Intramembrane charge movement (Q) and sodium current (INa) were monitored in isolated voltage-clamped frog nodes of Ranvier. ON charge movements (QON) for pulses from the holding potential (-100 mV) to potentials V \leq 0 mV followed single exponential time courses, whereas two exponentials were found for pulses to V \geq 20 mV. The voltage dependence of both QON and its time constant TON indicated that the two ON components resolved at V \geq 20 mV were also present, though not resolvable, for pulses to V \leq 0 mV. OFF charge movements (QOFF) monitored at various potentials were well described by single exponentials. When QOFF was monitored at -30 or -40 mV after a 200-μs pulse to +20 mV and QON was monitored at the same potential using pulses directly from -100 mV, TON/TOFF = 2.5 ± 0.3. At a set OFF potential (-90 to -70 mV), TOFF first increased with increasing duration ION of the preceding pulse to a given potential (0 to +30 mV) and then decreased with further increases in ION. The declining phase of TOFF followed a time course similar to that of the decline in QOFF with ION. For the same pulse protocol, the OFF time constant τNa for INa also first increased with ION but then remained constant over the ION interval during which TOFF and QOFF were declining. After 200- or 300-μs pulses to +20, +30, or +50 mV, TOFF/τNa at -70 to -90 mV was 1.2 ± 0.1. Similar TOFF/τNa ratios were predicted by channel models having three identical charged gating particles that can rapidly and reversibly form an immobile dimer or trimer after independently crossing the membrane from their OFF to their ON locations.

INTRODUCTION

In the original characterization of the voltage- and time-dependent ionic conductance changes that underlie the squid axon action potential, Hodgkin and Huxley (1952) suggested that the conductance state of individual membrane ionic channels might be determined by the intramembrane location of charged or dipolar molecules that could change position or orientation in response to changes in membrane electric field. Some 20 years later, charge displacement currents presumably due to movement of intramembrane...
charges were recorded from both muscle fibers (Schneider and Chandler, 1973) and nerve axons (Armstrong and Bezanilla, 1973). Most of the intramembrane charge movement recorded from axons appears to be related to sodium channels (Yeh and Armstrong, 1978; Cahalan and Almers, 1979; Almers, 1978).

It is now generally agreed (Armstrong and Bezanilla, 1974; Neumcke et al., 1976; Keynes, 1980) that gating current is not simply proportional to the rate of change of the $m$ parameter in the Hodgkin-Huxley (1952) model. Although alternative models for sodium channel gating have been suggested (cf. Armstrong, 1978), none has as yet been exhaustively tested. The present report represents an attempt to quantitatively characterize various kinetic properties of intramembrane charge movement and sodium current in frog node of Ranvier. Any satisfactory model for sodium channel gating in node should be capable of accounting for and reproducing such observations.

An abstract of some of the present results was presented to the VII International Biophysics Congress (Schneider and Dubois, 1981).

METHODS

Experiments were carried out on nodes of Ranvier in isolated myelinated nerve fibers from the frog Rana esculenta. Fibers were bathed in Ringer's solution for dissection and mounting in the experimental chamber. The end pools were then changed to isotonic CsCl (120 mM) and both fiber ends were recut. Isotonic CsCl was used at the cut ends during all experiments unless otherwise specified. The Ringer's solution contained 111.5 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl$_2$, and 2.4 mM NaHCO$_3$, and its pH was 7.4. Ionic current through potassium channels was suppressed by the internal cesium (Dubois and Bergman, 1975) and by 10 mM tetraethylammonium (TEA) ion (Hille, 1967; Koppenhöfer, 1967) added as TEA chloride to the Ringer's solution bathing the node. All results were obtained at 8–12°C.

The nodal membrane was voltage-clamped using the method of Nonner (1969). Membrane currents were calculated arbitrarily assuming an exoplasmic resistance of 10 MΩ. A membrane potential of $-70$ mV was defined as that potential corresponding to 30% "fast" inactivation of the sodium current (Nonner et al., 1975). The nodes were routinely maintained at a holding potential of $-100$ mV. For each voltage pulse, the time-independent linear leakage currents and two components of exponentially decaying linear capacity current were approximated by an analog circuit driven by the command pulse (Dubois and Bergman, 1977$^a$) and were subtracted from the total current by a differential amplifier.

Sodium currents ($I_{Na}$) were obtained after low-pass filtering with an active Bessel filter with cut-off frequency set at 20, 30, or 40 kHz. Residual linear current components were generally removed by digitally summing currents for a given number of depolarizing pulses of amplitude $P$ with those for twice that number of hyperpolarizing pulses of amplitude $-P/2$. This protocol will be referred to as the "$-P/2$ routine." Whenever the depolarizing pulse had multiple steps, each step was present at half amplitude in $-P/2$. Both $P$ and $-P/2$ were applied from the holding potential.

Charge movement was recorded after blocking ionic current through sodium channels by also adding $10^{-6}$ M tetrodotoxin (TTX) to the Ringer's solution bathing the node. To minimize noise and obtain direct estimates of charge transfer, the current remaining after subtracting linear components was integrated by an operational...
amplifier circuit. The integrated signal was digitized and summed as for \( I_{Na} \), but without prior filtering. To minimize drift and prevent saturation, the operational amplifier integrator was AC-coupled at its input (600 Hz) and its feedback capacitor was shunted until 0.5 ms before the start of each record using a transistor switch. Charge movement records were routinely obtained using 24 depolarizing pulses in the \(-P/2\) routine. To estimate possible errors in \( Q \) due to the apparatus, tests were carried out using a dummy circuit representing the nerve fiber and external resistances. For a 1-ms pulse \( P \) that moved 100 fC of charge, the mean \( \pm \) SEM charge remaining after the \(-P/2\) routine was 1.8 \( \pm \) 0.3 fC (\( N = 50 \); maximum = 6 fC).

Analog-to-digital conversion (8-bit) and digital summing (16-bit capacity) were carried out by a single averager (Memoscope RE10; R2E, Orsay, France) that sampled 1,000 successive points at 4-\( \mu \)s intervals. In the few experiments in which \( I_{Na} \) inactivation during a pulse was monitored, 16-\( \mu \)s sampling intervals were used. After completion, each sum record was converted to analog form, displayed on an oscilloscope, and photographed. The photographed records were subsequently projected and traced continuously by hand because individual points were not resolvable over most of the record. Such tracings were then measured at 12.5- or 25-\( \mu \)s intervals to give the data actually analyzed. Time constants and initial amplitudes of apparently exponential components were determined by a linear regression analysis (9810A; Hewlett-Packard Co., Palo Alto, Calif.) using logs of the measured values. Sodium tail current records obtained with the \(-P/2\) routine generally appeared to be distorted during the initial 25 or 35 \( \mu \)s, perhaps due to oscillation or amplifier saturation, so that the 37.5- or 50-\( \mu \)s value was the first used in analyzing \( I_{Na} \) tails. For the same fibers in TTX, charge movement records obtained with the same pulses but using integrated currents appeared to have less initial distortion and were analyzed starting either 12.5 or 25 \( \mu \)s after pulse \textit{on} or \textit{off}.

RESULTS

Direct Recording of Charge Movement

Various types of recordings of gating current (\( I_{Q} \)) or its associated gating charge movement (\( Q \)) for a given pulse are illustrated in Fig. 1. Trace \( a \) is a standard recording of gating current, obtained here using the \(-P/2\) routine. It shows a transient outward current during the pulse and transient inward current after the pulse. The fact that the steady current during the pulse was below the initial baseline indicates a slightly lower conductance for the depolarizing than for the hyperpolarizing pulses. Gating current is assumed to constitute all current above this steady level during the pulse and all current after the pulse. Trace \( b \) of Fig. 1 was obtained for pulses to the same potentials as in \( a \), but by using analog integration before digitization and summing by the \(-P/2\) routine. As expected for the integral of \( I_{Q} \), trace \( b \) rose at pulse \textit{on} and fell at pulse \textit{off}. After its initial rising phase, trace \( b \) declined approximately linearly with time during the pulse. This also was to be expected because the current record (trace \( a \)) had a negative steady “\textit{on}” level that, when integrated, would produce a linearly declining phase. Trace \( b \) was corrected for time-independent nonlinear currents by fitting straight sloping baselines by eye to the latter parts of its \textit{on} and \textit{off} segments, extrapolating the baselines to the preceding pulse edge, and subtracting them from \( b \) to give \( c \). The fact that trace \( c \) does not return to the initial baseline after the pulse
illustrates the well-established finding that the charge carried by on gating current exceeds that carried back by off gating current if the off potential is not considerably negative to -100 mV (Armstrong and Bezanilla, 1977; Nonner et al., 1978).

In principle, gating current and gating charge movement traces contain the same information. For the pulse used in Fig. 1, off gating current in node of

![Graph of gating current and charge movement traces.](image)

**Figure 1.** Gating current and gating charge movement traces. (a) Gating current recorded in response to 24 120-mV depolarizing pulses and 48 60-mV hyperpolarizing pulses (-P/2 routine). (b) Gating charge movement recorded in response to 24 120-mV depolarizing pulses and 48 60-mV hyperpolarizing pulses (-P/2 routine). (c) Gating charge movement trace presented in b after correction for time-independent nonlinear currents. (d) Gating charge movement recorded in response to 24 120-mV depolarizing pulses and 24 120-mV hyperpolarizing pulses and corrected for time-independent nonlinear currents. Temperature: 11°C. Fiber: 8-1-80.

Ranvier is well described by a single exponentially decaying component (Neumcke, et al., 1976; Dubois and Bergman, 1977b) so that

\[ I_Q(t) = I_Q(0) \exp(-t/\tau_Q), \]  

(1)

where \( t \) is time and \( \tau_Q \) is the gating current time constant. The gating charge movement is by definition the integral of the gating current so that if Eq. 1 described \( I_Q(t) \), then \( Q(t) \) will be

\[ Q(t) = \tau_Q I_Q(0) [1 - \exp(-t/\tau_Q)]. \]  

(2)

\( Q(t) \) thus rises to its final level \( \tau_Q I_Q(0) \) along a single exponential time course having the same time constant as the decline of \( I_Q(t) \). If \( I_Q \) were to be composed of multiple exponential components with different \( I_Q(0) \) and \( \tau_Q \) values but with each following Eq. 1, then \( Q \) would also be composed of the same number of components, each following Eq. 2 with corresponding \( I_Q(0) \) and \( \tau_Q \). Similar equations apply to the off charge except that the initial value of \( Q \) is nonzero.
Eqs. 1 and 2 allow quantitative comparison of \( I_Q \) and \( Q \) records for a given pulse. Considering traces \( a \) and \( c \) in Fig. 1, the \( \tau_Q \) values obtained from the \( I_Q \) and \( Q \) traces were 92 and 87 \( \mu \)s, and the final \( Q_{ON} \) values were 83 fC for \( \tau_Q I_Q(0) \) from \( I_Q \) and 80 fC from \( Q \). Other paired \( I_Q \) and \( Q \) records showed similarly good agreement of parameters. Since the \( Q \) traces were considerably less noisy than \( I_Q \), they were used for the studies presented here. Gating current in node has previously been analyzed using \( Q \) traces generated from digital \( I_Q \) records (Neumcke et al., 1976; Nonner et al., 1978).

Trace \( d \) of Fig. 1 presents a control for nonlinear charge displacement over the voltage range negative to \(-100 \) mV. It was obtained from currents for the same size depolarizing pulses as in \( c \) but here summed with currents for the same number of hyperpolarizing pulses of equal amplitude (\( \pm P \) routine). The similarity of \( c \) and \( d \) and of other pairs of records comparing the \(-P/2 \) and \( \pm P \) routines indicates that charge movement recordings are minimally influenced by nonlinearities in the hyperpolarizing voltage range. To minimize the possibility of membrane damage by large hyperpolarizing pulses, charge movements were generally recorded with the \(-P/2 \) routine. The \( \pm P \) routine was used for a few fibers that were found to tolerate large hyperpolarizing pulses.

**Two Components of ON Charge Movement**

on charge movement records obtained by the integral method for pulses to several different membrane potentials in the same fiber are presented in the upper part of Fig. 2. In the lower part of Fig. 2 the \( Q_{ON} \) time courses are analyzed using normalized semilog plots of the fraction \( 1 - [Q_{ON}(t)/Q_{ON}(\infty)] \) of ON charge remaining to be moved for each record. For depolarizing pulses to and below 0 mV, the ON charge movement is well described by a single exponential, whereas for pulses to and beyond \(+20 \) mV, two exponential components are required. The single integrated ON displacement current record at a positive potential presented by Neumcke et al. (1976, Fig. 3D) deviates appreciably from its single exponential fit and thus also indicates the presence of more than one component of \( Q_{ON} \) at positive potentials in node of Ranvier. After correcting \( I_{Na} \) turn-on in one fiber for a two-exponential inactivation process in parallel (Chiu, 1977), \( I_{Na} \) activation at \(+20 \) mV approached its final level at about the same time as the slow component of \( Q_{ON} \) in the same fiber. This would tend to indicate that the present slow component of \( Q_{ON} \) may be similar to the "intermediate" component in squid axons (Armstrong and Gilly, 1979) rather than the squid axon "slow" component (Armstrong and Bezanilla, 1977).

The charge-voltage relationship for the experiment of Fig. 2 is analyzed in Fig. 3A. The filled circles present values of total \( Q_{ON} \) as a function of pulse membrane potential. At positive potentials the amounts of charge carried by the fast (squares) and slow (triangles) kinetically separable components are also indicated. The values of total \( Q_{ON} \) at 0 mV are larger than the \( Q \) values for either the fast or the slow components at positive potentials. Therefore, if there were only a single component of \( Q \) for pulses to and below 0 mV and if a second component began to contribute to \( Q \) only for pulses beyond 0 mV,
the charge moved by the first component would have to attain a maximum at about 0 mV and then decline for pulses beyond 0 mV. Such behavior would not be expected for a voltage-dependent redistribution of charges or dipoles within the membrane. A more likely hypothesis is that there are multiple components of charge movement even at negative potentials but that their time constants are too similar to allow kinetic separation using these data and techniques.

**Figure 2.** Charge movement kinetics. (A) Traces of charge movement recorded at different membrane potentials and corrected for time-independent nonlinear currents. (B) Semilogarithmic plots of charge movement traces presented in part A. Filled circles give the overall charge movement and open circles (for pulses to and beyond +20 mV) give the charge movement remaining after subtracting the slow exponential component (straight lines through filled circles). Temperature: 8°C. Fiber: 10-4-80.
The relationship between $\tau_{ON}$ and pulse membrane potential (Fig. 3B) for the experiment of Figs. 2 and 3A appears to be consistent with the latter hypothesis. Within the scatter in the data, neither the faster nor the slower of the two time constants at and beyond +20 mV appears to continue the trend of the voltage dependence of the single time constants observed up to 0 mV.

**Figure 3.** Voltage dependence of $ON$ charge movement. (A) $ON$ charge movement vs. voltage relationship. The circles represent the overall charge movement. For membrane potentials equal to and more positive than +20 mV, the triangles and squares represent, respectively, the slow and fast charge movement components. The curves present the nonlinear least-squares fit of Eq. 3 to all data points (solid curve) or to only the data points at or below 0 mV (dashed curve). Parameter values obtained from the fits were $Q_{max} = 100$ or 96 fC, $V = -31.6$ or $-33.4$ mV, and $k = 18.1$ or 16.8 mV for the solid or dashed curves, respectively. (B) $ON$ charge movement time constants vs. voltage. For membrane potentials more negative than +20 mV, the charge movement kinetics were described by one exponential (circles). For membrane potentials equal to or more positive than +20 mV, the charge movement kinetics were described by the sum of a slow (triangles) and a fast (squares) exponential component. Curves were drawn by eye. Same fiber and run as for Fig. 2.
Thus neither of the two components at positive potentials appears to correspond to a single process occurring at and below 0 mV.

Table I presents values of time constants and relative amplitudes for the two ON charge movement components resolved for pulses to +20 mV in four fibers. Based on exponential extrapolation to pulse ON, the slow component carried 0.66 ± 0.04 (mean ± SEM) of the total QON at +20 mV. It should be noted that this charge assignment assumes that both components of QON follow exponential time courses without delay. If the slow component actually began only after a delay, as suggested by Armstrong and Gilly (1979) for the intermediate component in squid axons, we would be overestimating the charge transfer caused by the slow component and underestimating that caused by the fast component. In one of the fibers in Table I, QON was also monitored for pulses to 0 mV and found to be well described by a single exponential. In this fiber the mean value of QON at 0 mV was 87% of QON at +20 mV (Fig. 3A).

### Table I

<table>
<thead>
<tr>
<th>Fiber</th>
<th>N*</th>
<th>Qslow/Qtotal</th>
<th>τmax</th>
<th>τslow</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-4-80</td>
<td>4</td>
<td>0.75</td>
<td>65</td>
<td>180</td>
</tr>
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<td>28-4-80</td>
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<td>0.63</td>
<td>80</td>
<td>220</td>
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<td>10-4-80</td>
<td>2</td>
<td>0.66</td>
<td>43</td>
<td>170</td>
</tr>
<tr>
<td>21-2-80A</td>
<td>6</td>
<td>0.58</td>
<td>52</td>
<td>152</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td></td>
<td>0.66±0.04</td>
<td>60±8</td>
<td>180±14</td>
</tr>
</tbody>
</table>

* Number of records of QON at +20 mV analyzed to give the mean values presented for each fiber.

The solid line in Fig. 3A presents the nonlinear least-squares fit (Horowicz and Schneider, 1981a) of the total QON data by the equation

\[ Q_{ON} = Q_{max}/[1 + \exp ((\bar{V} - V)/k)] \]

(3)

for the steady-state transfer of charge due to redistribution of charged intramembrane particles between two sites according to the Boltzmann relationship (Schneider and Chandler, 1973; Keynes and Rojas, 1974; Nonner et al., 1975). The adjusted parameters were the maximum charge displaced \( Q_{max} \), the membrane potential for 50% charge displacement \( \bar{V} \) and the steepness factor \( k \) (equal to \( RT/az \), with \( R \) being the gas constant, \( T \) the absolute temperature, \( z \) the valence of the charged particle, and \( a \) the fraction of the membrane field that the particles traverse). The fact that Eq. 3, which describes a single charge transition, can closely approximate the \( Q \) vs. \( V \) data does not rule out the possibility of multiple \( Q \) components. Horowicz and Schneider (1981b and unpublished calculations) have found that \( Q \) vs. \( V \) relationships predicted for a variety of multi-transition models for charge movement may be experimen-
tally indistinguishable from Eq. 3. When Eq. 3 was fit only to the data points at or below 0 mV in Fig. 3A, the theoretical curve (dashed line) was quite close to the one obtained when all points were considered. This also tends to argue against a second \( Q \) component appearing only for pulses to positive potentials because the results in the negative voltage range, where such a second component would make no contribution, allow close prediction of the \( Q \) vs. \( V \) behavior at positive potentials, where the second component comprised at least about one-third of \( Q_{\text{ON}} \) (squares or triangles in Fig. 3A).

In the course of these and other (Dubois and Schneider, 1981) studies, \( Q_{\text{ON}} \) vs. \( V \) data were obtained from nine fibers at 7.5-13°C. The parameter values and their standard errors obtained from the fit of Eq. 3 to the pooled data were \(-33.1 \pm 1.0 \text{ mV for } \bar{V} \) and \(13.3 \pm 0.9 \text{ mV for } k \). The relative standard error in \( Q_{\text{max}} \) was \( \pm 2.0\% \). The mean \( \pm \text{SEM} \) of the parameter values obtained from fits to data from individual fibers were \(-32.1 \pm 2.0 \text{ mV for } \bar{V} \) and \(13.0 \pm 0.9 \text{ mV for } k \). Our \( Q_{\text{ON}} \) results thus agree closely with those of Nonner et al. (1975), who obtained \(-33.7 \text{ mV for } \bar{V} \) and \(14.9 \text{ mV for } k \) in frog node of Ranvier.

**Kinetics of \( \text{on} \) and \( \text{off} \) Charge Movement at the Same Potential**

The results in Figs. 2 and 3 indicate the presence of multiple components of gating charge movement at all potentials, even though the components may not always be separable kinetically. If, on the contrary, all charge movement were generated by a system having only a single voltage- and time-dependent transition, then at any given potential the \( \text{on} \) and \( \text{off} \) charge movements should have the same time constants (e.g., Hodgkin and Huxley, 1952). We therefore examined \( \text{on} \) and \( \text{off} \) \( Q \) time courses at the same potential to see whether such data could rule out the possibility of a single transition system. Fig. 4A presents records from one such experiment. The right-hand traces in each panel give the \( Q \) time courses for the voltage step sequences shown diagrammatically on the left. The \( \text{on} \) charge movement at \(-30 \text{ mV} \) (record \( b \)) was considerably slower than the \( \text{off} \) at the same potential (record \( a \)), which was recorded after first having moved the charge in the \( \text{on} \) direction by a 200-\( \mu \text{s} \) prepulse to \(+20 \text{ mV} \). Semilog plots of \( \text{on} \) and \( \text{off} \) charge movements at \(-30 \text{ mV} \), each obtained by averaging measurements from tracings of four records such as those in Fig. 4A, are presented in Fig. 4B. Both \( \text{on} \) and \( \text{off} \) charge movements closely followed single exponentials, with time constants \( \tau_{\text{ON}} \) of 250 \( \mu \text{s} \) and \( \tau_{\text{OFF}} \) of 62 \( \mu \text{s} \). Single exponential fits to the individual traces that were used for Fig. 4B gave \( \tau_{\text{ON}} \) values ranging from 216 to 277 \( \mu \text{s} \) (mean \( \pm \text{SE} = 250 \pm 14 \mu \text{s} \)) and \( \tau_{\text{OFF}} \) values from 50 to 79 \( \mu \text{s} \) (61 \( \pm 7 \mu \text{s} \)).

\( \text{on} \) and \( \text{off} \) charge movement time constants obtained at the same potential in each of several fibers are presented in Table II. For the potentials tested, which were all in the neighborhood of \( \bar{V} \) for these fibers, \( \tau_{\text{ON}} \) was 1.21-4.17 times larger than \( \tau_{\text{OFF}} \) (mean \( \pm \text{SE}, 2.52 \pm 0.29 \)). Such a discrepancy between \( \text{on} \) and \( \text{off} \) time constants is incompatible with a system having only a single-voltage and time-dependent transition, even though a single exponential does provide a good description of these \( \text{on} \) and \( \text{off} \) charge movement time courses.
The voltage dependencies of \( \tau_{\text{ON}} \) and \( \tau_{\text{OFF}} \) were also investigated. Results from one fiber are presented in Fig. 5. Over the voltage range where both time constants could be determined, \( \tau_{\text{ON}} \) (filled circles) was two to three times larger than \( \tau_{\text{OFF}} \) (open circles). Considering the scatter in the measurements, it is difficult to tell whether \( \tau_{\text{ON}}/\tau_{\text{OFF}} \) was constant or varied with the voltage.

Since \( \tau_{\text{OFF}} \) was always measured after a 200-\( \mu \)s prepulse to positive potentials, it might be argued that the voltage dependence of the charge movement

\[ \text{Figure 4. ON and off charge movement kinetics at the same potential.} \]

(A) Traces of charge movement recorded during pulses to \(-30\) mV applied either after 200 \( \mu \)s depolarizations to \(+20\) mV (a, off response) or from the holding potential (b, on response). Pulse programs shown on the left. (B) Semilogarithmic plot of average ON and OFF charge movements (four recordings of each) at \(-30\) mV obtained using pulse programs shown in part A. The time constants for ON and OFF charge movements are, respectively, 250 and 62 \( \mu \)s. Temperature: 7.5°C. Fiber: 22-1-80.
process had been modified by the prepulse, as, for example, accompanying “charge immobilization” (Armstrong and Bezanilla, 1977; Nonner, 1980). Figs. 6A and B, which present the \( Q_{ON} \) and \( Q_{OFF} \) values corresponding to the \( \tau_{ON} \) and \( \tau_{OFF} \) data in Fig. 5, indicate that this was not the case. Both the charge \( Q_{ON} \) moved outward during a single step and the charge \( Q_{OFF} \) moved back after a 200-\( \mu \)s on prepulse to +20 mV gave essentially identical values of \( \bar{V} \) and \( k \) when fit by Eq. 3 or its complement for \( Q_{OFF} \). The simplest interpretation of this result is that the relative energy levels of the various potential energy wells that the charges occupy in the membrane were not significantly altered by the prepulse.

The \( Q_{OFF} \) value for \( Q_{\text{max}} \) obtained from the data in Fig. 6B was only 68% of the \( Q_{\text{OFF}} \) value obtained from the data in Fig. 6A. At the prepulse voltage of +20 mV only 86% of the steady-state value of \( Q_{ON} \) was found to be moved during the first 200 \( \mu \)s of the prepulse. Since the fit of Eq. 3 to the steady-state \( Q_{ON} \) data indicated that at +20 mV \( Q_{ON} \) was 99% of \( Q_{\text{max}} \), the \( Q_{OFF} \) value for \( Q_{\text{max}} \) could be at most only 85% of the \( Q_{ON} \) value. If the remaining deficit is attributed to charge immobilization, the percent of the 200-\( \mu \)s \( Q_{ON} \) that was not immobilized during the prepulse was 80% \( \left[\frac{100(0.68/0.85)}{100}\right] \).

For a single energy barrier located between two possible sites that the intramembrane charged particles can occupy, the time constant vs. voltage relationships shown in Fig. 5 should be described by the equation

\[
\tau = 2\tau_0/\left(\exp[\eta(V - \bar{V})/k] + \exp \left[(1 - \eta)(\bar{V} - V)/k\right]\right)
\]

(Adrian, 1978; Horowicz and Schneider, 1981b), where \( \tau_0 \) is the value of \( \tau \) at \( \bar{V} \) and \( \eta \) is the fraction of the total field between sites that appears between the barrier peak and the resting site. Values of the parameters \( \bar{V} \) and \( k \) in Eq. 4 are given in Table II.

### Table II

<table>
<thead>
<tr>
<th>Fiber</th>
<th>( V )</th>
<th>( \tau_{ON} )</th>
<th>( \tau_{OFF} )</th>
<th>( \tau_{ON}/\tau_{OFF} )</th>
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<tr>
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</tbody>
</table>

Mean±SEM  2.52±0.29

\( Q_{OFF} \) charge movements were recorded after 200-\( \mu \)s on prepulses to +20 mV, whereas \( Q_{ON} \) charge movements were recorded using pulses directly from the -100-mV holding potential.
4 were determined from the fits to the charge vs. voltage relationships presented in Fig. 6. Assuming the barrier to be symmetrically positioned between the two sites, η is 1/2 and τ\text{max} is the maximum value τ\text{max} of τ (Keynes and Rojas, 1974). For this case the value of τ\text{max} was adjusted to give the best fits to either the τ\text{ON} or τ\text{OFF} data. One may observe that the resulting theoretical curves (solid in Fig. 5) poorly describe the experimentally determined τ-V relationships. The τ\text{OFF} values are in rough agreement with the theoretical τ\text{OFF}-V curve over a voltage range positive to about -60 mV, but at the most negative potentials they are closer to the theoretical curve for τ\text{ON} than to that for τ\text{OFF}. This may explain why previous determinations of τ\text{ON} and τ\text{OFF} over non-overlapping voltage ranges were thought to follow a single τ vs. V relationship (Keynes and Rojas, 1974). Allowing the barrier to be asymmetrically located and using a linearized form of Eq. 4 to determine η
and τν (Horowicz and Schneider, 1981b) gave the dashed theoretical curves, which correspond more closely to the data in Fig. 5.

**Effect of ON Pulse Duration on OFF Charge Movement**

Using a holding potential at the negative end of the Q vs. V relationship, QON is measured using a single pulse, whereas QOFF must be measured after a prepulse that moves some or all charge in the on direction. It has been reported that τOFF at a given potential depends on the duration of the preceding pulse (Armstrong and Bezanilla, 1977; Nonner et al., 1978). In view of our observation that on and off Q time constants are not equal at the same potential, we reinvestigated the effect of the on prepulse duration on off charge movement.

**Figure 6.** ON and OFF charge movement vs. voltage relationships. (A) ON charge movement recorded during pulses to various potentials applied from the holding potential. (B) OFF charge movement recorded during pulses to various potentials preceded by 200-μs depolarizations to +20 mV. Mean values and standard errors of two to six determinations. The curves are nonlinear least-squares fit of Eq. 3. The values of the parameters Qmax, V, and k and their standard errors are, respectively: 130 ± 6 fC, -33.7 ± 1.7 mV, and 11.6 ± 2.1 mV (on response), and 89 ± 3 fC, -33.1 ± 1.8 mV, and 11.0 ± 2.4 mV (off response). Same fiber and run as for Fig. 5.

Two parameters of charge movement are known to change with time during relatively large depolarizing pulses: QON increases with time to its final value and charge immobilization develops (Armstrong and Bezanilla, 1977; Nonner, 1980). Fig. 7 presents the time course of charge immobilization. QOFF was monitored at -80 mV after on pulse durations of up to 3 ms at +20 mV and normalized to the final value QON of QON at +20 mV in each of four fibers. The time course of normalized QOFF in Fig. 7 was described by the equation

\[
\frac{Q_{OFF}}{Q_{ON}} = \frac{Q_{ON}}{Q_{ON\infty}} \left[ \frac{Q_{OFF\infty}}{Q_{ON\infty}} + \left( 1 - \frac{Q_{OFF\infty}}{Q_{ON\infty}} \right) e^{-t_{ON}/\tau_O} \right],
\]

where QON and QOFF are both functions of the on pulse duration tON, QOFF∞/
QPFF represents the immobilization-resistant fraction of charges, and $\tau_i$ is the immobilization time constant. In agreement with the observations of Armstrong and Bezanilla (1977) and Nonner (1980), $\text{QPFF}/\text{QON} = 0.33$. Also in agreement with Nonner (1980), the value of $\tau_i$ used in the fit of Fig. 7 was 800 $\mu$s, somewhat larger than the mean value of the fast inactivation time constant for $I_{Na}$ of $480 \pm 21 \mu$s determined in the same fibers at the same ON potential of $+20 \text{mV}$.

The influence of ON pulse duration on the kinetics of QOFF are considered in Fig. 8. Part A presents semilog plots of QOFF at $-80 \text{ mV}$ in one fiber after pulses of 100 (a), 600 (b), and 2,000 $\mu$s (c) to $+20 \text{ mV}$. The three time courses are all quite well described by a single exponential, as was the case in other experiments. $\tau_{\text{OFF}}$ after the 600- $\mu$s pulse was larger than $\tau_{\text{OFF}}$ after either the 100- or 2,000- $\mu$s pulses. Fig. 8B presents $\tau_{\text{OFF}}$ at $-80 \text{ mV}$ as a function of the duration $t_{\text{ON}}$ of the preceding pulse at $+20 \text{ mV}$. $\tau_{\text{OFF}}$ first increased with increasing $t_{\text{ON}}$; then for $t_{\text{ON}}$ values greater than $\sim 600-1,000 \mu$s, $\tau_{\text{OFF}}$ decreased with increasing $t_{\text{ON}}$.

It is tempting to relate the initial rise in $\tau_{\text{OFF}}$ to increasing QON and to relate the later decline in $\tau_{\text{OFF}}$ to charge immobilization, which has been shown to be accompanied by increased speed of movement of the remaining nonimmobilized charge (Nonner, 1980). Fig. 8C presents QON at $+20 \text{ mV}$ and QOFF.

![Figure 7](image-url)
Figure 8. Effect of on pulse duration on off charge movement kinetics. (A) Semilogarithmic plots of \( Q_{\text{OFF}} \) at \(-80 \text{ mV}\) after on pulses of 100 (a), 600 (b), and 2,000 \( \mu \text{s} \) (c) at +20 mV. (B) Off time constant at \(-80 \text{ mV}\) as a function of the duration of the on prepulse at +20 mV. (C) \( Q_{\text{ON}} \) at +20 mV and \( Q_{\text{OFF}} \) at \(-80 \text{ mV}\) relative to \( Q_{\text{ON\ max}} \) as a function of the duration of the on prepulses to +20 mV. In B and C most of the points are mean values of two determinations. Numbers of determinations differing from two appear in brackets. Curves were drawn by eye. Temperature: 8.5°C. Fiber: 21-2-80 A.
at -80 mV, both as a function of $t_{ON}$ at +20 mV. The late declining phase of $Q_{OFF}$ does indeed seem to parallel the late decline in $\tau_{OFF}$. However, the early rise of $Q_{ON}$ is relatively more rapid than the more prolonged early rising phase of $\tau_{OFF}$.

The relationship between on charge movement and the increasing phase of $\tau_{OFF}$ is further explored in Fig. 9, which presents $\tau_{OFF}$ as a function of $Q_{ON}$.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig9.png}
\caption{off charge movement time constant as a function of the relative quantity of charge moved during the preceding on pulse. $\tau_{OFF}$ was measured in four fibers (different symbols) at -80 mV after on prespulses of varying duration at either 0 or +20 mV. The filled symbols give results for $t_{ON} \leq 300 \mu s$ at 0 mV or $\leq 200 \mu s$ at +20 mV. The open symbols are for longer $t_{ON}$ values for which $\tau_{OFF}$ is probably underestimated because of the onset of charge immobilization. $Q_{ON_{\text{max}}}$ for each fiber was calculated from the steady value of $Q_{ON}$ at 0 or +20 mV in the same fiber using Eq. 3 with $V = -33.1$ mV and $t = 13.3$ mV. Curve was drawn by eye. Temperature: 7-10°C. Fibers: 21-2-80 A (■), 22-2-80 (●), 7-3-80 (▲), and 28-4-80 (▲).}
\end{figure}

$Q_{\text{max}}$ for the experiment of Fig. 8 and for three other experiments in which $\tau_{OFF}$ was measured at -80 mV after on prespulses of varying duration at either 0 or +20 mV. The filled symbols give results for $t_{ON} \leq 300 \mu s$ at 0 mV or for $t_{ON} \leq 200 \mu s$ at +20 mV. Open symbols are for longer $t_{ON}$ values at the same potentials. They include results for on durations up to and including those giving the maximum $\tau_{OFF}$. The increase of $\tau_{OFF}$ with $Q_{ON}$ occurs over the
entire range of $Q_{ON}/Q_{max}$ studied, but is clearly most marked as $Q_{ON}$ nears $Q_{max}$. In fact, the pronounced increase in $\tau_{OFF}$ with movement of the last part of $Q_{ON}$ was probably even more striking than Fig. 9 indicates because for the open symbols $\tau_{OFF}$ was most likely underestimated to an unknown extent by the onset of charge immobilization. If all of the increase in $\tau_{OFF}$ were a direct consequence of on charge movement, the last 5–10% of $Q_{ON}$ must clearly have a much more marked effect on $\tau_{OFF}$ than the preceding 90–95%. Alternatively, if two different processes were involved, one process might be directly related to the amount of on charge movement and might give rise to the gradual phase of increasing $\tau_{OFF}$ with $Q_{ON}/Q_{max}$. A second parallel voltage- and time-dependent process, which is independent of the amount of charge moved but which alters the rate of charge movement and sets in more slowly than on charge movement, might give rise to the apparent steep phase of increasing $\tau_{OFF}$ with the last 5–10% of $Q_{ON}/Q_{max}$ (see Discussion).

**ON and OFF Charge Movement Kinetics in the Absence of Charge Immobilization**

The increase in $\tau_{OFF}$ with on pulse duration was apparently normally obscured by the onset of effects most likely associated with $I_{Na}$ inactivation and charge immobilization. It was therefore of interest to study $\tau_{OFF}$ in the absence of the inactivation process. Since iodate has been shown to remove $I_{Na}$ inactivation (Stampfli, 1974), its effect on charge movement was investigated. Fig. 10 presents results from an experiment in which on and off charge movements were monitored before and after changing the cut end solution to one in which 30 mM NaIO$_3$ replaced an equimolar amount of CsCl in the normal cut end solution. Traces on the left were obtained under control conditions and those on the right were recorded 14–24 min after changing the cut end pools to the IO$_3^-$ solution. The upper traces indicate that IO$_3^-$ treatment almost completely eliminated charge immobilization. For 1.5-ms pulses from $-100$ to 0 mV, the ratio of mean $Q_{OFF}$ to mean $Q_{ON}$ was 0.58 for five control traces and 0.99 for three traces after IO$_3^-$ treatment. IO$_3^-$ also decreased the mean value of $Q_{ON}$ for these pulses to 59% of the control value, which is indicative of general fiber deterioration. In fact, the fibers were quite unstable once iodate became effective, and many $Q$ records had to be discarded because of obvious changes in leakage current during the summing process or even during individual pulses. This made the iodate effect quite difficult to study and only two experiments were successful. The iodate treatment was, however, preferable to the use of either sea anemone (Anemone sulcata) or scorpion (Androctonus australis Hector) toxins, which slow but do not eliminate $I_{Na}$ inactivation (Bergman et al., 1976; Romey et al., 1976; Bernard et al., 1977; unpublished personal observations).

The effect of internal IO$_3^-$ on on and off charge movement time courses at $-30$ mV is shown by traces $b$ and $c$ in Fig. 10. The on time course was little affected by iodate, whereas the off was considerably slowed. This is shown more clearly in Fig. 11, which presents semilog plots of normalized average values obtained from two or three traces as in Fig. 10$ b$ or $c$. At $-30$ mV, $\tau_{ON}$ was 246 $\mu$s in control and 245 $\mu$s after iodate, whereas $\tau_{OFF}$ increased from 83 to 269 $\mu$s after iodate treatment. The interesting finding that after iodate
Figure 10. Effects of internal iodate on charge movement. The charge movement was recorded during and after pulses to 0 mV applied from the holding potential (a, on and off responses), during pulses to −30 mV applied from the holding potential (b, on response) and during pulses to −30 mV applied after 1,500-μs depolarizations to 0 mV (c, off response) in control conditions (left) and 14–24 min after replacing 30 mM CsCl by 30 mM NaIO₃ in the side pools' solution. In the presence of IO₃, the charge immobilization was almost eliminated and the off charge movement was slowed down. Temperature: 13°C. Fiber: 5-6-80 A.

Figure 11. Semilogarithmic plots of on (A) and off (B) charge movements at −30 mV in control conditions (filled circles) and during diffusion of iodate in the axoplasm (open circles). The off charge movement was recorded after 1,500-μs prepulses to 0 mV. The points are average values obtained from two or three traces. In control and iodate, respectively, τ₀ on is 246 and 245 μs, τ₀ off is 83 and 269 μs. Temperature: 13°C. Fiber: 5-6-80 A.
treatment $\tau_{OFF}$ following a relatively long ON prepulse became equal to $\tau_{ON}$ at the same potential was confirmed in the one other fiber in which the iodate effect was successfully studied. Fiber instability unfortunately prevented determination of the voltage and time dependencies of $\tau_{ON}$ and $\tau_{OFF}$ with charge immobilization eliminated by iodate.

**OFF Time Course of Sodium Current**

Data regarding the relative kinetics of charge movement and sodium conductance may prove to be important for distinguishing between alternative models for sodium channel gating. We have therefore re-examined the relationship between the off kinetics of $I_{Na}$ and $Q$, devoting special attention to the effects of on pulse duration.

In five out of a total of seven fibers in which the OFF time courses of $I_{Na}$ and $Q$ were both successfully monitored, the $I_{Na}$ tails were completely characterized by a single exponentially decaying component. Sodium tail currents obtained from one such fiber are presented in part A of Fig. 12, which gives $I_{Na}$ at OFF potentials of $-100$, $-80$, and $-60$ mV after 300-μs pulses to $+20$ mV. Semi-log plots (part B) of values measured from tracings of the records in part A show that these $I_{Na}$ tails followed single exponential time courses from 37.5 μs after pulse OFF at $-100$ or $-80$ mV and from 50 μs after pulse OFF at $-60$ mV. For comparison, records and semilog plots of $Q_{OFF}$ obtained for the same pulses in the same fiber after the addition of TTX are presented as parts C and D of Fig. 12. $Q_{OFF}$ was well described by a single exponentially decaying component from 12.5 μs after pulse OFF at each OFF voltage. In the two fibers in which $I_{Na}$ tails did not follow a single exponential decay, a second smaller and slower exponentially decaying component was also present (see also Neumcke et al., 1976; Goldman and Hanin, 1978). In these two fibers the slower $I_{Na}$ component had a time constant 3.2 or 3.1 times that of the faster component and, based on extrapolation to the start of pulse OFF, comprised only 3.9 or 9.5% of the initial OFF amplitude of $I_{Na}$. Only the time constant of the faster component of $I_{Na}$ was used for comparison with $Q_{OFF}$. Since the remainder of the results deal exclusively with OFF time constants for $I_{Na}$ and $Q$, these will simply be denoted here by $\tau_{Na}$ and $\tau_{Q}$ without any specific indication for pulse OFF.

**Voltage Dependence of OFF Time Constants for Sodium Current and Charge Movement**

Sodium current tails and OFF charge movements were recorded at several OFF membrane potentials in each of three fibers, giving the results in Fig. 13. In each fiber OFF pulses at various membrane potentials were preceded by a pulse of a set duration to a given positive potential. The OFF time constants $\tau_{Na}$ and $\tau_{Q}$ both tended to become smaller as the OFF voltage was made more negative. $\tau_{Q}$ was always equal to or slightly larger than $\tau_{Na}$. No consistent effect of $V_{OFF}$ on $\tau_{Q}/\tau_{Na}$ is apparent in the data in Fig. 13. These results are different from those obtained by Neumcke et al. (1976), who found $\tau_{Q}/\tau_{Na}$ to be $\sim 3$ for OFF
FIGURE 12. OFF time course of sodium current and charge movement. (A) Tails of sodium current recorded at different membrane potentials (−100, −80, −60 mV) after activation of the conductance by 300 μs depolarizations to +20 mV terminated at the arrows. (B) Semilogarithmic plots of values obtained from tracings of the records shown in part A. (C) OFF charge movement for the same pulses as in part A. The arrow marks pulse off; the four points before the arrow give final values of charge during the pulse. (D) Semilogarithmic plots of values obtained from tracings of the records in part C. Temperature: 8.5°C. Fiber: 20-2-80.
voltages near $-100 \text{ mV}$ in frog node of Ranvier under similar conditions. The source of this discrepancy is not clear (see Discussion).

One possible problem with comparing time courses of $I_{N_a}$ and $Q$ is that during large sodium currents, significant voltage drops may occur across any resistance in series with the membrane, causing differences between the

![Graph A]

**Figure 13.** Voltage dependence of off time constants for sodium current and charge movement. Off time constants for sodium current (open circles) and charge movement (filled circles) were determined on three different fibers (A, B, and C) during pulses to various potentials preceded by on pulses to $+30 \text{ mV}$ for 200 $\mu$s (A) or to $+20 \text{ mV}$ for 300 $\mu$s (B and C). Mean values and standard errors of several determinations at each potential (three or four for $Q$, two or three for $I_{N_a}$). Curves were drawn by eye. Temperature: 9.5°C (A), 9°C (B), 8.5°C (C). Fibers: 6-2-80 A (A), 21-2-80 (B), 20-2-80 (C).
apparent and true transmembrane potential (Drouin and Neumcke, 1974). Such series resistance artifacts should not pose any problem for gating charge measurements that involve only small currents. Fig. 14 presents a test for the effect of series resistance artifacts in measuring off time constants of \( I_{Na} \). Sodium current tails were measured in the standard control solution and then in the same solution plus 4 nM TTX, which diminished \( I_{Na} \) to \( \sim 40\% \) of control. Normalized semilog plots of Na tail currents at \(-70 \text{ mV}\) in the two solutions are presented in part A of Fig. 14 and appear to be virtually identical. Part B of Fig. 14 presents the off voltage dependence of \( \tau_{Na} \) obtained in the two solutions. At and negative to \(-70 \text{ mV}\) the off time constants were the same for control and partially depressed sodium currents, indicating no significant effect of the series resistance artifact on the determination of \( \tau_{Na} \).

![Figure 14. Test for series resistance artifact. Sodium tail currents were recorded in standard control solution (filled circles) and then in the same solution plus 4 nM TTX (open circles) during off pulses to various potentials preceded by on depolarizations to 0 mV lasting 400 \( \mu \text{s} \). (A) Semilogarithmic plot of normalized sodium tail currents recorded at \(-70 \text{ mV}\). (B) off time constants for \( I_{Na} \) as a function of off membrane potential. Curves were drawn by eye. Temperature: 8°C. Fiber: 25-2-80 A.](image)

**Effects of on Pulse Duration on off Time Constants for Sodium Current and Charge Movement**

Fig. 15 presents off time constants \( \tau_Q \) and \( \tau_{Na} \) at \(-80 \text{ mV}\) as a function of the preceding on prepulse duration \( t_{ON} \) at +20 mV. As in Fig. 8B, \( \tau_Q \) first increased as \( t_{ON} \) was increased up to \( \sim 0.7 \) to 1 ms and then decreased for longer \( t_{ON} \)’s. In contrast, \( \tau_{Na} \) increased with \( t_{ON} \) toward a maximum level, which then remained approximately constant for on durations from \( \sim 1.5 \) to 2.5 ms. The monotonic rise in \( \tau_{Na} \) with \( t_{ON} \) generally paralleled the rising phase of \( \tau_Q \). However, the declining phase of \( \tau_Q \) at longer on durations, which paralleled the fall in \( Q_{OFF} \) in this fiber as in the fiber shown in Fig. 8, was not reflected by a decline in \( \tau_{Na} \).
Mean normalized $\tau_{Na}$ values from the fiber in Fig. 15 and from two other fibers are presented in Fig. 16A, also as a function of prepulse $t_{ON}$. These data confirm that the sodium current off time constant at $-80$ mV exhibits no decline for on pulse durations longer than 1 ms. Since $\tau_Q$ declines with the development of charge immobilization, whereas $\tau_{Na}$ does not, it must be concluded that this alteration in $Q$ kinetics cannot be due to a nonspecific change in membrane properties but must be attributed specifically to the nonimmobilized gating charges of inactivated sodium channels.

The effect of $t_{ON}$ on the ratio of off time constants $\tau_Q/\tau_{Na}$ is examined for the same three fibers plus one other in Fig. 16B. For on prepulse durations up to $\sim 0.5$ ms the ratio appeared to be almost independent of $t_{ON}$, supporting the parallel rising phase of $\tau_Q$ and $\tau_{Na}$ with $t_{ON}$. Then for pulses longer than $\sim 0.5$ ms $\tau_Q/\tau_{Na}$ decreased with increasing $t_{ON}$ due to declining $\tau_Q$ and constant $\tau_{Na}$.

To highlight trends in $\tau_Q/\tau_{Na}$ as a function of $t_{ON}$, fiber to fiber variation in absolute levels of $\tau_Q/\tau_{Na}$ were minimized by using the following normalization routine for Fig. 16B: the data from each fiber were scaled so that the mean of all values of $\tau_Q/\tau_{Na}$ in that fiber for on pulse durations between 100 and 500 $\mu$s was equal to the mean of the individual means from the four fibers for the same range of pulse durations.

All values of $\tau_Q/\tau_{Na}$ obtained in these experiments for 200- or 300-$\mu$s pulses to $+20$ to $+50$ mV followed by off's at $-70$ to $-90$ mV are given in Table III. For such pulses, which should be little affected by the inactivation process, the off time constant for charge movement was $\sim 20\%$ larger than the time constant for decay of $I_{Na}$.
FIGURE 16. Effect of on pulse duration on the off time constant for sodium current and on the ratio of off time constants $\tau_{Q}/\tau_{Na}$. (A) Sodium tail currents were recorded in three different fibers at -80 mV after on depolarizations to +20 mV of various durations ($t_{ON}$). For each fiber, the off time constants were normalized to their mean values for $t_{ON}$ between 1 and 2 ms (filled circles). Temperature: 8°C. Fibers: 18-2-80, 25-4-80, 28-4-80. (B) Sodium current and charge movement were recorded in four different fibers. For each fiber, the off time constants $\tau_{Q}$ and $\tau_{Na}$ were determined at the same potential (-80 to -60 mV) after depolarizations of various durations ($t_{ON}$) to constant voltage (+20 or +30 mV) and their ratio was calculated. Ratios from different fibers were normalized as described in the text. Curve was drawn by eye. Temperature: 8–9.5°C. Fibers: 6-2-80 (●), 18-2-80 (■), 25-4-80 (▲), 28-4-80 (▼).
DISCUSSION

Comparison with Previous Observations

Generalizing from studies on squid axons, almost all of the charge movement recorded from nerve fibers appears to be involved with gating sodium channels (Yeh and Armstrong, 1978; Cahalan and Almers, 1979; Almers, 1978). Any acceptable model for sodium channel gating should therefore be capable of reproducing all properties of both sodium currents and charge movements in nerve. In an effort to further define some of these properties, present observations will be compared with previously published results.

To our knowledge, the present data concerning ON and OFF charge movement time constants at the same potential are the first such results reported in the literature. In several previous studies, $\tau_{\text{OFF}}$ was determined over a voltage range negative to about $-50$ to $-70$ mV, whereas $\tau_{\text{ON}}$ was determined at more positive voltages (Keynes and Rojas, 1974 and 1976; Bullock and Schauf, 1978). Considering the scatter in the data, a single bell-shaped $\tau$ vs. $V$ relationship appeared to be in reasonable agreement with both the $\tau_{\text{ON}}$ and $\tau_{\text{OFF}}$ values in such data sets. However, the present determinations of $\tau_{\text{ON}}$ and $\tau_{\text{OFF}}$ over an overlapping voltage range clearly show that in node of Ranvier $\tau_{\text{ON}}$ and $\tau_{\text{OFF}}$ cannot be described by the same $\tau$ vs. $V$ relationship.

A further complication with some of the previous $\tau$ vs. $V$ results might also be noted here, namely that changes in the holding potential were used as the method for altering $V_{\text{OFF}}$ (Keynes and Rojas, 1974 and 1976). Such a procedure will cause $\tau_{\text{OFF}}$ to vary due to the combined results of (a) a varying degree of partial inactivation of the sodium channel gating mechanism and (b) the direct effect of $V_{\text{OFF}}$ on $\tau_{\text{OFF}}$. Since $\tau_{\text{OFF}}$ decreases with increasing inactivation (Nonner, 1980 and above), the increase of $\tau_{\text{OFF}}$ with increasingly positive values of $V_{\text{OFF}}$ observed in such cases must have been less steep than would have been observed under the condition of constant inactivation.

The effects of ON pulse duration and amplitude on the OFF time constants for charge movement have been noted or studied in a number of reports.

\begin{table}[h]
\centering
\caption{RELATIVE OFF TIME CONSTANTS FOR CHARGE MOVEMENT AND SODIUM CURRENT}
\begin{tabular}{|c|c|c|c|c|}
\hline
Fiber & $V_{\text{ON}}$ & $\tau_{\text{ON}}$ & $V_{\text{OFF}}$ & $\tau_{\text{ON}}/\tau_{\text{OFF}}$ \\
& mV & $\mu$s & mV & \\
\hline
20-2-80 & +20 & 300 & -90 & 1.52 \\
& & & -80 & 1.39 \\
& & & -70 & 1.18 \\
21-2-80 & +20 & 300 & -80 & 1.37 \\
28-4-80 & +20 & 300 & -80 & 1.34 \\
6-2-80A & +30 & 200 & -90 & 1.05 \\
& & & -80 & 0.98 \\
& & & -70 & 1.03 \\
18-2-80 & +50 & 200 & -70 & 0.99 \\
& & & 300 & 0.95 \\
\hline
Mean±SEM & & & & 1.18±0.07 \\
\hline
\end{tabular}
\end{table}
Using pulses producing relatively little inactivation, it has previously been observed that for given on and off voltages, $\tau_{\text{OFF}}$ increases with on pulse duration in both squid axons (Armstrong and Bezanilla, 1974 [Fig. 5], 1977; Keynes and Rojas, 1974) and node of Ranvier (Nonner et al., 1978). The fact that $\tau_{\text{OFF}}$ increases with $V_{\text{ON}}$ for pulses of set duration has also been noted for both squid axons (Keynes and Rojas, 1974; Meves, 1974) and node of Ranvier (Nonner et al., 1978). Neither of these effects was detected in Myxicola axons (Schauf et al., 1977). The decline in $\tau_{\text{OFF}}$ with pulse durations that would have produced significantly increasing sodium inactivation has been previously observed in both squid axons (Fig. 2 of Armstrong and Bezanilla, 1977) and node of Ranvier (Nonner et al., 1978; Nonner, 1980), but not in Myxicola axons (Table I of Bullock and Schauf, 1979). These comparisons seem to indicate that the sodium channel gating processes may be similar in squid and frog axons, at least insofar as can be determined from gating charge movement measurements, but that both may differ from the gating process in Myxicola axons. Further comparisons of present with previous results will therefore be restricted to studies on squid or frog axons.

In the present experiments it was observed that for pulses giving relatively little sodium inactivation the mean (± SEM) ratio of off time constants $\tau_{\text{Q}}/\tau_{\text{Na}}$ was 1.18 ± 0.07 for $\text{VOFF}$ between −70 and −90 mV. This is in excellent agreement with the values of 1.18 ± 0.04 and 1.22 ± 0.03 obtained by Bezanilla and Armstrong (1975a, Table I) and by Armstrong and Bezanilla (1977, Table IV) from squid axons at $\text{VOFF} = -70$ mV. However, it is considerably less than the values of 2.55 ± 0.05, 2.32 ± 0.06, and 1.92 ± 0.15 obtained by Neumcke et al. (1976, Table III) from three frog nodes at off voltages of −90, −82, and −74 mV, respectively. Separate comparison of off time constants at voltages near −90 mV shows good agreement of the present $\tau_{\text{Q}}$ values with those reported by Neumcke et al. (1976), whereas the present $\tau_{\text{Na}}$ values tended to be two to three times larger than those of Neumcke et al. One difference in procedure that might be thought to account for the different $\tau_{\text{Na}}$ values is that the first off points used in the present $I_{\text{Na}}$ tail analyses occurred 37.5 µs after pulse off, whereas Neumcke et al. used points starting 15 µs after the pulse. Fast components of $I_{\text{Na}}$ resolved by Neumcke et al. might thus conceivably have been missed in the present analysis. However, the $I_{\text{Na}}$ tail records presented by Neumcke et al. (1976, Figs. 4B and 5B) were well described by a single exponential component from 15 to 100 µs after pulse off so that sampling starting at 15 or 37.5 µs should have resulted in similar values for $\tau_{\text{Na}}$. As a further indication that time resolution was probably not a limiting factor in the present experiments, $\tau_{\text{Na}}$ was determined to be 24 µs at −110 mV in one fiber, in which case $\tau_{\text{Q}}/\tau_{\text{Na}}$ was still only 1.39 (Fig. 13C). Since Neumcke et al. (1976) used 50-ms prepulses to the same potential as $\text{VOFF}$, their $\tau_{\text{Q}}$ values for the more positive $\text{VOFF}$ levels are probably underestimated due to the effect of inactivation. Correcting for this effect would tend to increase their $\tau_{\text{Q}}/\tau_{\text{Na}}$ values at the more positive $\text{VOFF}$ levels so that the discrepancy between our $\tau_{\text{Q}}/\tau_{\text{Na}}$ values and those of Neumcke et al. should probably apply to all off voltages. The source of this discrepancy is not
known. In the experiments of Armstrong and Bezanilla (1977), inactivation was negligible at the holding potential of -70 mV so that no correction need be applied to the $\tau_Q$ values reported by those authors.

The present observation that the ratio of off time constants $\tau_Q/\tau_{Na}$ changes minimally with increasing on pulse durations $t_{ON}$ giving little sodium inactivation confirms the observations of Armstrong and Bezanilla (1977, Table IV). In both cases there was a considerable increase in the off time constants for both $I_{Na}$ and $Q$ with $t_{ON}$, but both increased in parallel so that their ratio remained about constant.

**Kinetic Models for Sodium Channel Activation**

Previous studies of sodium and gating currents have already indicated that models for Na channel activation based on multiple identical and independent gating particles per channel are inadequate (Armstrong and Bezanilla, 1974; Armstrong, 1978; Neumcke et al., 1976). Our observations of two components of $Q_{ON}$, of unequal on and off time constants for $Q$ at a given voltage, of off $\tau_Q$ values that depend on the preceding on pulse duration and of off $\tau_Q/\tau_{Na}$ ratios of $\sim 1.2$ are also all inconsistent with such models. Armstrong and Gilly (1979) have recently proposed a simplified version of the Armstrong and Bezanilla (1977) sequential state scheme that accounts for two components of $Q_{ON}$, a rising phase in on gating current and a delay in $I_{Na}$ activation. However, our simulations with the model and parameter values given by Armstrong and Gilly (1979) indicate that the ratio of off time constants $\tau_Q/\tau_{Na}$ predicted by their model at -70 mV following 200- to 1,000-μs pulses to +50 mV varies from 0.30 to 1.13, respectively, due to increasing $\tau_Q$ and constant $\tau_{Na}$. The Armstrong-Gilly scheme thus does not appear to account for the observed approximately constant $\tau_Q/\tau_{Na}$ of $\sim 1.2$ found by Armstrong and Bezanilla (1977, Table IV) and ourselves.

The observed similarity of off time constants for $I_{Na}$ and $Q$ was one of the first indications that the unmodified Hodgkin-Huxley (1952) formalism could not account for both $I_{Na}$ and $Q$ (Armstrong and Bezanilla, 1974). It was suggested originally that this observation might be accounted for on the basis of a closed-loop scheme whereby channel deactivation proceeded by a path different from channel activation (Bezanilla and Armstrong, 1975a and b). However, Oxford (1981) recently demonstrated that a separate deactivation path is inconsistent with $I_{Na}$ time courses during multiple step pulses, and Armstrong and Bezanilla (1977) and Armstrong and Gilly (1979) eliminated the separate deactivation path from their models.

Several relatively simple modifications of independent particle models can give rise to similar off time constants for $I_{Na}$ and $Q$. Consider the model in which channel opening requires movement of three identical charged gating particles associated with the channel, with the rate constants for movement of each particle being voltage dependent but independent of the location of the other particles. This model, which corresponds exactly to the Hodgkin-Huxley (1952) equations for Na channel activation, is represented by the upper line
in the kinetic scheme A (Armstrong and Bezanilla, 1974)

\[
\begin{array}{c}
X_4 \leftrightarrow X_3 \leftrightarrow X_2 \leftrightarrow X_1 \\
3\alpha & 2\alpha & \alpha \\
\beta & 2\beta & 3\beta \\
\end{array}
\]

(A)

In this case \( X_1 \) is the conducting state and each transition contributes an equal amount of charge movement.

Now assume that once all three of the gating particles have crossed the membrane they can form a trimer \( X_1T \) that cannot cross the membrane. Such trimer formation by the \( X_1 \) to \( X_1T \) transition is represented perpendicular to the other transitions in scheme A to emphasize that it involves no charge movement but only interactions of charges that have already moved from their resting locations. In contrast to the other rate constants, \( \gamma_1 \) and \( \delta_1 \) would thus be independent of potential. If only \( X_1 \) were conducting, trimer formation would constitute opening of the channel; if both \( X_1 \) and \( X_1T \) were conducting, trimer formation would promote the open condition. Scheme A constitutes a greatly simplified version of a recent model involving four gating particles with dimer, trimer, and tetramer formation after the individual particles cross the membrane (Baumann and Easton, 1981).

If it is assumed that \( \gamma \) and \( \delta \) are both large compared with \( \alpha \) and \( \beta \), \( X_1 \) and \( X_1T \) would be in equilibrium so that \( X_1/X_1T = \delta/\gamma \). Scheme A could then be reduced to an equivalent four-state scheme under appropriate adjustment of the rate constants for the transition from \( X_1 \) to \( X_2 \). Setting \( f_1 = X_1/(X_1 + X_1T) \) in scheme A, one obtains

\[
\begin{array}{c}
X_4 \leftrightarrow X_3 \leftrightarrow X_2 \leftrightarrow X_1' \\
3\alpha & 2\alpha & \alpha \\
\beta & 2\beta & 3\beta \\
\end{array}
\]

(B)

\( X_1' \) of scheme B equals \( X_1 + X_1T \) of scheme A. The fraction of conducting channels would be \((1 - f_1)X_1'\) or \(X_1'\), depending on whether only \(X_1\) or both \(X_1\) and \(X_1T\) were conducting.

Scheme B with \( f = 0.5 \) has been used to simulate OFF \( I_{Na} \) and \( Q \) records at a potential where \( \beta = 20 \text{ ms}^{-1} \) and \( \alpha = 1 \text{ ms}^{-1} \) after ON responses of various durations at a potential where \( \alpha/\beta = 10 \). Using single exponential fits starting 30 \( \mu \text{s} \) after the start of the simulated records, the ratio of OFF time constants \( \tau_Q/\tau_{Na} \) ranged from 1.3 to 1.7 for \( Q_{ON}/Q_{max} \) values ranging from 0.38 to 0.95, respectively. These values seem acceptably close to those in Fig. 16B because inactivation, which would tend to decrease \( \tau_Q \) but not \( \tau_{Na} \) for the larger \( Q_{ON}/Q_{max} \) values (Figs. 15 and 16A), has not been considered in the simulation.

The presence of two components of ON charge movement for relatively large depolarizations (Figs. 2 and 3 and Table I) is not predicted by scheme B. This can be simulated while maintaining similar OFF \( \tau_Q \) and \( \tau_{Na} \) values by an alternative particle interaction step. Assume that once two of the three identical particles have crossed the membrane independently, they must form
a dimer before the third particle can cross. This is diagrammed in scheme C.

\[
\begin{align*}
X_4 &\xrightleftharpoons[3\alpha]{2\beta} X_3 \xrightleftharpoons[\gamma_x]{\delta_x} X_2 \\
X_2 D &\xrightleftharpoons[\alpha]{\beta} X_1
\end{align*}
\]
(C)

Assuming the dimerization step to be sufficiently rapid so as to always be at equilibrium, scheme C can be reduced to scheme D.

\[
\begin{align*}
X_4 &\xrightleftharpoons[3\alpha]{2\beta} X_3 \xrightleftharpoons[a(1-f_2)]{2\beta f_2} X_2' \xrightleftharpoons[\beta]{\beta} X_1.
\end{align*}
\]
(D)

Here \( f_2 = X_2 / (X_2 + X_2D) \) and \( X_2' = X_2 + X_2D \). Simulations with scheme D with \( f_2 = \frac{2}{3} \), \( \alpha_{ON}/\beta_{ON} = 20 \), \( \alpha_{OFF} = 20 \text{ ms}^{-1} \), and \( \alpha_{OFF} = 1 \text{ ms}^{-1} \) gave \( \tau_{ON}/\tau_{Na} \) values of 1.0–1.6 for \( Q_{ON}/Q_{max} \) values of 0.43–0.95, again acceptably close to those observed. \( Q_{ON} \) records were also simulated with scheme D with \( f_2 = \frac{2}{3} \). For \( \alpha_{ON}/\beta_{ON} \) values giving \( Q_{ON}/Q_{max} \) greater than about 0.7, such simulated \( Q_{ON} \) records were resolvable into two exponential components with the slower component carrying 46–63% of the total \( Q_{ON} \). For \( \alpha_{ON}/\beta_{ON} \) values giving \( Q_{ON}/Q_{max} \) less than about 0.7, the simulated \( Q_{ON} \) records were well described by single exponentials.

Both schemes B and D can likely provide good approximations to the general time course of \( I_{Na} \) because it has already been shown that \( I_{Na} \) can be closely reproduced using a wide variety of relative values both for individual forward rate constants and for individual reverse rate constants in the four-state model (Bezanilla and Armstrong, 1975b). Although neither scheme predicts a voltage-dependent delay before the start of \( m^a \) activation kinetics (Neumcke et al., 1976) nor a rising phase in \( ON \) gating current (Armstrong and Gilly, 1979), both observations might be accounted for by including formation of the trimer \( X_4T \) from the state \( X_4 \), where all charges are in their resting location. With rapid equilibration, this trimerization reaction would result in reducing the effective rate constants for the \( X_4 \) to \( X_3 \) transition from \( 3\alpha \) to \( 3\alpha f_4 \) in schemes B or D. \( X_4 \) and \( f_4 \) would be analogous to \( X_1 \) and \( f_1 \).

The inequality of \( \tau_{ON} \) and \( \tau_{OFF} \) at a given voltage and the steep variation of \( OFF \) time constants for \( Q \) with movement of the last 5–10% of \( Q_{ON} \) are not predicted by schemes B or D. These observations might perhaps be explained on the basis of state \( Y \) beyond \( X_1 \) and states \( X_5 \) and \( X_6 \) before \( X_4 \) (Armstrong and Bezanilla, 1977; Armstrong and Gilly, 1979). Alternatively, they might both be due to changes in apparent rate constants for transitions between states. Such changes might develop and/or decay with time after a step change in voltage. If for each transition the fractional change in forward and reverse rate constants were the same, the steady charge vs. voltage relationship would not be altered. This would be in agreement with the similar observed \( Q \) vs. \( V \) relationships but different time constants for \( Q_{ON} \) and \( Q_{OFF} \). In terms
of Eyring rate theory, identical fractional changes in forward and reverse rate constants would correspond to identical changes in energy barrier heights both for forward and reverse transitions. Starting from the general representation of any linear sequential model given by the upper line of scheme E (cf. Armstrong, 1978), a kinetic representation of this type of mechanism would be

\[
\begin{align*}
X_n \overset{\alpha_{n-1}}{\Rightarrow} X_{n-1} \overset{\alpha_{n-2}}{\Rightarrow} \cdots \overset{\alpha_2}{\Rightarrow} X_2 \overset{\alpha_1}{\Rightarrow} X_1 \\
\beta_{n-1} \quad \beta_{n-2} \quad \beta_2 \quad \beta_1 \\
\varepsilon_n \parallel \omega_n \quad \varepsilon_{n-1} \parallel \omega_{n-1} \quad \varepsilon_2 \parallel \omega_2 \quad \varepsilon_1 \parallel \omega_1 \\
\alpha'_{n-1} \quad \alpha'_{n-2} \quad \alpha'_2 \quad \alpha'_1 \\
X'_n \overset{\beta'_{n-1}}{\Rightarrow} X'_{n-1} \overset{\beta'_{n-2}}{\Rightarrow} \cdots \overset{\beta'_2}{\Rightarrow} X'_2 \overset{\beta'_1}{\Rightarrow} X'_1
\end{align*}
\]

To preserve the \( Q \) vs. \( V \) relationship, \( \alpha_i/\beta_i = \alpha'_i/\beta'_i \). This condition together with consideration of microscopic reversibility for each circular path requires that \( \varepsilon_i/\omega_i \) be the same for all values of \( i \). In the simplest case the individual rate constants \( \varepsilon_i \) and \( \omega_i \) would each be the same for all values of \( i \). Allowing voltage dependence of \( \varepsilon \) and \( \omega \), scheme E would then represent a voltage- and time-dependent transition of the overall Na channel gating mechanism between two parallel sets of states. Admitting more than one parallel set of states for a channel might decrease the number of sequential states required in each set to describe channel behavior. At present there appear to be too many free parameters to distinguish models with parallel sets of states from models having only a single but likely more complex set of sequential states.

We thank Dr. C. Bergman for stimulating discussion during the course of this work and for help in some experiments. Dr. Schneider is grateful to Dr. Bergman and Dr. P. Ascher for their hospitality during his stay at the Laboratoire de Neurobiologie, Ecole Normale Superieure, and to Drs. C. M. Armstrong and T. Begenisich for helpful discussion of kinetic models for Na channels. We thank Drs. Armstrong and W. F. Gilly for providing the computer program for their Na channel model (1979) and Dr. W. Nonner for a preprint of his paper (1980).

MFS was supported by Research Career Development Award KO5-NS00078 from the U. S. Public Health Service.

Received for publication 5 June 1981 and in revised form 5 November 1981.

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