

## ANALYSIS OF 'OFF' TAILS OF INTRAMEMBRANE CHARGE MOVEMENTS IN SKELETAL MUSCLE OF *RANA TEMPORARIA*

By C. L.-H. HUANG

*From the Physiological Laboratory, Downing Street, Cambridge CB2 3EG*

(Received 28 February 1984)

### SUMMARY

1. Components of non-linear charge were isolated in transients obtained in response to hyperpolarizing ('off') voltage-clamp steps in frog muscle fibres.

2. 'Off' currents of the  $q_\gamma$  charge were isolated by comparing records obtained from long depolarizing steps with those resulting from short steps which intercepted the 'on' currents of the  $q_\gamma$  charge. The 'off' transients of the  $q_\gamma$  component so deduced were rapid decays lasting 10–15 ms, in contrast with their prolonged time course in the preceding 'on' steps.

3. 'Off' responses were rapid even at voltages when the 'on' current, obtained from imposed 10 mV steps made at a series of closely incremented conditioning voltages, was delayed and prolonged. Such slow transfers of charge could not be demonstrated in 'off' tails.

4. Large depolarizing steps resulted in rapid  $q_\gamma$  transients in 'on' currents not distinguishable from the rest of the charge movement. Nevertheless, by separating the  $q_\gamma$  component through its inactivation by prolonged depolarization, it was possible to show that  $q_\gamma$  currents in the 'off' tails were still rapid decays.

5. It is concluded that in contrast to the varied pattern shown by  $q_\gamma$  in 'on' transients, its 'off' responses are everywhere relatively fast decays. These features can be predicted by a simple two-state model in which the forward and backward rate constants depend upon the amount of charge moved, as well as the membrane voltage.

### INTRODUCTION

It is known from earlier experiments that intramembrane charge movements in skeletal muscle obtained in response to depolarizing ('on') voltage steps are often complex in form. This finding was the basis of a separation of the non-linear membrane capacitance into at least three different components (Adrian & Peres, 1979; Huang, 1980, 1981*a*, 1982). Of these, one particular species  $q_\gamma$  (termed the  $Q_\gamma$  charge by Adrian & Peres, 1979) has been of particular interest owing to the resemblance of its kinetic features, steady-state distribution and sensitivity to pharmacological agents to those shown by the activation of contraction (Huang, 1982; Hui, 1983). It is also likely that the  $q_\gamma$  system is independent from the remaining ( $q_\alpha$  and  $q_\beta$ ) components of charge observed in the non-linear transients (Adrian & Huang, 1984*b*).

However, in contrast to the existing reports concerning the nature of 'on' responses of the voltage-dependent charge, similar analyses of currents obtained in response to hyperpolarizing ('off') steps have not been attempted. Such 'off' records do not show the complex features that facilitated resolution of the different components during the 'on' transients. The experiments in this paper were performed to ascertain the nature of  $q_\gamma$  currents in 'off' steps. This entailed recognizing the corresponding  $q_\gamma$  responses in the preceding 'on' traces, and consequently using different pulse procedures to vary the form of the  $q_\gamma$  displacement current at different potentials. Nevertheless, all the experiments indicate that  $q_\gamma$  charge movements in 'off' responses are rapid decays, often complete within 10–15 ms.

These, and earlier findings, have important theoretical implications for the kind of mechanisms that could underlie the transfers of  $q_\gamma$  charge in response to voltage change. They are compatible with a situation in which the rate constants governing transitions in the systems concerned are dependent not only upon voltage, but also upon the distribution of the charge in the imposed field. Thus, even the simplest form of this class of hypothesis made predictions consistent with all the major features of the  $q_\gamma$  component. Accordingly, schemes of this kind might form a useful basis for further testing.

#### METHODS

A three-micro-electrode voltage clamp of the pelvic end of frog (*Rana temporaria*) sartorius muscle fibres employed experimental apparatus fully described elsewhere (Adrian & Rakowski, 1978), and so what follows is only an outline of the experimental procedure. Glass micro-electrodes, resistance 4–10 M $\Omega$ , were inserted at positions  $l = 375 \mu\text{m}$  (voltage control electrode  $V_1$ ),  $750 \mu\text{m}$  (second voltage electrode  $V_2$ ) and  $1000 \mu\text{m}$  (current injection electrode  $I_0$ ) from the end of the fibre respectively. The electrodes used to record  $V_1$  and  $V_2$  were filled with 3 M-KCl, and the current injection electrode was filled with 2 M-K citrate. Membrane currents ( $i_m$ ) obtained in response to applied voltage-clamp steps were examined. Each record was an average of four to six sweeps.

$$i_m(t) = \frac{d}{6l^2 R_1} [V_2(t) - V_1(t)].$$

The linear membrane and cable constants were ascertained from the analysis of the transients obtained in response to 10 mV steps applied from the holding potential of  $-90$  mV. The records obtained from this procedure will be referred to as the control records. The values of the length constant,  $\lambda$ , the longitudinal internal resistance,  $r_i$ , and the resistance of unit length of membrane,  $r_m$ , were calculated from the steady values of the potentials,  $V_1$  and  $V_2$ , and the injected current  $I_0$  at the end of the 10 mV step. The diameter,  $d$ , of the fibre studied, and its specific membrane constants,  $R_m$  and  $C_m$ , were computed employing a value of the internal sarcoplasmic resistivity  $R_1$  of 391  $\Omega$  cm in hypertonic solution at 2 °C and with a  $Q_{10}$  of 1.37 (Hodgkin & Nakajima, 1972). The capacitive charge moved in response to the imposed potential steps was computed using Simpson's rule as the integral of the transient parts of the respective currents obtained in response to the depolarizing ('on') and hyperpolarizing ('off') parts of the step, leak conductances having been corrected for (Adrian & Almers, 1974).

The above computations were performed on records of  $V_1(t)$ ,  $V_2(t) - V_1(t)$  and  $I_0(t)$ , obtained by 12-bit analog-to-digital conversion after filtering through three-pole Butterworth filters set at a corner frequency of 1.0 kHz, then sampled using a PDP 11/10E computer (Digital Equipment Corporation, Maynard, MA) with a Model 502 interface (Cambridge Electronic Design, Cambridge). Repeated control records (defined above) in the course of the experiments checked the condition and the stability of the fibre. The sampling interval,  $T$ , varied according to the pulse procedure used but was always sufficiently rapid to result in a Nyquist frequency ( $= 1/2T$ ) (Papoulis, 1977) higher than that set by the filters: usually the sampling interval was 200  $\mu\text{s}$ /point. The non-linear charge movements were deduced by taking the difference between currents obtained to test voltage

steps and the responses to the 10 mV control steps imposed at the  $-90$  mV reference potential, scaled, where appropriate, by the ratio of the sizes of the test to the control steps.

Fibres were studied at  $3-5^\circ\text{C}$  in the following solutions at neutral pH:  $\text{Rb}_2\text{SO}_4$ , 2.5 mM; tetraethylammonium  $(\text{TEA})_2\text{SO}_4$ , 80 mM;  $(\text{TEA})\text{Cl}$ , 15 mM;  $\text{CaSO}_4$ , 8 mM; tetrodotoxin,  $2 \times 10^{-7}$  M; Tris buffer, 3 mM; sucrose, 310 mM;  $\text{CoSO}_4$ , 20 mM. The  $\text{CoSO}_4$  was added to suppress any small  $\text{Ca}^{2+}$  currents (Horowitz & Schneider, 1981).

## RESULTS

The aim of the experiments was to identify that component of the current resulting from a hyperpolarizing ('off') step attributable to the  $q_\gamma$  component of the voltage-dependent charge. In order to accomplish this through a wide voltage range, different kinds of pulse procedures had to be adopted.

### *Separation of 'off' components in time*

The most straightforward instance in which it was possible to isolate  $q_\gamma$  currents in 'off' records was where  $q_\beta$  and  $q_\gamma$  transients were clearly distinguishable in the preceding 'on' responses. The voltages at which this occurred were determined by exploring the membrane using 10 mV steps imposed 500 ms after potential steps to a range of conditioning voltages from the  $-90$  mV holding level, incremented in 2-3 mV intervals. Fig. 1*A* shows the charge movements to 10 mV steps of two different durations from a level of  $-53$  mV, at which the initial rapid  $q_\beta$  decay and the slower, delayed  $q_\gamma$  current were clearly distinct, the  $q_\gamma$  transient extending well over 50 ms in the 'on' part of the record (Fig. 1*Aa*). The 'on' pulse of 124 ms was long enough to allow all the charging current to flow before the end of the step. Therefore the 'off' current, separately displayed in Fig. 1*Ab*, reflects redistributions of both  $q_\beta$  and  $q_\gamma$  components of voltage-dependent charge. In contrast, the shorter (22 ms) depolarizing step allowed only the initial current, mostly attributable to the  $q_\beta$  charge, to flow. Consequently, the 'off' current (shown separately for clarity in Fig. 1*Ac*) would be the result of the movement of mainly  $q_\beta$  charge, with little if any contribution from  $q_\gamma$ . Thus, in Fig. 1*Ac*, the size of the 'off' current was reduced.

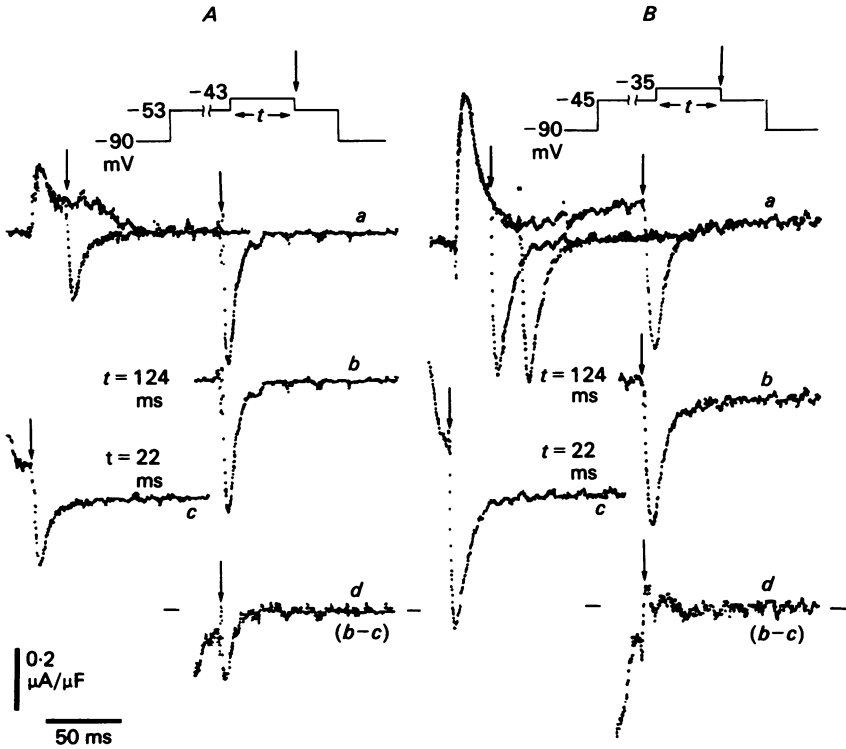
The time course of the  $q_\gamma$  current after the long voltage step could be obtained by taking the difference between the 'off' records *b* and *c*. The subtracted record that resulted (Fig. 1*Ad*) shows that the  $q_\gamma$  contribution to the 'off' response of the intramembrane charge under these conditions was a rapidly decaying current that lasted around 10-15 ms. This is in contrast to the substantially longer time course in the corresponding 'on' records.

These findings most likely result from changes in a capacitive charge rather than changes in ionic currents during the 'on' part of the step. Thus, earlier papers have reported the conservation of charge in this voltage range even as pulse durations were varied (Schneider & Chandler, 1973; Chandler, Rakowski & Schneider, 1976; Huang, 1983*a*).

Fig. 1*B* shows results, similar to those shown in Fig. 1*A*, from voltage excursions between  $-45$  and  $-35$  mV. At  $-35$  mV, the 'on' transients were almost complete by 22 ms. In contrast to the findings in Fig. 1*A*, there was little difference between 'off' currents after 22 ms steps and those after 124 ms steps (Fig. 1*Bc* and *Bb*), and so the subtracted record (Fig. 1*Bd*) was flat. These results are as would be expected

had the currents here been the result of redistribution of a finite amount of conserved charge all of which had reached the steady state by the end of the imposed voltage steps.

In any event, any activation of ionic currents in the course of the 'on' steps did



Fibre E01

Fig. 1. *Aa*, charge movements obtained in response to 10 mV steps of different lengths between voltages of -53 and -43 mV (pulse procedure in inset). Times of onset of 'off' steps marked by arrows. *b*, the 'off' recovery from a 124 ms 'on' step: the amount of 'off' charge was 4.4 nC/μF. *c*, the 'off' recovery from a 22 ms 'on' step gave an 'off' charge of 2.9 nC/μF. *d*, the  $q_v$  current in the 'off' step to the long 'on' pulse is the difference ( $b - c$ ). In *d*, horizontal bars denote zero current. *Ba*, charge movements in response to voltage steps between -45 and -35 mV of durations 22, 42 and 124 ms. 'Off' responses from the 'on' pulses of duration 124 ms (*b*), 42 and 22 ms (*c*) gave quantities of non-linear charge moved of 6.35, 7.09 and 6.65 nC/μF, respectively. *d*, the subtraction trace resulting from the difference ( $b - c$ ) was flat. Fibre cable constants:  $R_1 = 345 \Omega \text{ cm}$ ; temp. = 3 °C;  $\lambda = 4.8 \text{ mm}$ ;  $r_1 = 9394 \text{ k}\Omega/\text{cm}$ ; diam. = 68 μm;  $r_m = 2197 \text{ k}\Omega \text{ cm}$ ;  $R_m = 47.23 \text{ k}\Omega \text{ cm}^2$ ;  $C_m = 7.7 \mu\text{F}/\text{cm}^2$ .

not appear to result in appreciable contributions to the 'off' tails, at least for the potentials investigated here. For example, Fig. 2 shows that when the duration of a depolarizing step from -47 to -27 mV was varied between 50 and 370 ms, the 'off' tails retained a similar form despite the development of some delayed outward current to varying degrees (Fig. 2*A*). Furthermore, the 'off' charge remained relatively constant (Fig. 2*B*).

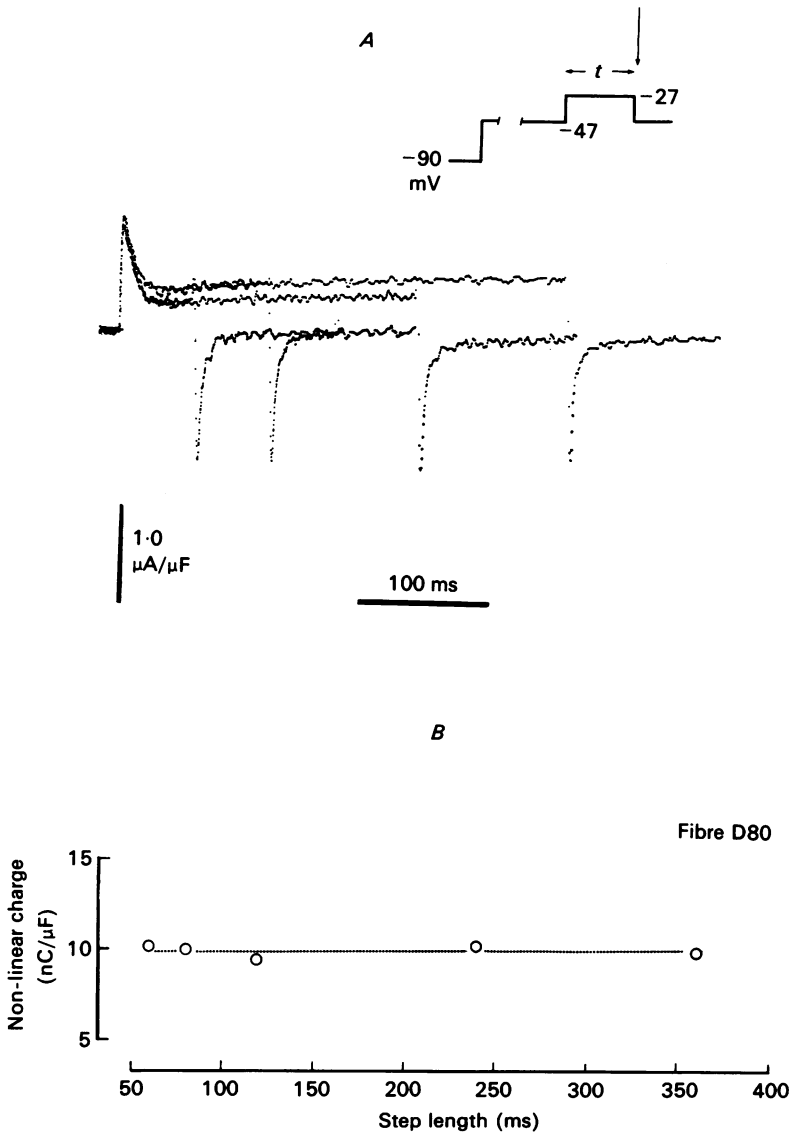


Fig. 2. *A*, 'off' tails obtained after 'on' pulses of varying lengths between 50 and 370 ms, and different degrees of activation of delayed ionic current. *B*, non-linear charge moved in each 'off' response plotted against 'on' pulse duration. Fibre cable constants:  $R_1 = 344 \Omega \text{ cm}$ ; temp. =  $3.1^\circ \text{C}$ ;  $\lambda = 4.9 \text{ mm}$ ;  $r_1 = 7358 \text{ k}\Omega/\text{cm}$ ; diam. =  $82 \mu\text{m}$ ;  $r_m = 1586 \text{ k}\Omega \text{ cm}$ ;  $R_m = 41.48 \text{ k}\Omega \text{ cm}^2$ ;  $C_m = 3.0 \mu \text{ F}/\text{cm}^2$ .

The simplest interpretation of the results presented therefore is that the current records obtained by the subtraction procedure in Fig. 1 *Ad* reflect the rearrangement of a conserved amount of voltage-dependent charge attributable to  $q_\gamma$ , and that these currents decay with a relatively rapid time course, not dissimilar to the 'off' tails shown by the other major,  $q_\beta$ , component of charge.

*Separation of 'off' components using voltage*

At potentials close to the foot of the dependence of the non-linear charge upon voltage, it is the  $q_\gamma$  rather than the  $q_\beta$  component which contributes most to the steepness of the  $Q-V$  (charge-membrane potential) function (Adrian & Peres, 1979; Huang, 1981*a*). This means that one would expect relatively little change in the  $q_\beta$

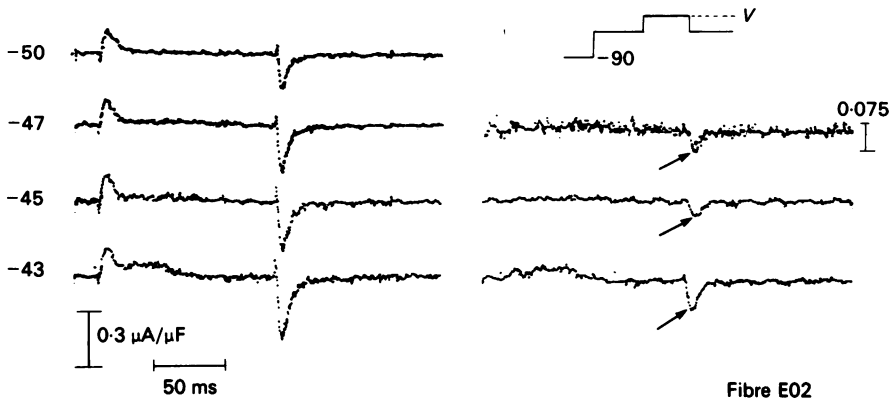


Fig. 3. Left: charge movements examined at close voltage increments close to the foot of the dependence of non-linear charge upon membrane potential (pulse procedure in inset). Right:  $q_\gamma$  currents deduced from the differences between traces on the left, and the charge movement obtained at  $-50$  mV. The  $q_\gamma$  currents in the 'off' tails are arrowed. Cable constants:  $R_1 = 345 \Omega \text{ cm}$ ; temp. =  $3^\circ\text{C}$ ;  $\lambda = 4.3 \text{ mm}$ ;  $r_1 = 8411 \text{ k}\Omega/\text{cm}$ ; diam. =  $79.5 \mu\text{m}$ ;  $r_m = 1206.3 \text{ k}\Omega \text{ cm}$ ;  $R_m = 32.75 \text{ k}\Omega \text{ cm}^2$ ;  $C_m = 6.3 \mu\text{F}/\text{cm}^2$ .

response to imposed  $10 \text{ mV}$  steps from a selection of voltages close to such levels. Therefore, it was possible to isolate the wave form of the  $q_\gamma$  current alone under these conditions, by comparing charge movements that contain both  $q_\beta$  and  $q_\gamma$ , with records obtained where the voltage was slightly more hyperpolarized, but nevertheless at a level before the threshold of appearance of the  $q_\gamma$  charge movement. The latter traces then would contain only the  $q_\beta$  current.

Displacement currents were obtained in response to  $10 \text{ mV}$  steps of duration  $125 \text{ ms}$ , which were imposed  $500 \text{ ms}$  after the membrane was taken to one of a series of conditioning voltages. These were altered in  $2\text{--}3 \text{ mV}$  increments, and fell in the potential region close to the foot of the non-linear  $Q-V$  curve, and extending on either side of the level at which  $q_\gamma$  was first visible in the non-linear records. Thus, Fig. 3 (left) shows that at a potential of  $-50 \text{ mV}$ , the charge movement consisted mainly of the rapid decay reflecting redistributions in the  $q_\beta$  system. At more depolarized potentials (beyond  $-47 \text{ mV}$ ) through this range of voltage levels, which is close to the contractile rheobase,  $q_\gamma$  currents were represented as small but prolonged transients. These have been shown on an earlier occasion (Adrian & Huang, 1984*a*) to last as long as  $300 \text{ ms}$  under some conditions, and result in the transfer of appreciable quantities of charge. Fig. 3 shows that the appearance of  $q_\gamma$  currents was accompanied by an increase in the initial size of the 'off' responses (Fig. 3: left

records), which nevertheless remained rapid decays. Since the  $-50$  mV record (Fig. 3: top) contains  $q_\beta$  but no noticeable  $q_\gamma$ , the  $q_\gamma$  component in the remaining traces could be isolated by comparing them with traces obtained at a  $-50$  mV membrane potential. The subtraction records derived from such a procedure (Fig. 3: right) show that appreciable fast decays were absent in the 'on' parts of the responses. This is consistent with the  $q_\beta$  component having been successfully eliminated from the traces obtained. The  $q_\gamma$  currents were delayed and prolonged and often lasted over 80–100 ms; notice that the record at  $-47$  mV is displayed at a higher gain to demonstrate the delayed  $q_\gamma$  current. However, no matter how slow the  $q_\gamma$  charge movement in the 'on' records, its 'off' responses remained relatively fast decays complete within 15–20 ms (Fig. 3: arrows).

#### *Prolonged currents are absent in 'off' responses*

The preceding results suggest that most of the  $q_\gamma$  current in the 'off' tails decays rapidly over 10–20 ms at temperatures close to  $4^\circ\text{C}$ . However, as mentioned above, 'on' currents at some voltages may include a prolonged current extending 100–400 ms (Adrian & Huang, 1984a). The experiments in this section were carried out to see whether similar prolonged transients could occur in the 'off' tails. These currents were sought by interrupting 'off' tails at different times with a 10 mV depolarizing step. Any slow current so intercepted would then result in a decrease in the amount of charge movement seen following the 10 mV depolarization.

Fig. 4A shows charge movements resulting from hyperpolarizing steps of various durations from  $-42$  to  $-52$  mV. There is a small prolonged tail in the 'on' currents (arrowheads: Fig. 4A) which did not vary with pulse length until the preceding 'off' interval had been shortened to 10–20 ms. Fig. 4A (right side) shows differences between the various 'on' (depolarizing) records, and the 'on' records obtained when the 'off' interval was 116 ms. The subtracted records were relatively flat until the 'off' interval was shortened to 10 ms.

Fig. 4B plots the amount of 'on' charge moved against the preceding 'off' interval. There was little change in the amount of charge until the interval became shorter than 20 ms. This agrees with the earlier findings suggesting that the 'off' response is largely complete within 10–15 ms.

Similar experiments were performed over a wide range of voltages. Fig. 5 summarizes results from determining the charge from 'on' (depolarizing) pulses when the preceding 'off' intervals were 124 ms ( $\circ$ ), and 40 ms ( $\triangle$ ) respectively. The amount of non-linear charge moved in the two cases did not significantly differ at each voltage examined. These procedures, therefore, do not unmask prolonged currents in 'off' tails, whereas they do so for 'on' responses at some voltages (Adrian & Huang, 1984a).

#### *Large voltage steps*

At strong depolarizations, the  $q_\gamma$  currents assume rapid kinetics and consequently merge with  $q_\beta$  currents. Neither of the protocols above could then distinguish  $q_\beta$  and  $q_\gamma$  transients even in 'on' responses. To isolate the time course of  $q_\gamma$ , it was then necessary to compare charge movements before and after  $q_\gamma$  was selectively immobilized by prolonged depolarization (Adrian & Peres, 1979; Huang, 1981a).

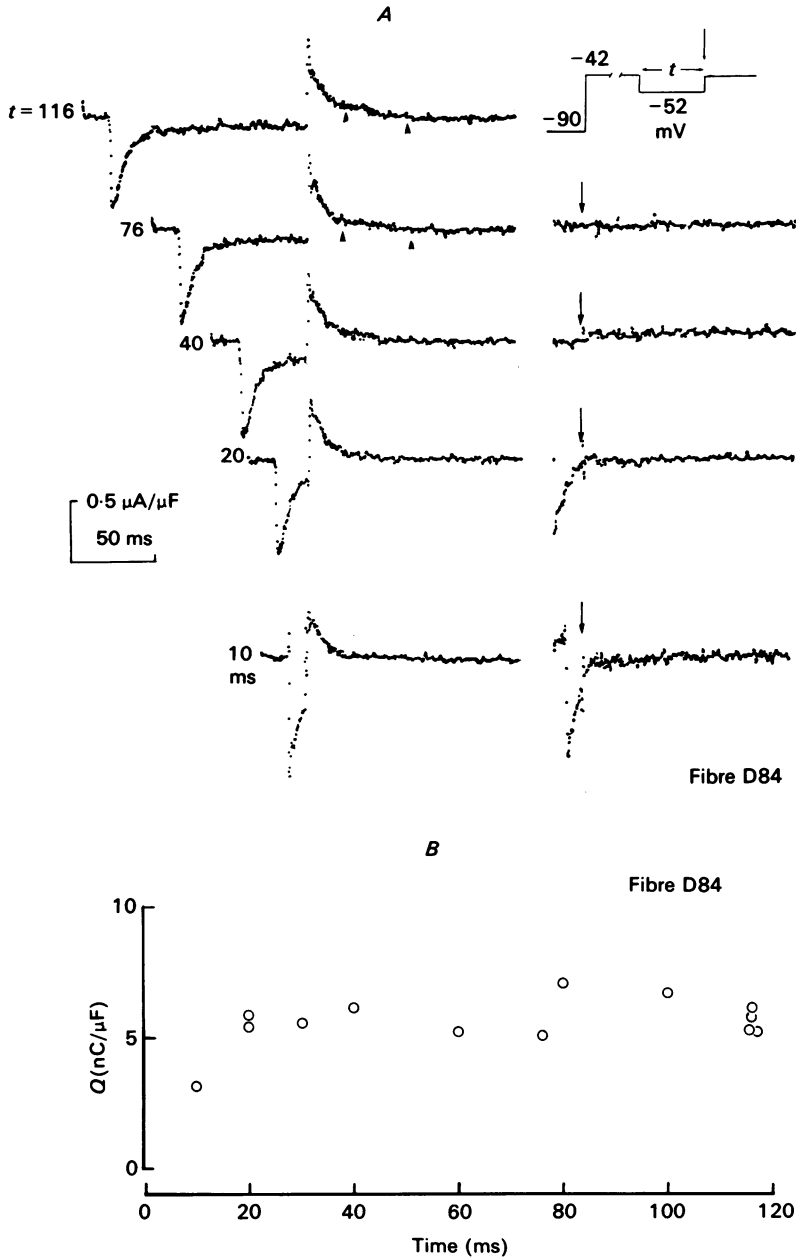


Fig. 4. Experiments intercepting 'off' tails after varying times; pulse procedure shown in inset. *A*, left: hyperpolarizing steps of different lengths between  $-42$  and  $-90$  mV gave charge movements in which  $q_{\gamma}$  occurred in the 'on' (depolarizing) responses (marked by arrowheads). Right: subtraction traces obtained by comparing the different 'on' responses with the 'on' responses obtained when the preceding 'off' voltage step was 116 ms. The vertical arrows mark the time of imposition of the 'on' step. *B*, 'on' (depolarizing step) charge ( $Q$ ) plotted against previous 'off' interval. Fibre cable constants:  $R_i = 337 \Omega \text{ cm}$ ; temp. =  $3.8^\circ\text{C}$ ;  $\lambda = 4.6 \text{ mm}$ ;  $r_i = 4272 \text{ k}\Omega/\text{cm}$ ; diam. =  $100 \mu\text{m}$ ;  $r_m = 893.7 \text{ k}\Omega \text{ cm}$ ;  $R_m = 28.13 \text{ k}\Omega \text{ cm}^2$ ;  $C_m = 7.4 \mu\text{F}/\text{cm}^2$ .



'Off' responses were obtained in response to voltage steps of 105 ms duration to a constant level of  $-20$  mV: these were imposed 500 ms after the fibre was taken to the reference voltage of  $-90$  mV from a range of holding potentials (Fig. 6A). A steep dependence of available non-linear charge upon holding voltage was observed (Fig. 6B:  $\circ$ ). The control procedure performed in four further fibres in the presence

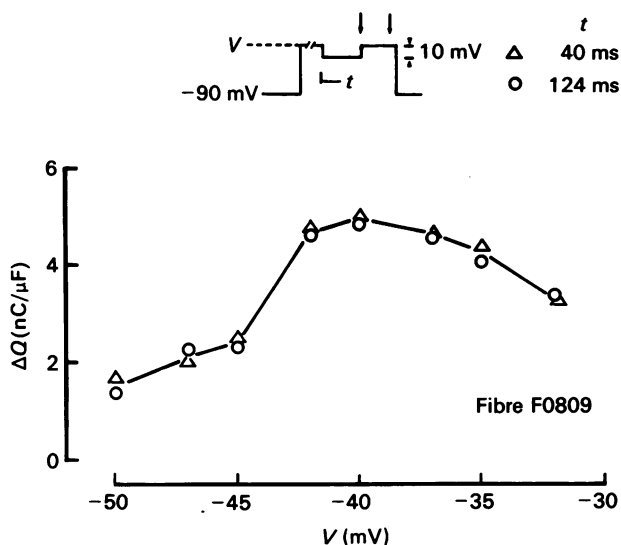


Fig. 5. Pulse procedure as in Fig. 4 (inset), but in which the non-linear charge moved in the depolarizing ('on') step was investigated using preceding 'off' pulses of duration 124 ms and 40 ms, and size 10 mV through a wide range of test voltages.

of 2 mM-tetracaine to abolish the  $q_\gamma$  charge movement (Huang, 1981*a*, 1982; Hui, 1983), resulted in a considerably less steep voltage dependence (Fig. 6B:  $\bullet$ ; mean  $\pm$  s.e. of the mean). The difference between the two curves in Fig. 6B provides an estimate of the voltage dependence of the  $q_\gamma$  system. Thus, at  $-40$  mV the two curves merged, corresponding to a more or less complete inactivation of  $q_\gamma$ ; this finding is in agreement with earlier work (Adrian & Peres, 1979; Huang, 1981*a*), although it appears here that  $q_\beta$  is also partly inactivated at  $-40$  mV. 'Off' responses at the different holding voltages (Fig. 6C) were always rapid decays with magnitudes which decreased with inactivation.

The  $q_\gamma$  current was isolated by comparing charge movement records obtained when the holding voltage was  $-90$  mV (Fig. 7A,  $q_\beta + q_\gamma$ ) with those obtained when  $q_\gamma$  was inactivated by holding at  $-40$  mV (Fig. 7B,  $q_\beta$ ). Fig. 7C shows the difference ( $A - B$ ), which represents the  $q_\gamma$  charge movement. The 'on' response appeared monotonic rather than forming a 'hump', and the 'off' response showed the usual rapid decay.

#### *Kinetic description of the $q_\gamma$ charge movement*

The steady-state features of the  $q_\gamma$  system have been shown earlier to be consistent with a system possessing two major energy levels  $S_1$  and  $S_2$  each producing different amounts of displaced charge. The levels have a steep but approximately linear

dependence of free energy upon the voltage (Duane & Huang, 1982). The result is a variation of non-linear charge due to  $q_\gamma$  that has a transition voltage  $\bar{V}$  of  $-35$  mV, and a steepness consistent with transfer of a charge of valency 4.5 as the system moves from state  $S_1$  to state  $S_2$  as the membrane is depolarized. On the other hand, the

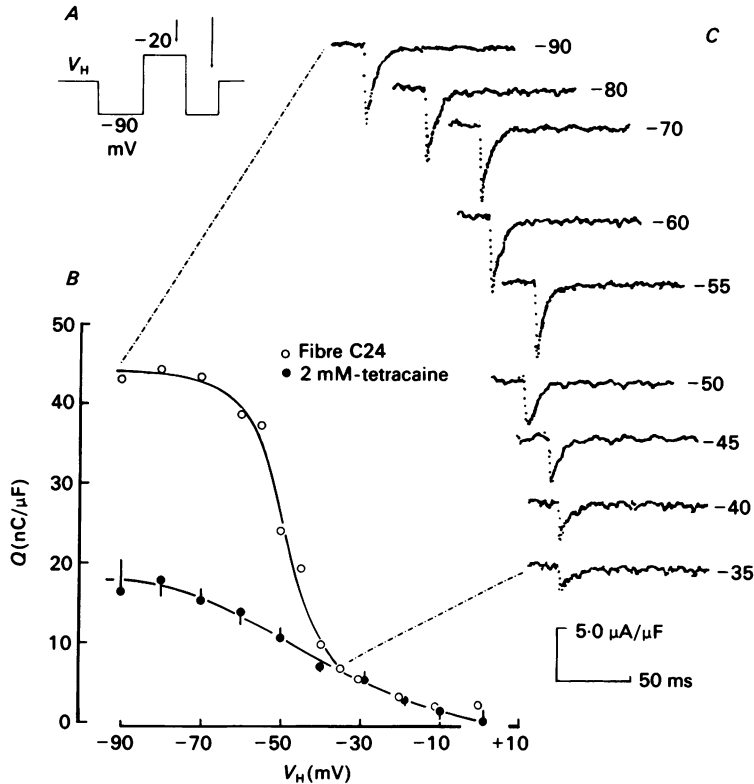


Fig. 6. Recovery of  $q_\gamma$  charge from large depolarizations at different holding potentials  $V_H$ . A, pulse procedure. B, non-linear charge at different holding potentials in the absence (○) and the presence (●: mean  $\pm$  s.e. of the mean in four fibres) of 2 mM-tetracaine. C, 'off' charge movements at each holding level.

complex kinetics of  $q_\gamma$  currents cannot be explained by the transition of the system from one state to the other across a simple energy barrier (Huang, 1983b). However, it is shown here that many features of the  $q_\gamma$  charge movement are predicted by a model which assumes that the rate constants that govern forward and reverse reactions depend not only upon the voltage  $V$  across the membrane but also upon the degree to which the non-linear charge has moved into state  $S_2$ . If one adopts the convention that the normalized charge  $q = 0$ , where all the charge resides in state  $S_1$ , and where  $q = 1$  if all the charge has moved into state  $S_2$ , then the capacitive current can be written:

$$\left(\frac{dq}{dt}\right)_V = \alpha(1-q) - \beta q. \quad (1)$$

In the simplest case, the rate constants are linear functions of the occupancy of state  $S_2$  in addition to their dependence upon voltage:

$$\alpha = \alpha_0(V) [1 + \zeta q(V, t)], \tag{2}$$

$$\beta = \beta_0(V) [1 + \zeta q(V, t)]. \tag{3}$$

These equations describe the behaviour of a system of two levels separated by an energy barrier which sees a fraction  $\delta < 1$  of the field between  $S_1$  and  $S_2$  (Adrian, 1978).

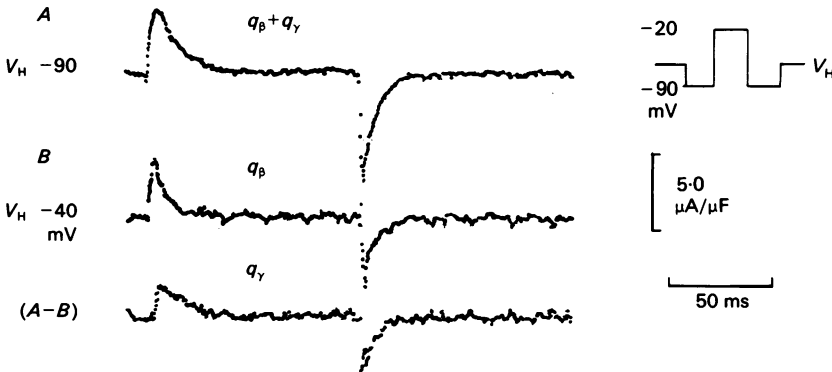


Fig. 7. Isolating the  $q_\gamma$  current in response to large depolarizing steps; same fibre as Fig. 6. *A*, charge movement at a holding potential of  $-90$  mV: both  $q_\beta$  and  $q_\gamma$  would be present. *B*, charge movement at a holding level of  $-40$  mV:  $q_\gamma$  inactivated. *C*, the  $q_\gamma$  transient deduced by taking the difference ( $A - B$ ). Note the fast 'off' current. Fibre cable constants:  $R_i = 335 \Omega \text{ cm}$ ; temp. =  $4.0^\circ\text{C}$ ;  $\lambda = 5.46 \text{ mm}$ ;  $r_i = 3799.3 \text{ k}\Omega/\text{cm}$ ; diam. =  $106 \mu\text{m}$ ;  $r_m = 1132.2 \text{ k}\Omega \text{ cm}$ ;  $R_m = 37.67 \text{ k}\Omega \text{ cm}^2$ ;  $C_m = 7.5 \mu\text{F}/\text{cm}^2$ .

The height of this barrier varies with the amount  $q(V, t)$  of product  $S_2$  to a degree specified by coefficient  $\zeta$ . The system consequently is 'co-operative' in the sense that a previous transition from  $S_1$  to  $S_2$  would enhance the reaction rates of the transitions that result in the observed charge movement, possibly involving mechanisms discussed on an earlier occasion (Huang, 1983*b*). The rest of the description parallels the simple scheme of Adrian (1978) in which the rate constants  $\alpha_0(V)$  and  $\beta_0(V)$  have the following dependences upon voltage:

$$\alpha_0(V) = A \exp(-zeV\delta/kT), \tag{4}$$

$$\beta_0(V) = A \exp\{-[\bar{V} - (1 - \delta)V]ze/kT\}. \tag{5}$$

The symbols  $e$ ,  $k$ , and  $T$  have their usual physical meanings. Values of the transition potential  $\bar{V}$  ( $= -35$  mV) and the valence  $z$  ( $= 4.5$ ) are already available from earlier steady-state analyses (Adrian & Peres, 1979; Duane & Huang, 1982) so that the only free parameters are the constant quantities  $\zeta$ ,  $\delta$  and  $A$ . Finally, in the steady state, the charge  $q(V, \infty)$  in this scheme reduces to a single valued function of potential,  $q(V, \infty) = \alpha_0(V)/[\alpha_0(V) + \beta_0(V)]$ , in agreement with earlier experimental results (Huang, 1983*a*; Adrian & Huang, 1984*a*).

The calculations presented in Fig. 8 show that even this admittedly over-simplified

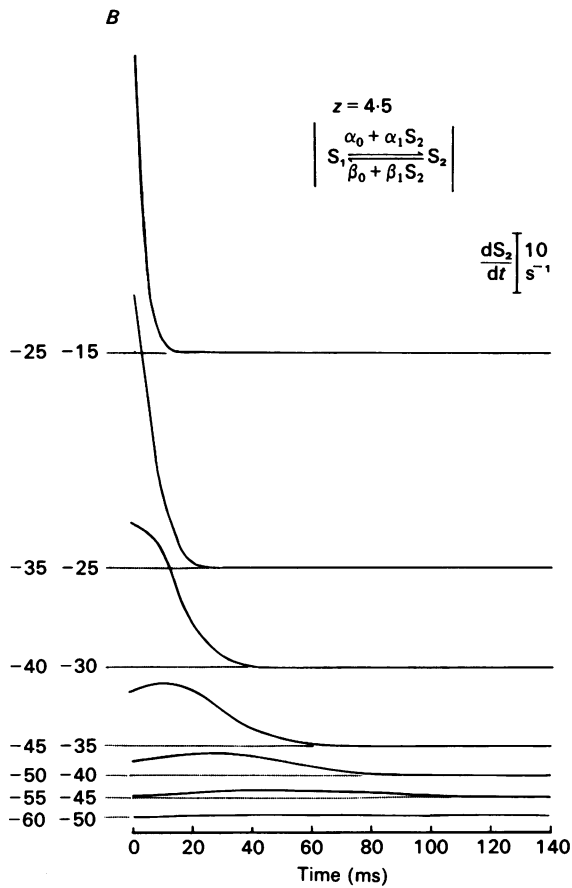
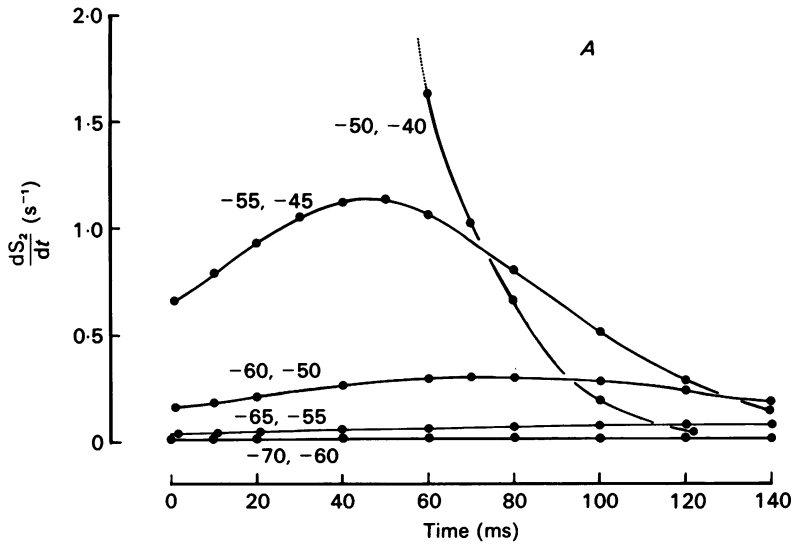


Fig. 8. For legend see opposite.

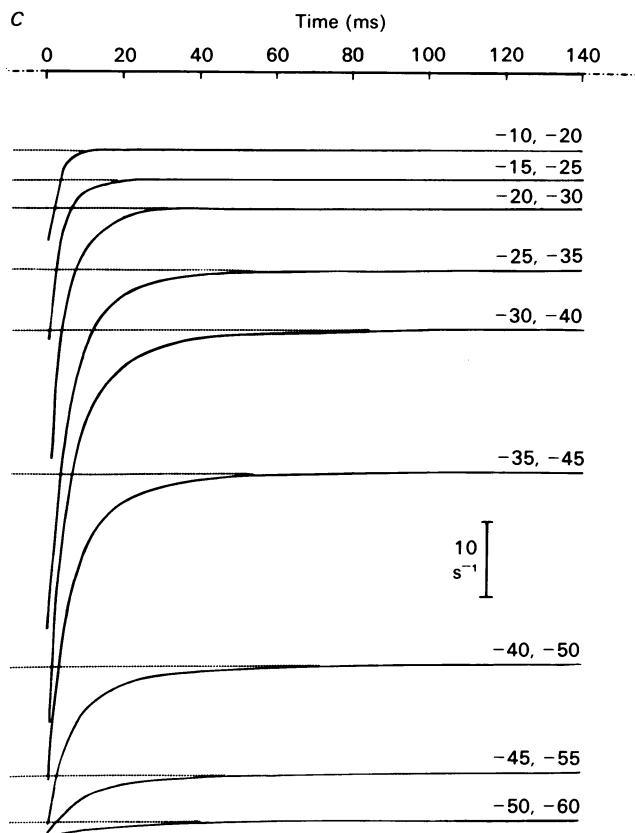


Fig. 8. The simplest form of non-linear system that assumes reaction rate constants dependent upon both charge and voltage. *A*, 'on' responses to 10 mV steps close to the contractile threshold, shown at high gain. *B*, predicted 'on' responses compared with *C*, 'off' responses to 10 mV steps over a wide voltage range at lower gain. The dotted lines denote zero current for each trace. Currents are normalized to the maximum available  $q_y$  charge, and so have units of  $s^{-1}$ .

non-linear scheme can successfully predict the major features of the response of  $q_y$  charge to applied 10 mV steps from a range of depolarized voltages. In the calculations,  $\delta = 0.5$ ,  $\zeta = 10^4$  and  $A = 0.245 s^{-1}$ . A Gill modification of the Runge-Kutta method that incorporated internal stepsize control identical to the algorithm used by Adrian & Peachey (1973), kindly supplied by Dr R. H. Adrian, was used to calculate the predicted transients. These are plotted normalized to the maximum available displaced  $q_y$  charge, in units of  $s^{-1}$  in Fig. 8.

Fig. 8*A* plots predicted traces proportional to 'on'  $q_y$  currents at high gain. There was little or no detectable current at  $-60$  mV, but 10 mV steps to  $-50$  and  $-55$  mV (close to the contractile rheobase for muscles in tetraethylammonium solutions: Huang, 1981*b*), resulted in the small and prolonged transients similar to those reported by Adrian & Huang (1984*a*). Depolarizations to around  $-45$  mV gave 'humps' resembling those reported earlier (Adrian & Peres, 1979; Huang, 1982), and

depolarizations to voltages 5–10 mV more positive resulted in currents with substantially faster kinetics. These properties of the computed curves therefore agree with experimental measurements. In addition, the model also successfully predicts ‘off’ responses that are relatively rapid decays through the entire voltage range: Fig. 8*B* and *C* compare ‘on’ and ‘off’ responses at low gain at most of the depolarizing levels of potential that have been studied experimentally.

Eqns. (2) and (3) above express the forward and return rate constants  $\alpha$  and  $\beta$ , as virial expansions in the charge  $q(V, t)$ , dropping higher than first-order terms to leave the interactions resulting from formation of product  $S_2$  to be described by a single coefficient,  $\zeta$ . The analysis that resulted provided a satisfactory description of the behaviour of the  $q_\gamma$  charge as available so far. However, information about certain aspects of this species of the charge movement is as yet incomplete: this is in particular at those times where  $q_\beta$  and  $q_\gamma$  currents overlap. It is possible that the inclusion of higher-order coefficients may therefore be justified as further details of  $q_\gamma$  kinetics become available.

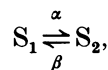
#### DISCUSSION

This paper describes a series of experiments carried out in order to gain an indication of the nature of the  $q_\gamma$  component of the charge movement in response to the hyperpolarizing (‘off’) part of a voltage step. By using a range of pulse procedures, it was shown that despite the wide variations in the form of  $q_\gamma$  currents in ‘on’ responses as voltage was varied, the corresponding ‘off’ decays were always relatively rapid.

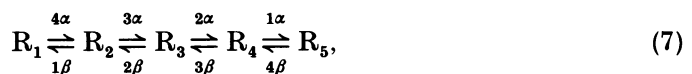
Adrian & Peres (1977) have commented upon the similarities between the ‘on’ kinetics of the  $q_\gamma$  charge movement and that of the first derivative of the potassium current  $d(g_K)/dt$ . In a Hodgkin–Huxley scheme,  $g_K$  would be given by the expression  $\bar{g}_K n^4$ , where  $n$  is defined by a first-order rate equation (Hodgkin & Huxley, 1952):

$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n. \quad (6)$$

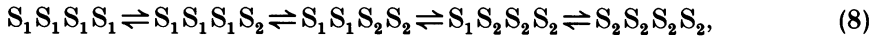
The terms  $\alpha_n$  and  $\beta_n$  are single-valued functions of voltage. The results in this paper suggest that the ‘off’ tails may also be amenable to such a description. Therefore, in so far as the over-all equations for  $dn^4/dt$  generates a higher-order function of the appropriate shape, formalisms of this kind might continue to be useful to describe the kinetics and steady-state features of the  $q_\gamma$  charge (Adrian & Peres, 1979). However, such models are not amenable to simple physical interpretation. For example, the most simple mechanisms that might underlie a system showing over-all  $dn^4/dt$  kinetics would be in terms of either four parallel first-order processes of the type:



or in terms of series systems of the kind:



in which the rate of formation of final product ( $R_5$  in the second case) would obey  $dn^4/dt$  kinetics, despite individual reactions in each scheme being first-order. However, a realistic capacitative system would require the voltage-sensitive steps, i.e. those reactions the rate constants of which depend upon voltage, to be the ones that produce the charge movement. The first scheme would therefore produce exponential charge movements obeying eqn. (6). The sequential scheme (7) has the same canonical form as the system:



in which reactions between each intermediate involve simply one reaction between  $S_1$  and  $S_2$ . The coefficient in front of each rate constant and between intermediates in the scheme (7) would then simply reflect the number of each kind of subunit  $S_1$  or  $S_2$  in each stage in the sequence (8). The voltage-dependent component of each reaction would therefore be identical to that described for the parallel model. A sequential system of this kind would then give charge movements that also have exponential kinetics, and these will have time constants the same as those predicted for the parallel system. Therefore, a Hodgkin-Huxley-type system is not an appropriate physical interpretation of the capacitive currents produced by the  $q_y$  charge.

However, a simple alternative scheme in which transitions between two available states in the system resulting from imposed depolarizing steps would be enhanced by movement of non-linear charge that had taken place previously, was considered. This offered a physically reasonable description of the observed features of the  $q_y$  current. The basis of the model was the dependence of the rate constants describing forward and backward reactions upon distribution of charge in the field, in addition to their dependence upon membrane potential. Possibilities of this kind have been discussed on earlier occasions in more general terms (Huang, 1983*b*). The steady-state features of such a system were obtained from earlier results (Adrian, 1978; Adrian & Peres, 1979; Duane & Huang, 1982). Even the most straightforward form of such a hypothesis gave predictions that agreed with empirical observations, and so therefore could offer a basis for further experimental testing.

The author thanks Dr R. H. Adrian (Physiological Laboratory) and Dr R. M. Lynden-Bell (University Chemical Laboratories, Cambridge) for helpful discussions, and Mr W. Smith for skilled assistance.

#### REFERENCES

- ADRIAN, R. H. (1978). Charge movement in the membrane of striated muscle. *Annual Review of Biophysics and Bioengineering* **7**, 85–112.
- ADRIAN, R. H. & ALMERS, W. (1974). Membrane capacity measurements on frog skeletal muscle in media of low ionic content. *Journal of Physiology* **237**, 573–605.
- ADRIAN, R. H. & HUANG, C. L.-H. (1984*a*). Charge movements near the mechanical threshold in skeletal muscle of *Rana temporaria*. *Journal of Physiology* **349**, 483–500.
- ADRIAN, R. H. & HUANG, C. L.-H. (1984*b*). Experimental analysis of the relationship between charge movement components in skeletal muscle of *Rana temporaria*. *Journal of Physiology* **353**, 419–434.
- ADRIAN, R. H. & PEACHEY, L. D. (1973). Reconstruction of the action potential in frog skeletal muscle. *Journal of Physiology* **235**, 103–131.

- ADRIAN, R. H. & PERES, A. R. (1977). A 'gating' signal for the potassium channel? *Nature* **267**, 800-804.
- ADRIAN, R. H. & PERES, A. (1979). Charge movement and membrane capacity in frog skeletal muscle. *Journal of Physiology* **289**, 83-97.
- ADRIAN, R. H. & RAKOWSKI, R. F. (1978). Reactivation of membrane charge movement and delayed potassium conductance in skeletal muscle fibres. *Journal of Physiology* **278**, 533-557.
- CHANDLER, W. K., RAKOWSKI, R. F. & SCHNEIDER, M. F. (1976). A non-linear voltage-dependent charge movement in frog skeletal muscle. *Journal of Physiology* **254**, 245-283.
- DUANE, S. & HUANG, C. L.-H. (1982). A quantitative description of the voltage-dependent capacitance in frog skeletal muscle in terms of equilibrium statistical mechanics. *Proceedings of the Royal Society B* **215**, 75-94.
- HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology* **117**, 500-544.
- HODGKIN, A. L. & NAKAJIMA, S. (1972). The effects of diameter on the electrical constants of frog skeletal muscle fibres. *Journal of Physiology* **221**, 105-120.
- HOROWICZ, P. & SCHNEIDER, M. F. (1981). Membrane charge movement in contracting and non-contracting skeletal muscle fibres. *Journal of Physiology* **314**, 565-593.
- HUANG, C. L.-H. (1980). Charge movement components in skeletal muscle. *Journal of Physiology* **305**, 31-32P.
- HUANG, C. L.-H. (1981*a*). Dielectric components of charge movements in skeletal muscle. *Journal of Physiology* **313**, 187-205.
- HUANG, C. L.-H. (1981*b*). Effects of local anaesthetics on the relationship between charge movements and contractile thresholds in frog skeletal muscle. *Journal of Physiology* **320**, 381-391.
- HUANG, C. L.-H. (1982). Pharmacological separation of charge movement components in frog skeletal muscle. *Journal of Physiology* **324**, 375-387.
- HUANG, C. L.-H. (1983*a*). Experimental analysis of alternative models of charge movements in frog skeletal muscle. *Journal of Physiology* **336**, 527-544.
- HUANG, C. L.-H. (1983*b*). Time domain spectroscopy of the membrane capacitance in frog skeletal muscle. *Journal of Physiology* **341**, 1-24.
- HUI, C. S. (1983). Pharmacological studies of charge movements in frog skeletal muscle. *Journal of Physiology* **337**, 509-552.
- PAPOULIS, A. (1977). *Signal Analysis*. New York: McGraw-Hill.
- SCHNEIDER, M. F. & CHANDLER, W. K. (1973). Voltage dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature* **242**, 244-246.