.cm. C_m = 4·539 μF/cm² at the beginning of the experiment. The product $R_i.C_m$ decreased monotonically so that 1 μA/μF corresponds to 0·798 mV (beginning) and 0·618 mV (end). Fibre 4211-77; 5·8 °C; sarcomere length = 3·0 μm; solution E in Table 1; feed-back voltage at V_2. Trace *c* is average of sixteen sweeps; others eight. The fifty-first point in trace *d* has been omitted. *B*, voltage dependence of partially inactivated charge movement. Data points represent time integrals of charge movements, some of which are shown in *A*. First and last sequences of test pulses were elicited 26 and 79 min after V_H was set. Zero reference of data points has been shifted to $Q(-97)$ for fitting the sigmoid curve. The best fit parameters were $\bar{V}$ = −55·2 mV, k = 16·2 mV, Q_{max} = 4·2 nC/μF. Vertical bar to the right is ±1 S.D. of the measurement, which is ±0·248 nC/μF. *C*, pulse protocol. From left to right are hyperpolarizing control pulse, hyperpolarizing test pulse, depolarizing control pulse, depolarizing test pulse, all separated by dashes. V_H = holding potential, T = magnitude of test pulses.

Inactivation of charge movement

Chandler, Rakowski & Schneider, (1976*b*) found that when twitch fibres were depolarized to −21 mV at 1 °C, charge movements inactivated completely with a time constant of about 20 sec. The amount of steady-state inactivation increases sigmoidally with voltage (Adrian & Almers, 1976). In addition, repriming of charge movement and recovery of contractility share some similar properties (Adrian, Chandler & Rakowski, 1976). These observations suggest that charge movement

may regulate Ca release from the sarcoplasmic reticulum. Since tension in a depolarized slow fibre can be maintained for a very long time (Gilly & Hui, 1980*a*), it was of interest to determine whether the charge movements in slow fibres inactivate during long depolarizations.

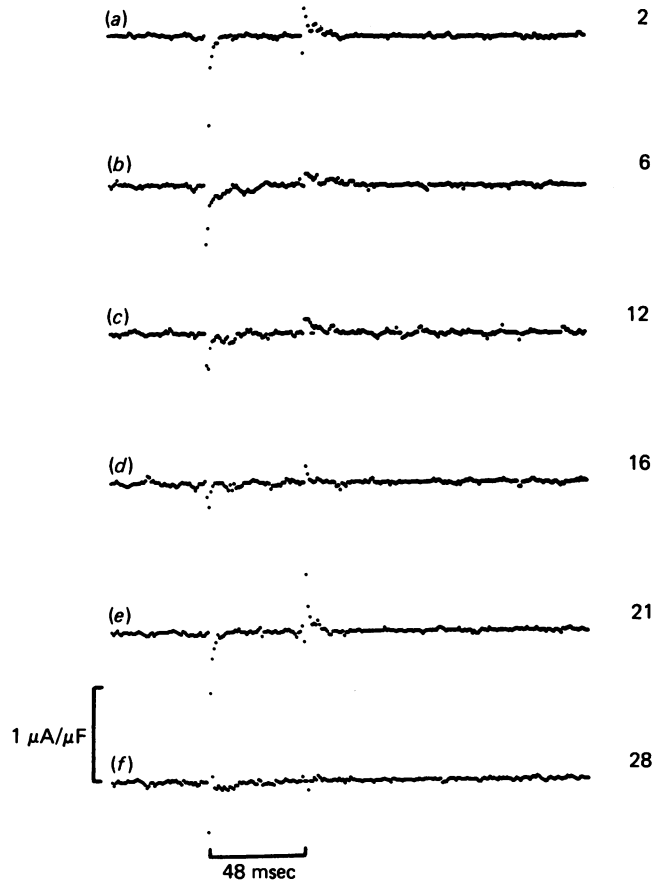

Fig. 7. Charge movement in a slow fibre depolarized to 0 mV. ΔV(test − control) traces for hyperpolarizations to −95 mV. Same fibre as in Fig. 6. C_m has diminished further so that 1 μA/μF corresponds to 0·495 mV (beginning) and 0·407 mV (end). Traces are averages of eight sweeps. Numbers at the right indicate the time (min) measured from the instant the 0 mV holding potential was turned on to the middle of the train of test pulses. The following points have been omitted: 51, 52, 101 (*a*), 101 (*b–d*), 51 (*e*), 52, 101, 102 (*f*). The arithmetic means of values of Q_{on} and Q_{off} in nC/μF are: (*a*) 2·09, (*b*) 2·86, (*c*) 3·39, (*d*) 1·25, (*e*) 3·04, (*f*) 1·43.

We tried to depolarize slow fibres by changing the holding potential, V_H, in the depolarizing direction. This caused strong contraction and, very often, fibre damage. To avoid this problem, we bathed slow fibres in a depolarizing solution in which TEA^+ was partially replaced by Rb^+ (solution D or E). This served to activate some level of contracture with minimal ionic currents. The electrodes were then inserted and V_H was set near the resting potential, typically −40 to −20 mV. Charge move-

ment was measured by applying test pulses in both depolarizing and hyperpolarizing directions and control pulses of identical sizes and polarities. The latter were superimposed on conditioning pulses covering the same voltage range as in experiments on normally polarized fibres (Fig. 6*C*).

Two experiments with a slow fibre held at −40 mV showed that a substantial fraction of charge movement was still present with Q_{max} at least 70% of that at

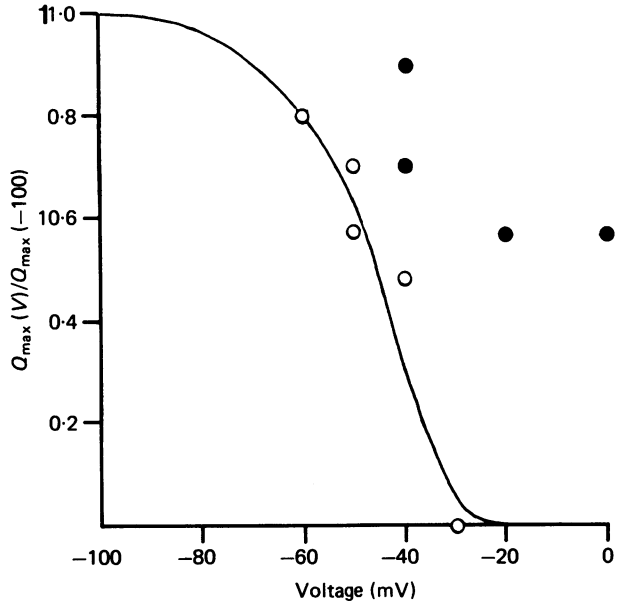

Fig. 8. Comparison of steady-state inactivation of charge movement in slow and twitch fibres. Filled circles represent Q_{max} (V) from slow fibres, bathed in isotonic solutions containing 2 mM-tetracaine and chronically depolarized to various holding potentials; 4–6 °C. Open circles and the solid curves were obtained by Adrian & Almers (1976) from twitch fibres bathed in a hypertonic solution.

−80 mV. The Q–V distribution of the remaining charge did not differ appreciably from that obtained in normally polarized fibres.

Fig. 6*A* shows the results of an experiment with a fibre held at −20 mV. The Q–V curve obtained (Fig. 6*B*) has a slope somewhat less steep than, but still within the scatter of, the values measured in normally polarized fibres. $\overline{V}$ was shifted to the left, to −55 mV, and Q_{max} was reduced to 4·2 nC/μF. At the end of the run, it was possible to change V_H to −80 mV without dislodging the electrodes. After a 20 min repriming period, the value of Q_{max} had increased to 7·7 nC/μF, implying that about 44% of charge had apparently been inactivated at −20 mV.

Next, the holding potential was changed to 0 mV very slowly. As mentioned above, this would result in contraction in most slow fibres. In this particular case, however, there was relatively little movement and the electrodes remained in place. Charge movements were measured over a course of 30 min by applying hyperpolarizing test and control pulses. As shown in Fig. 7, charge movement at 0 mV is not totally inactivated even after 30 min. The quantity of charge decreased slightly, but the

scatter of values is larger than the decrease. It is possible that the partial inactivation had already been achieved within the period during which the holding potential was slowly changed to 0 mV and the first block of control and test pulses was taken, and the slow decay was due to fibre deterioration. The average value of $Q(-95)-Q(0)$ during this period was $-2{\cdot}67$ nC/μF. Later, $Q(+57)-Q(0)$ was measured by applying depolarizing test and control pulses (not shown), and was $+1{\cdot}57$ nC/μF. Thus, the value of Q_{max} at 0 mV was 4·2 nC/μF, again corresponding to 43 % inactivation, the same as at $V_H = -20$ mV.

Fig. 8 shows values of steady-state Q_{max} plotted against the holding potential. Filled symbols were obtained from three slow fibres and open symbols and continuous curve were obtained by Adrian & Almers (1976) from twitch fibres bathed in a hypertonic solution. The solution they used gave a value of $\bar{V}$ that was 15 mV more negative than that obtained by us in isotonic solution. It is obvious that the slow fibre data cannot be fitted by the twitch fibre inactivation curve even if the curve is shifted 15 mV to the right.

DISCUSSION

Our experiments showed that charge movements do exist in slow fibres and follow a steady-state voltage distribution similar to that in twitch fibres. The time constants of charge rearrangement are similar in the two fibre types, differing at most by a factor of 2–4. The values of the best fit parameters for the Q–V distribution given in Table 2 show substantial scatter. This is attributed to the degree of difficulty in performing the slow fibre experiments, primarily because high resistance electrodes had to be used, resulting in noisy records.

Q_{max} in slow fibres is about 7 nC/μF, which is about a quarter of that in twitch fibres. It has been estimated that Q_{max} should be increased by about 6 % to account for the charge at the holding potential, and by about 10 % for the omission of two erratic points. Even with the corrections, Q_{max} in slow fibres is only 1/4 to 1/3 of that in twitch fibres. Since the density of delayed rectifier channels in slow fibres is probably less than 10 % of that in twitch fibres (Gilly & Hui, 1980*b*), it seems unlikely that all the movable charge is involved in gating potassium channels (Adrian & Peres, 1977, 1979).

Another difference between the charge movements in slow and twitch fibres is that when a slow fibre was chronically depolarized to 0 mV in tetracaine solution, charge movement does not inactivate completely. It is quite unlikely that the remaining charge is the same as Q_2 in depolarized twitch fibres (Adrian & Almers, 1976; Adrian *et al.* 1976), because the Q–V distribution we obtained was different from that of Q_2, and the control pulses we used were elicited in the symmetric region of Q_2–V curve such that roughly equal amounts of Q_2 would have moved during a matching pair of test and control pulses, and thus be cancelled out.

We have reported (Gilly & Hui, 1980*a*) that slow and twitch fibres share the same strength–duration curves and similar latencies in the rise in tension. These similarities are consistent with the existence of some common mechanisms in the early steps of excitation–contraction coupling leading to Ca^{2+} release from the sarcoplasmic reticulum. If charge movement indeed plays a role in tubule:sarcoplasmic reticulum coupling, the finding that charge movements in both fibre types are similar would be

TABLE 4. Comparison of physiological properties of frog slow and twitch fibres

Properties	Twitch	Slow
	Contractile	
*External Ca^{2+} required?	No	No
Threshold		
Long pulse (800 msec)	−47 mV[a]	−55 mV[a]
*Short pulse (2 msec)	+15 mV[a]	Same as twitch[a]
*Latency for rise in tension (step V change)	5–10 msec[b,c]	10 msec[b]
Shortening velocity and rate of relaxation	Fast	Much slower than twitch
*Steady-state tension *vs.* V	Sigmoidal, 3-fold in < 5 mV[d]	Same as twitch
Inactivation of tension	Complete	Slight within hundreds of seconds
Decay in mechanical excitability		
*Fast component	τ = 1–2 msec[a]	Same as twitch[a]
Slow component	τ = 5–6 msec[a]	> 300 msec[a]
	Electrical	
Resting membrane		
Specific resistance	~ 10^3 Ω.cm²[e]	~ 10^5 Ω.cm²[f]
*Specific linear capacitance (50 μm dia.)	4–6 μF/cm²[e]	4·4 μF/cm²
Space constant, λ	~ 1 mm[e]	~ 1 cm[f]
Inward rectification	Yes	Negligible
Chloride conductance	Large	Negligible[g]
Action potential?	Yes	No
Sodium conductance, $\bar{G}_{Na}$	~ 50 mS/cm²[e]	< 0·02 mS/cm²
*Delayed rectifier, G_K	Two components	Two components
G_K *vs.* V (fast component)	Sigmoidal, e-fold in < 5 mV	Sigmoidal, e-fold in ~ 15 mV
$\bar{G}_K$ (fast component)	6–20 mS/cm²	0·3–0·9 mS/cm²
*Kinetics of G_K change	Voltage-dependent	Similar dependence (within a factor of 2–4)
Inactivation?	Yes, both components	Fast: unknown Slow: no
*Effect of TEA	Blocks	Blocks
Charge movement		
*Q *vs.* V	Sigmoidal, e-fold in 8–13 mV	Similar to twitch
Q_{max}	25–30 nC/μF	7 nC/μF
*Kinetics	Voltage-dependent	Probably similar
Inactivation?	Complete in 1 min at 0 mV	Partial after 30 min at 0 mV
Q_2	Yes[h]	Unknown

Notes: *a*, measured at 9 °C; *b*, measured at 20 °C; *c*, from Heistracher & Hunt (1969); *d*, from Hodgkin & Horowicz (1960); *e*, from Adrian, Chandler & Hodgkin (1970); *f*. agree with Stefani & Steinbach (1969); *g*, from Stefani & Steinbach (1969); *h*, from Adrian & Almers (1976), Adrian *et al.* (1976); * properties that are similar in both fibre types.

consistent with this hypothesis. Diversities in the subsequent steps could then lead to the drastically different rates of rise in tension and rates of relaxation in the two fibre types (see Discussion in Gilly & Hui, 1980*a*).

Franzini-Armstrong (1970) has proposed that the 'feet' structures in triads of twitch fibres are possible locations for tubule:sarcoplasmic reticulum coupling. For twitch fibres in hypertonic solution, Chandler *et al.* (1976*a*) found a qualitative agreement between the density of charge groups and the density of 'feet', which supports that proposal. Similar 'feet' structures have been seen in the dyads and triads of slow fibres (Franzini-Armstrong, 1973). C. H. Bailey & L. D. Peachey (in preparation) found that the transverse tubules in slow fibres dilate to the shape of a pancake at the dyadic and triadic junctions. If the 'pancakes' only form dyads with the sarcoplasmic reticulum, the density of 'feet' would be about $250/\mu m^2$ of tubular and surface membrane, whereas if they all form triads the density would be about $500/\mu m^2$. The actual value should be closer to the former, because most contacts in slow fibres are dyads.

A value of 7 nC/μF for Q_{max} and 13 mV for k corresponds to approximately 200 charge groups/μm^2, assuming 1 $\mu F/cm^2$ in the surface and tubular membranes. Thus, it appears that, within the framework of the two-state model, the density of charge groups and 'feet' may also be in qualitative agreement in slow fibres, pointing toward the possibility that most of the movable charge might be involved in contractile activation. The charge might open and close the calcium channels in the sarcoplasmic reticulum membrane via some mechanical linkages (Chandler *et al.* 1976*b*), or via some dipole chains (Hui, 1977), located in the 'feet'.

Results from this series of papers have elucidated some interesting physiology of slow muscle and confirmed some of the earlier findings. Slow and twitch fibres share many similarities and differences in contractile and electrical properties as shown in Table 4. We feel that this pair of fibre types would be very useful for comparative studies of excitation–contraction coupling in skeletal muscle.

This work was supported by a grant from the U.S. National Institutes of Health (PHS NS 07474) to Dr W. K. Chandler and by a NIH Postdoctoral Fellowship to C.S.H. We would like to thank Dr Chandler for continuous encouragement and helpful advice throughout the course of this work, Drs R. W. Tsien, S. M. Baylor, and M. W. Marshall for suggestions on the manuscript, and Mr H. Fein and staff for construction and maintenance of equipment.

REFERENCES

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

ADRIAN, R. H., CAPUTO, C. & HUANG, C. L.-H. (1978). Effect of stretch on intramembrane charge movement in striated muscle. *J. Physiol.* **284**, 151–152*P*.

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., CHANDLER, W. K. & RAKOWSKI, R. F. (1976). Charge movement and mechanical repriming in skeletal muscle. *J. Physiol.* **254**, 361–388.

ADRIAN, R. H. & PERES, A. R. (1977). A gating signal for the potassium channel. *Nature, Lond.* **267**, 800–804.

ADRIAN, R. H. & PERES, A. R. (1979). Charge movement and membrane capacity in frog muscle. *J. Physiol.* **289**, 83–97.

ALMERS, W. (1976). Differential effects of tretracaine on delayed potassium channels and displacement currents in frog skeletal muscle. *J. Physiol.* **262**, 613–637.

ALMERS, W. & BEST, P. M. (1976). Effects of tetracaine on displacement currents and contraction of frog skeletal muscle. *J. Physiol.* **262**, 583–611.

ARMSTRONG, C. M. & BEZANILLA, F. (1974). Charge movement associated with the opening and closing of the activation gates of the Na channels. *J. gen. Physiol.* **63**, 533–552.

CHANDLER, W. K., GILLY, W. F. & HUI, C. S. (1978). Electrical properties of amphibian slow muscle fibres. In *Biophysical Aspects of Cardiac Muscle*, ed. MORAD, M., pp. 31–44. New York: Academic Press.

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.

DE MELLO, W. C. (1973). Membrane sealing in frog skeletal muscle fibres. *Proc. natn. Acad. Sci. U.S.A.* **70**, 982–984.

FLITNEY, F. W. (1971). The volume of the T-system and its association with the sarcoplasmic reticulum in slow muscle fibres of the frog. *J. Physiol.* **217**, 243–257.

FRANZINI-ARMSTRONG, C. (1970). Studies of the triad. I. Structure of the junction in frog twitch fibres. *J. cell Biol.* **47**, 488–499.

FRANZINI-ARMSTRONG, C. (1973). Studies of the triad. IV. Structure of the junction in frog slow fibres. *J. cell Biol.* **56**, 120–128.

GILLY, W. F. & HUI, C. S. (1978). Charge movement in frog slow and twitch muscle fibers. *Biophys. J.* **21**, 167a.

GILLY, W. F. & HUI, C. S. (1980*a*). Mechanical activation in slow and twitch skeletal muscle fibres of the frog. *J. Physiol.* **301**, 137–156.

GILLY, W. F. & HUI, C. S. (1980*b*). Membrane electrical properties in frog slow muscle fibres. *J. Physiol.* **301**, 157–173.

HEISTRACHER, P. & HUNT, C. C. (1969). The relation of membrane changes to contraction in twitch muscle fibres. *J. Physiol.* **201**, 589–611.

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

HUI, C. S. (1977). Possible origin of gating current in nerve membrane. *Biosystems* **8**, 207–212.

HUI, C. S. & GILLY, W. F. (1979). Charge movement and mechanical activation in striated muscle fibers. *Biophys. J.* **25**, 200a.

LÜTTGAU, H. C. & OETLIKER, H. (1968). The action of caffeine on the activation of the contractile mechanism in striated muscle fibres. *J. Physiol.* **194**, 51–74.

NASLEDOV, G. A., MANDELSTAM, J. E. & RADZJUKEWICH, T. L. (1972). A study of excitation–contraction coupling in frog tonic muscle fibers of *Rana Temporaria*. *Experientia* **28**, 1305–1306.

PAGE, S. G. (1965). A comparison of the fine structures of frog slow and twitch muscle fibres. *J. cell Biol.* **26**, 477–497.

PEACHEY, L. D. & HUXLEY, A. F. (1962). Structural identification of twitch and slow striated muscle fibers of the frog. *J. cell Biol.* **13**, 177–180.

SCHLEVIN, H. H. (1978). Effects of external Ca^{2+} and pH on muscle charge movement. *Biophys. J.* **21**, 168a.

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 fibers. *J. gen. Physiol.* **67**, 125–163.

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