VOLTAGE CLAMP EXPERIMENTS IN STRIATED MUSCLE FIBRES

BY R. H. ADRIAN, W. K. CHANDLER* AND A. L. HODGKIN

From the Physiological Laboratory, University of Cambridge

(Received 1 December 1969)

SUMMARY

1. Membrane currents during step depolarizations were determined by a method in which three electrodes were inserted near the end of a fibre in the frog's sartorius muscle. The theoretical basis and limitations of the method are discussed.

2. Measurements of the membrane capacity \( (C_M) \) and resting resistance \( (R_M) \) derived from the current during a step change in membrane potential are consistent with values found by other methods.

3. In fibres made mechanically inactive with hypertonic solutions (Ringer solution plus 350 mm sucrose) step depolarizations produced ionic currents which resembled those of nerve in showing \((a)\) an early transient inward current, abolished by tetrodotoxin, which reversed when the depolarization was carried beyond an internal potential of about +20 mV, \((b)\) a delayed outward current, with a linear instantaneous current–voltage relation, and a mean equilibrium potential with a normal potassium concentration (2.5 mm) of −85 mV.

4. The reversal potential for the early current appears to be consistent with the sodium equilibrium potential expected in hypertonic solutions.

5. The variation of the equilibrium potential for the delayed current \( (V'_K) \) with external potassium concentration suggests that the channel for delayed current has a ratio of potassium to sodium permeability of 30:1; this is less than the resting membrane where the ratio appears to be 100:1. \( V'_K \) corresponds well with the membrane potential at the beginning of the negative after-potential observed under similar conditions.

6. The variation of \( V'_K \) with the amount of current which has passed through the delayed channel suggests that potassium ions accumulate in a space of between \( \frac{1}{3} \) and \( \frac{1}{4} \) of the fibre volume. If potassium accumulates in the transverse tubular system (T system) much greater variation in \( V'_K \) would be expected.

* Present address: Department of Physiology, Yale University, New Haven, Connecticut, U.S.A.
7. The delayed current is not maintained but is inactivated like the early current. The inactivation is approximately exponential with a time constant of 0.5 to 1 sec at 20°C. The steady-state inactivation of the potassium current is similar to that for the sodium current, but its voltage dependence is less steep and the potential for half inactivation is 20 mV rate more positive.

8. Reconstructions of ionic currents were made in terms of the parameters \((m, n, h)\) of the Hodgkin–Huxley model for the squid axon, using constants which showed a similar dependence on voltage.

9. Propagated action potentials and conduction velocities were computed for various conditions on the assumption that the T system behaves as if it were a series resistance and capacity in parallel with surface capacity and the channels for sodium, potassium and leak current. There was reasonable agreement with observed values, the main difference being that the calculated velocities and rates of rise were somewhat less than those observed experimentally.

INTRODUCTION

The aim of this investigation is to describe the changes in ionic conductance which occur when a muscle fibre is depolarized. Since the changes occur over a range of voltages where a muscle fibre would normally be contracting vigorously, most of the experiments were carried out on muscle fibres immersed in Ringer fluid containing 350 mM sucrose, in addition to the normal electrolytes. Such solutions greatly reduce mechanical movement, but have relatively little effect on the action potential (Hodgkin & Horowicz, 1957).

The method of controlling membrane potential and measuring membrane current, which has been described briefly in preliminary communications (Adrian, Chandler & Hodgkin, 1966, 1968), depends on inserting three micro-electrodes near the end of a fibre in the frog's sartorius muscle. One micro-electrode delivers current and the other two measure membrane potential and membrane current. The method works well for delayed currents, and seems to give consistent information about inactivation of the sodium current. It fails in the presence of a large regenerative inward current and is clearly less satisfactory for investigating early currents than the sucrose-gap method described by Rougier, Vassort & Ildefonse (1968).

METHODS

Three micro-electrodes were inserted into a muscle fibre near the pelvic end of the sartorius muscle of *Rana temporaria* (Fig. 1). The micro-electrodes were of the conventional type and had resistances of 4–10 MΩ and tip potentials of less than 4 mV; the voltage electrodes were filled with 3 M-KCl and the current electrode with 2–3
m-K citrate. The current electrode was screened with a metal tube. The feed-back amplifier consisted of a pair of cathode followers feeding into the Y-amplifier of a Tektronix 502 oscilloscope; the output was taken from the cathode follower at the final stage of the amplifier. The voltage gain of the amplifier was normally 5000 but occasionally gains of 2000 or 10,000 were employed.

The voltage across the membrane was amplified in one channel of a Tektronix 502 or 565 oscilloscope. The total electrode current was recorded as a potential difference across a resistance of 2350 Ω shunted by a condenser of 0.25 μF. The condenser improved stability but rounded off the total current with a time constant of 0.6 msec. However, since it is only in the steady state that the total current bears a simple

Fig. 1. Diagram of arrangement of micro-electrodes and feed-back amplifier. The approximate equivalent circuit of the muscle fibre is shown below. The diagram illustrates the case where the electrode separation $l$ is 125 μ.

relation to membrane current, little was lost by adding the condenser. The range over which the membrane potential could be controlled was limited by the ability of the current electrode to pass large currents; the maximum current varied greatly but was sometimes as high as 6 μA.

As can be seen from Fig. 1, the voltage control was taken from the electrode nearest the end of the fibre (electrode 1 potential $V_1$). It would have been easier to control the amplifier from the voltage $V_2$ at electrode 2, and this procedure was used occasionally (see pp. 614, 617). However, when using the method normally, the voltage $V_1$ was applied to the feed-back amplifier. The reason is brought out in the theoretical section. Qualitatively one may say that $V_2 - V_1$ is proportional to the current into
the region around electrode 1, so that $V_1$ rather than $V_2$ is the appropriate control voltage.

The membrane current per unit length at electrode 1 was taken to be proportional to the potential difference $V_2 - V_1$ and calculated by eqn. (1) of the theoretical section. Choice of an appropriate electrode distance was determined by the magnitude of the currents to be recorded. If a high membrane conductance was expected, a short length, 125 $\mu$, was employed, since a short length demands less from the feedback system and improves accuracy and time-resolution. A distance of 125 $\mu$ is inappropriate if the membrane conductance is small, since $V_1$ and $V_2$ are then nearly equal and it is necessary that the recording of $V_1$ and $V_2$ should be highly differential. The differentiality of the amplifier was normally set to 0.1%, but the two electrodes could not be relied on to reject a common signal with this accuracy, since they may not seal perfectly into the membrane. Tests made with two electrodes inserted side by side at the same distance from the end of the muscle showed that the recording was differential to within about 0.5%. For a fibre of diameter 80 $\mu$, internal resistivity 250 $\Omega$ cm and membrane resistance 5000 $\Omega$ cm$^2$, a 0.5% lack of balance would introduce errors of 21, 5 or 1% for electrode separations of 250, 500 and 1000 $\mu$. In practice the lengths usually employed were 125 $\mu$ for large currents only, or 250 $\mu$ when both large and small currents were to be recorded. Table 1 gives details of some of the solutions employed. When studying delayed currents, tetrodotoxin, at a concentration of $10^{-4}$ g/ml., was added to the solution.

**Table 1. Composition of solutions**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Solution</th>
<th>K</th>
<th>Na</th>
<th>Ca</th>
<th>Cl</th>
<th>HPO$_4^{2-}$</th>
<th>H$_2$PO$_4^-$</th>
<th>SO$_4^{2-}$</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.5 K, Cl</td>
<td>2.5</td>
<td>120</td>
<td>1.8</td>
<td>121</td>
<td>2.15</td>
<td>0.85</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>2.5 K, SO$_4$</td>
<td>2.5</td>
<td>190</td>
<td>9</td>
<td>—</td>
<td>1.08</td>
<td>0.43</td>
<td>104</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>5 K, SO$_4$</td>
<td>5</td>
<td>188</td>
<td>9</td>
<td>—</td>
<td>1.08</td>
<td>0.43</td>
<td>104</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>2.5 K, Cl+S</td>
<td>2.5</td>
<td>120</td>
<td>1.8</td>
<td>121</td>
<td>2.15</td>
<td>0.85</td>
<td>—</td>
<td>350</td>
</tr>
<tr>
<td>E</td>
<td>10 K, Cl+S</td>
<td>10</td>
<td>113</td>
<td>1.8</td>
<td>121</td>
<td>2.15</td>
<td>0.85</td>
<td>—</td>
<td>350</td>
</tr>
<tr>
<td>F</td>
<td>2.5 K, SO$_4$+S</td>
<td>2.5</td>
<td>190</td>
<td>9</td>
<td>—</td>
<td>1.08</td>
<td>0.43</td>
<td>104</td>
<td>350</td>
</tr>
</tbody>
</table>

Solution A is isotonic Ringer, B is isotonic sulphate Ringer, D is hypertonic Ringer and F is hypertonic sulphate Ringer.

**Theory of the three-electrode method**

An approximate equivalent circuit of the recording arrangement is shown in Fig. 1. The end of the fibre is at $x = 0$; the recording electrodes are at $x = l$ and $x = 2l$ and the current electrode is at $x = 2l+l'$. The potentials of the points $x = 0$, $x = l$, $x = 2l$ and $x = 2l+l'$ are denoted by $V_0$, $V_1$, $V_2$ and $V_3$ respectively; $r_1$ is the resistance per unit length of the fibre. The element labelled 1 represents the membrane from $x = 0$ to $x = 3l/2$; 2 represents the membrane from $x = 3l/2$ to $x = 2l+l'/2$. The current crossing element 1 is $(V_2-V_1)/r_1 l$, and the length of the element is $3l/2$ so the membrane current per unit length $i_m(x)$ is taken as

$$i_m(l) = \frac{2(V_2-V_1)}{3l^2r_1}.$$

(1)
The expression can be made exact by introducing a correction factor defined by

\[ i_m(l) = \frac{p^2(V_2 - V_1)}{3l^2r_1}. \]  

(2)

To evaluate \( p \) we shall assume a linear cable with known values of \( r_1 \) and membrane conductance per unit length, \( g_m \). Between \( x = 0 \) and \( x = 2l + l' \) the potential is given by

\[ V = V_0 \cosh(x/\lambda) \]  

(3)

so that

\[ V_2 - V_1 = V_0 [\cosh(2l/\lambda) - \cosh(l/\lambda)], \]  

(4)

where \( \lambda = (g_m r_1)^{-\frac{1}{2}} \). Since the membrane current is given by

\[ i_m(l) = g_m V_1 \]  

(5)

\[ = g_m V_0 \cosh(l/\lambda) \]  

(6)

the factor \( p \) in eqn. (2) can be evaluated directly

\[ p = \frac{3}{2} \frac{g_m r_1 l^2}{\cosh(2l/\lambda) - \cosh(l/\lambda)} \cosh(l/\lambda) \]  

\[ = \frac{3}{2} \left( \frac{l}{\lambda} \right)^2 \frac{\cosh(l/\lambda)}{\cosh(2l/\lambda) - \cosh(l/\lambda)}. \]  

(7)

This is plotted in Fig. 2 from which it can be seen that the error is less than 5% if \( l/\lambda \) is less than 2. Eqns. (1) and (5) can be combined to give

\[ \frac{V_2 - V_1}{V_1} = \frac{3}{2} \left( \frac{l}{\lambda} \right)^2 \]  

\[ > 6 \]  

\[ \frac{V_2 - V_1}{V_1} < 6. \]  

(9)
With \( l = 125 \, \mu \) \( m \), a fibre diameter of 80 \( \mu \) and an internal resistivity of 300 \( \Omega \cdot \text{cm} \), \( l/\lambda = 2 \) corresponds to a membrane resistance of 5.9 \( \Omega \cdot \text{cm}^2 \)—roughly one-thousandth of the resting membrane resistance. In practice the membrane resistance varied between 5000 and about 20 \( \Omega \cdot \text{cm}^2 \), so that the 125 \( \mu \) spacing should be small enough to give reliable results, unless non-linearity or time-variation introduces some different kind of error. When measuring small currents or high membrane resistances, it was desirable to increase the distance between the recording electrodes, and values of 0.25, 0.5 or 1.0 mm were employed. This improved sensitivity and reduced the error introduced by lack of differentiability in the recording system.

\[ i_m = \frac{2(V_2 - V_1)}{3\rho_{\lambda} l}, \]  

where \( V_2 - V_1 \) has been calculated by cable theory for an 80 \( \mu \) fibre with \( R_i = 300 \, \Omega \cdot \text{cm} \) and \( l = 125 \, \mu \). The right hand scale gives \( V_2 - V_1 \) and the left hand scale gives \( i_m \). The two straight lines correspond to \( R_m = 1297 \, \Omega \cdot \text{cm}^2 \) and \( \lambda = 930 \, \mu \) for \( V < -40 \, \text{mV} \) and \( R_m = 126 \, \Omega \cdot \text{cm}^2 \) and \( \lambda = 290 \, \mu \) for \( V > -40 \, \text{mV} \).

Equation (1) is a less satisfactory approximation if there is a regenerative inward current. If \( g_m \) is assumed to have a fixed negative value, the expression for \( p \) is similar to eqn. (8) but \( (l/\lambda)^2 \) is negative and the \( \cosh \)’s are replaced by \( \cos \)’s. In that case \( p = 0.95 \) for \( l/|\lambda| = 0.65 \) and \( p = 0 \) when \( l/|\lambda| = 3\pi/4 \). In practice, smooth records of inward current could be obtained on either side of a range of voltages between -60 and -40 mV. Inside these limits there was often an all-or-nothing component of the inward current and the records were obviously unreliable.
In order to see how the approximate eqn. (1) worked in the non-linear region we assumed that the slope conductance of the membrane increased abruptly to a higher level at a critical potential. The theoretical distribution of potential was calculated by standard methods and values of \(2(V_2 - V_1)/3R_1\) were plotted against \(V_1\). It can be seen from Fig. 3 that the circles calculated by this method fall close to the two straight lines which represent the true current.

From eqn. (1) the membrane current density \(I_m\) in a cylindrical fibre is related to the voltage difference \(V_2 - V_1\) by

\[
I_m = \frac{a(V_2 - V_1)}{3R_1l^2},
\]

where \(a\) is the radius, and \(R_1\) is the internal resistivity. In some cases, \(R_1\) was assumed to have a value based on the unpublished results of Nakajima, i.e. \(180\ \text{Ω}\ \text{cm}\) at \(18.5^\circ\ \text{C}\) or \(303\ \text{Ω}\ \text{cm}\) at \(2^\circ\ \text{C}\) for fibres in isotonic solutions or \(400\ \text{Ω}\ \text{cm}\) at \(2^\circ\ \text{C}\) for fibres in Ringer fluid plus \(350\ \text{mm}\ \text{sucreose}\).

A more direct method of obtaining the membrane current density was to calculate \(r_1\) from measurements with small currents, for which the fibre can be regarded as a linear cable. The space constant \(\lambda\) was first calculated by eqns. (3) or (9) and \(r_1\) was then obtained from the relation between the potential \((V'_1)\) at \(x = l\) and the electrode current \(I_0\), which is applied at \(x = 2l + l'\). These quantities are related by

\[
I_0 = \frac{V'_1\cosh[(2l + l')/\lambda](1 + \tanh[(2l + l')/\lambda])}{r_1\lambda \cosh(l/\lambda)}.
\]

Since optical methods of measuring fibre diameter in whole muscle are unreliable, the most satisfactory way of obtaining the membrane current density \((I_m)\) from the membrane current per unit length \((i_m)\) is to calculate an effective diameter for a cylindrical fibre from the measured value of \(r_1\) and Nakajima's values of internal resistivity.

**RESULTS**

**Measurements of \(R_M\) and \(C_M\)**

It was important to make sure that the method gave reasonable values for the membrane resistance and capacity. The method of calculating \(\lambda\) and \(r_1\) was described in the previous section (eqns. 9 and 11); from these quantities \(r_m\) was calculated by \(r_m = r_1\lambda^2\) and \(R_M\) by \(R_M = 2\pi ar_m\) where \(a\) is the radius. Values of \(R_M\) for a number of different external solutions are given in Tables 2 and 3. These appear to be in reasonable agreement with those obtained by previous workers (Fatt & Katz, 1951).

In order to measure the membrane capacity, a series of experiments was carried out with the recording distance \(l\) equal to \(250\ \mu\) and with feed-back
control from the potential at \( x = 2l \) instead of \( x = l \) as in the standard method. The reason for making this change was that it improved the high frequency performance of the feed-back system, and avoided oscillations in the capacitative transient. In most cases the transient could be described as the sum of two exponentials. For example in fibre 58·3 in Table 3 the voltages at make were fitted by

\[
\frac{V_2 - V_1}{V_1(t = \infty)} = 0.32 \exp\left(\frac{-t}{0.17}\right) + 0.35 \exp\left(\frac{-t}{1.6}\right) + 0.028,
\]

where \( t \) is in msec. The finite rise time of \( V_2 \) accounts for the ratio being less than 1.028 when \( t = 0 \).

The membrane capacity was calculated by the following approximate method. For a membrane of capacity \( C_m \) and conductance \( g_m \) per unit length we have from eqn. (1)

\[
\frac{V(2l) - V(l, t)}{V(l, l)} = \frac{3}{2} \frac{g_m}{l r_1} \left[ V(l, t) + C_m \frac{dV(l, t)}{dt} \right],
\]

where \( V(l, t) \) and \( V(2l) \) are the potentials at \( l \) and \( 2l \) which are usually denoted by \( V_1 \) and \( V_2 \). In the steady state, eqn. (12) reduces to

\[
V(2l) - V(l, \infty) = \frac{3}{2} \frac{g_m}{l r_1} V(l, \infty).
\]

Elimination of \( g_m \) gives

\[
V(l, \infty) - V(l, t) = \frac{V(l, \infty)}{V(2l)} \frac{3}{2} \frac{g_m}{l r_1} \frac{dV(l, t)}{dt}.
\]

The current is recorded as the potential difference \( \Delta V \) where

\[
\Delta V = V(2l) - V(l, t)
\]

and the transient part of \( \Delta V \) is

\[
\Delta V' = [V(2l) - V(l, t)] - [V(2l) - V(l, \infty)]
\]

\[
= V(l, \infty) - V(l, t).
\]

Hence

\[
\Delta V' = \frac{V(l, \infty)}{V(2l)} \frac{3}{2} \frac{g_m}{l r_1} \frac{dV(l, t)}{dt}
\]

and since \( V(l, 0) = 0 \)

\[
c_m = \frac{2}{3l r_1} \frac{V(2l)}{V^2(l, \infty)} \int_0^\infty \Delta V' \, dt.
\]

The derivation is for the make of a voltage step but eqn. (19) applies equally to the break.

The same formula can be applied to any of the networks in Fig. 4 to give the total membrane capacity, for example, \( C_M' + C_1 + C_2 \ldots C_n \) in network \( d \). Values obtained by this method are given in Table 3 and may be seen to be
# Table 2. Mean values of constants in different solutions

<table>
<thead>
<tr>
<th>Row</th>
<th>Solution</th>
<th>Temperature (°C)</th>
<th>n</th>
<th>l (μ)</th>
<th>λ (mm)</th>
<th>Mean diameter (μ)</th>
<th>$R_t$ (Ω cm)</th>
<th>$R_M$ (Ω cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5 K, Cl</td>
<td>5</td>
<td>5</td>
<td>250–500</td>
<td>1.77 ± 0.02</td>
<td>56</td>
<td>169</td>
<td>3700</td>
</tr>
<tr>
<td>2</td>
<td>2.5 K, SO₄</td>
<td>3</td>
<td>8</td>
<td>250–1000</td>
<td>2.92 ± 0.27</td>
<td>78</td>
<td>360</td>
<td>15500</td>
</tr>
<tr>
<td>3</td>
<td>5 K, SO₄</td>
<td>5</td>
<td>8</td>
<td>250</td>
<td>2.53 ± 0.25</td>
<td>83</td>
<td>175</td>
<td>5000</td>
</tr>
<tr>
<td>4</td>
<td>80 K, SO₄</td>
<td>3</td>
<td>3</td>
<td>250</td>
<td>1.22 ± 0.06</td>
<td>75</td>
<td>274</td>
<td>2300</td>
</tr>
<tr>
<td>5</td>
<td>2.5 K, Cl+S</td>
<td>3</td>
<td>15</td>
<td>125–250</td>
<td>0.90 ± 0.08</td>
<td>59</td>
<td>304</td>
<td>1650</td>
</tr>
<tr>
<td>6</td>
<td>2.5 K, Cl+S</td>
<td>19</td>
<td>15</td>
<td>125–250</td>
<td>0.66 ± 0.09</td>
<td>63</td>
<td>264</td>
<td>740</td>
</tr>
<tr>
<td>7</td>
<td>2.5 K, SO₄+S</td>
<td>3</td>
<td>3</td>
<td>250</td>
<td>1.77 ± 0.30</td>
<td>65</td>
<td>214</td>
<td>3800</td>
</tr>
</tbody>
</table>

Constants were measured at the end of experiments; fibres in hypertonic solutions had been subjected to large depolarizing pulses. Variations in $R_t$ at constant temperature and tonicity may be due to errors in measuring the diameter.

# Table 3. Membrane capacities and other constants

<table>
<thead>
<tr>
<th>Fibre no.</th>
<th>$\lambda$ (mm)</th>
<th>$r_t$ (MΩ/cm)</th>
<th>$\tau$ (observed) (msec)</th>
<th>$\tau$ (calculated) (msec)</th>
<th>$\tau_s$ (msec)</th>
<th>$c_m$ (μF/cm)</th>
<th>$C_M$ (μF/cm²)</th>
<th>$R_M$ (Ω cm²)</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>58-1</td>
<td>1.8</td>
<td>3.8</td>
<td>0.81</td>
<td>0.47</td>
<td>0.34</td>
<td>0.125</td>
<td>4.2</td>
<td>3600</td>
<td>50</td>
</tr>
<tr>
<td>58-2</td>
<td>1.8</td>
<td>5.8</td>
<td>1.07</td>
<td>0.62</td>
<td>0.45</td>
<td>0.109</td>
<td>4.5</td>
<td>4400</td>
<td>75</td>
</tr>
<tr>
<td>58-3</td>
<td>1.8</td>
<td>3.7</td>
<td>1.58</td>
<td>0.69</td>
<td>0.89</td>
<td>0.189</td>
<td>6.3</td>
<td>3700</td>
<td>50</td>
</tr>
<tr>
<td>58-4</td>
<td>1.8</td>
<td>3.6</td>
<td>1.67</td>
<td>0.91</td>
<td>0.76</td>
<td>0.257</td>
<td>8.3</td>
<td>3600</td>
<td>55</td>
</tr>
<tr>
<td>58-5</td>
<td>1.7</td>
<td>18.0</td>
<td>3.57</td>
<td>3.00</td>
<td>0.57</td>
<td>0.047</td>
<td>3.4</td>
<td>7400</td>
<td>50</td>
</tr>
<tr>
<td>59-1</td>
<td>2.5</td>
<td>4.3</td>
<td>1.09</td>
<td>0.55</td>
<td>0.54</td>
<td>0.128</td>
<td>4.4</td>
<td>7600</td>
<td>95</td>
</tr>
<tr>
<td>59-2</td>
<td>2.7</td>
<td>4.6</td>
<td>1.47</td>
<td>0.72</td>
<td>0.75</td>
<td>0.156</td>
<td>5.5</td>
<td>9200</td>
<td>75</td>
</tr>
<tr>
<td>Mean</td>
<td>1.8³</td>
<td>6.3</td>
<td>1.28³</td>
<td>0.66</td>
<td>0.62</td>
<td>0.143</td>
<td>5.2</td>
<td>4500³</td>
<td>64</td>
</tr>
<tr>
<td>2.0⁴</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Muscle 58 in isotonic Ringer fluid, 5-5°C. Muscle 59 in isotonic sulphate Ringer, 3-4°C. Electrode spacing $l = 250 μ$ except in fibre 58-5 where $l = 500 μ$. Diameters in column 11 were calculated from $r_t$ and $R_t = 272 Ω \text{ cm at 5-5°C or } R_t = 290 Ω \text{ cm at 3-4°C}$. These values were used in calculating $C_M$ and $R_M$. Column 5 was calculated by eqn. (20) in text: $\tau_s = \tau$ (observed) − $\tau$ (calculated).

Notes: 1, the note book entry was ‘either 50 or 75 μ’; 2, excluding fibre 58-5 where $l = 500 μ$; 3, chloride Ringer; 4, sulphate Ringer. $c_m$ in column 7 was calculated from eqn. (19) and $\tau$ in column 5 from eqn. (20) using column 7 values for $c_m$.  

Muscle 58 in isotonic Ringer fluid, 5-5°C. Muscle 59 in isotonic sulphate Ringer, 3-4°C. Electrode spacing $l = 250 μ$ except in fibre 58-5 where $l = 500 μ$. Diameters in column 11 were calculated from $r_t$ and $R_t = 272 Ω \text{ cm at 5-5°C or } R_t = 290 Ω \text{ cm at 3-4°C}$. These values were used in calculating $C_M$ and $R_M$. Column 5 was calculated by eqn. (20) in text: $\tau_s = \tau$ (observed) − $\tau$ (calculated).

Notes: 1, the note book entry was ‘either 50 or 75 μ’; 2, excluding fibre 58-5 where $l = 500 μ$; 3, chloride Ringer; 4, sulphate Ringer. $c_m$ in column 7 was calculated from eqn. (19) and $\tau$ in column 5 from eqn. (20) using column 7 values for $c_m$.  

615
R. H. ADRIAN AND OTHERS

in reasonable agreement with those obtained by others (e.g. Katz, 1948; Fatt & Katz, 1951; Falk & Fatt, 1964).

A different method of analysing the records was to assume an equivalent circuit for the membrane and to calculate \( V(l, t) \) by cable theory. If the membrane is regarded as a resistance and capacity in parallel, \( V(l, t) \) should conform to eqn. 8, p. 135 of Carslaw & Jaeger (1959). From this formula the final time constant \( \tau_0(a) \) should be

\[
\tau_0(a) = \frac{16l^2r_1c_m}{16l^2/\lambda^2 + \pi^2}.
\]

As can be seen from Table 3 the recorded value of \( \tau_0 \) was consistently greater than predicted by eqn. (20). However, this discrepancy is to be

expected. For network \( b \) in Fig. 4, which Falk & Fatt (1964) regard as a low frequency approximation to a muscle fibre, it can be shown that the final time constant is

\[
\tau_0(b) = r_sc_m + \frac{16l^2r_1c_m}{16l^2/\lambda^2 + \pi^2}.
\]

On this basis the average values in Table 3 indicate that a total capacity of about 5 \( \mu F/cm^2 \) is charged through a series resistance of about 100 \( \Omega \) cm\(^2\). Preliminary calculations with network \( c \) indicate that the results could probably be fitted by values such as \( C_M' = 1 \mu F/cm^2 \), \( C_T = 4 \mu F/cm \), \( R_s = 150 \Omega \) cm\(^2\), but the records were not sufficiently accurate or consistent to justify an analysis along these lines.

The general conclusion from this section is that the method works reasonably well under transient conditions and that it gives values of membrane capacity and resistance which are consistent with those found by other methods.
Early ionic current

Although the method failed in the region of negative conductance it provided information about sodium currents at potentials between $-40$ and $+40$ mV. Figs. 5 and 6 show that the ionic current consists of 'sodium' and 'potassium' components of the same general type as those in nerve and that there is a fairly well defined sodium equilibrium potential. The right hand family of curves in Fig. 5 which reproduce the main features of the experimental records were calculated from equations similar to those used by Hodgkin & Huxley (1952d) with parameters given in Table 6. Fig. 7 shows early and late ionic currents as a function of membrane potential.

In five experiments with hypertonic Ringer fluid containing 350 mM sucrose and 120 mM-Na the equilibrium potential for the early currents ($V'_{Na}$) varied between 15 and 25 mV with a mean and s.e. of $20 \pm 1.8$ mV at $3^\circ$ C. Action potentials were measured on eight fibres in Ringer + 350 mM sucrose at $3^\circ$ C. The potential $V'_a$ at the crest of the spike varied between $+16$ and $+35$ mV with a mean and s.e. of $29 \pm 2.5$ mV. This is significantly greater than the estimate of the equilibrium potential, whereas one would expect a difference of a few millivolts in the opposite direction. The discrepancy may be attributed to some defect in the voltage clamp method which is suspect at times as short as 1 msec or to a difference in the two groups of fibres. Apart from any injurious effect, the hypertonic solutions should reduce $V'_{Na}$ by raising the internal concentrations of sodium and potassium. According to Chandler & Meves (1965) $V'_{Na}$ in squid axons obeys the equation

$$V'_{Na} = \frac{RT}{F} \ln \frac{[Na]_0 + b[K]_0}{[Na]_i + b[K]_i},$$  \hspace{1cm} (22)

where \( b = P_K/P_{Na} = \frac{1}{12}. \)

Since both $[Na]_i$ and $[K]_i$ should be increased about 2-4-fold in Ringer plus 350 mM sucrose, $V'_{Na}$ will be reduced by about $+20$ mV. For the purpose of reconstructing a normal action potential we have used a value of $+50$ mV in isotonic Ringer solution.

In order to obtain information about small sodium currents in the regenerative region, the method was modified by switching the feed-back control to the middle electrode, to improve stability, and increasing the electrode separations from 125 to 250 or 500 $\mu$, to increase sensitivity. In such experiments small graded inward currents were obtained up to a critical potential at which an all-or-nothing component appeared (Fig. 8). The results showed that $I_{Na}$ first becomes appreciable at about $-70$ mV and increased e-fold for a 2 mV change of $V_i$. These experiments were carried out in isotonic Ringer fluid.
Fig. 5. For legend see facing page.
Inactivation of sodium currents

Fig. 18 shows the steady-state inactivation curve determined with a voltage lasting 130 or 280 msec. The smooth curve was calculated by an equation similar to that used by Hodgkin & Huxley (1952d) which approximates to

\[ h_\infty = \frac{1}{1 + \exp\left(\frac{V - V_h}{k}\right)} \]  

(23)

with \( V_h = -70 \) mV and \( k = 5 \) mV. Hodgkin & Huxley (1952c) found \( V_h = -62 \) mV and \( k = 7 \) mV in squid axons.

The time course of inactivation on switching from \(-100 \) mV to \(-65 \) mV was approximately exponential with a time constant of about 30 msec at

Fig. 6. Currents near equilibrium potential of early current, \( l = 125 \mu \).
Fibre in Ringer + 350 mm sucrose, 1-9° C. Resting potential \(-90 \) mV. Holding potential \(-100 \) mV. Diameter 50 \( \mu \), \( R_1 = 400 \Omega \) cm, 1 mV \( \equiv 13 \) \( \mu \)A/cm².

---

Fig. 5. Left hand families. Tracings of records of the potential \( V_1 \) (above) and the voltage difference \( V_2 - V_1 \) (below) which is proportional to membrane current density. The scale for \( I_m \) depends on assuming an average fibre diameter of 59 \( \mu \) and an average \( R_1 \) of 304 \( \Omega \) cm; 0-1 mA/cm² \( \equiv 4-84 \) mV on the \( \Delta V \) records. Hypertonic Ringer fluid with 350 mm sucrose and no tetrodotoxin; \( l = 125 \mu \). Temperature 1° C; initial resting potential \(-91 \) mV. Holding potential \(-100 \) mV. Right hand families: above, potential; below, ionic currents calculated by the equations given on p. 632 with \( g_{Na} = 61 \) mmho/cm², \( \bar{g}_m = 0-042 \) msec⁻¹, \( \beta_m = 0-42 \) msec⁻¹, \( V_m = -48 \) mV, \( V'_n = +16-5 \) mV. \( g_K = 9-8 \) mmho/cm², \( \bar{g}_n = 0-00213 \) msec⁻¹, \( \beta_n = 0-009 \) msec⁻¹, \( V_n = -40 \) mV, \( V'_K = -73 \) mV. \( g_L = 0-55 \) mmho/cm². \( V_L = -100 \) mV.
Fig. 7. Early and late ionic currents as a function of membrane potential. Abscissa: membrane potential ($V_1$). Ordinate: ○ peak inward current density, ● peak outward current density. Hypertonic Ringer fluid (350 mM sucrose) without tetrodotoxin. Temperature 3° C; $I = 125 \mu$. Fibre diameter 55 $\mu$; initial resting potential (RP) – 91 mV; holding potential – 100 mV. The apparent value of $R_1$ at the end of the experiment was 140 $\Omega$ cm.

Fig. 8. A. Records of potential $V_2$ on left and membrane current ($V_2 - V_1$) on right. Electrode spacing 250 $\mu$; control on $V_2$ not on $V_1$ as in other Figs.; isotonic Ringer fluid without tetrodotoxin; resting potential = holding potential = – 100 mV; temperature 5·5° C; fibre diameter, 75 $\mu$; $r_1 = 5·8$ M$\Omega$/cm. On the membrane current records, 1 mV $\equiv 184$ nA/cm.

B. Relation between membrane current and membrane potential obtained from records such as those in A. Abscissa, membrane potential $V_1$ at 250 $\mu$ from end. Ordinate; membrane current ($V_2 - V_1$) at time of maximum inward current; 1 mV $\equiv 184$ nA/cm. The interrupted and dotted lines are estimates of leakage current and sodium current respectively. The vertical lines through the points ($\pm 0·1$ mV) are an indication of the error of measurement.
Fig. 8. For legend see facing page.
2° C. At $-100$ mV the time constant for removal of inactivation was 4–7 msec at the same temperature.

Depolarizations lasting several seconds gave a slowly developing inactivation of the kind described by Narahashi (1964). At $-100$ mV and 2° C the slow component disappeared with a time constant of about 1 sec.

**Delayed currents**

Fig. 9 shows the delayed currents at 3° C in hypertonic Ringer fluid containing tetrodotoxin, $10^{-6}$ g/ml. When the membrane was depolarized beyond $-40$ mV the outward current rose along a sigmoid curve in much the same way as it does in nerve. The right hand record, which was taken on a slower time base from a different fibre in the same muscle, shows that

![Diagram showing delayed currents](image)

**Fig. 9.** *A,* Membrane potentials, above, and currents, below, in two fibres at 2° C in Ringer + 350 mm sucrose and tetrodotoxin, $10^{-6}$ g/ml. *A,* fibre diameter 50 μ, resting potential $-97$ mV, holding potential $-97$ mV, 1 mV $\equiv 13$ μA/cm². *B,* fibre diameter 55 μ, resting potential $-89$ mV, holding potential $-100$ mV, 1 mV $\equiv 21$ μA/cm².

the outward current declined from its maximum with a time constant of about 2 sec. The decline in potassium conductance was much more rapid at room temperature, as may be seen in Fig. 15. Further details about potassium inactivation are given on p. 630.

There was considerable variation in the magnitudes of the delayed currents and in the rate at which they developed. Figs. 9 and 12 illustrate relatively rapid rates whereas Fig. 6 is an example of a slow rate. The cause of the variation is unknown but it seemed possible that prolonged immersions in hypertonic solutions might reduce the delayed currents and
possibly modify their time course. In fitting equations we have chosen experiments in which fibres gave large and rapidly developing outward currents.

The equilibrium potential of the delayed currents

The equilibrium potential was determined in the usual way by repolarizing the fibre to different levels after turning on the potassium conductance with a large initial depolarization (Fig. 10). At the end of the first rectangular pulse there is a tail of current which may be either outward or inward depending on the voltage of the second pulse. The rate at which the current decays is also dependent on voltage and increases rapidly between $-80$ and $-100$ mV (p. 630). With 2.5 mM-K the equilibrium potential in Fig. 10A was $-85$ mV. The record in Fig. 10B illustrates an equilibrium potential of $-52$ mV recorded in a different muscle immersed in 20 mM-K. Table 4 summarizes measurements of the equilibrium potential in 2-5, 10 and 20 mM-K. The mean values of the resting potential ($V_R$) and the equilibrium potential of the delayed currents ($V'_K$) are fitted by eqn. (22) if $[K]_o$ is taken as 220 mM and $P_{Na}/P_K$ is taken as 0.01 in the resting fibre and 0.03 in the delayed rectifying channel. This means that the delayed rectifying channel
Table 4. Effect of external potassium concentration on resting potential, $V_R$, and on equilibrium potential, $V'_K$, of delayed currents

**A. Experiments at 1–4°C**

<table>
<thead>
<tr>
<th>Fibre no.</th>
<th>$V_R$ (mV)</th>
<th>$V'_K$ (mV)</th>
<th>$V'_K - V_R$ (mV)</th>
<th>Mean ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1</td>
<td>97.5</td>
<td>83.5</td>
<td>14</td>
<td>6.1</td>
</tr>
<tr>
<td>8.3</td>
<td>82</td>
<td>74</td>
<td>8</td>
<td>6.4</td>
</tr>
<tr>
<td>8.4</td>
<td>94</td>
<td>87.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>11.5</td>
<td>89</td>
<td>84.5</td>
<td>4.5</td>
<td>6.6</td>
</tr>
<tr>
<td>11.9</td>
<td>90</td>
<td>80</td>
<td>10</td>
<td>7.1</td>
</tr>
<tr>
<td>12.6</td>
<td>89</td>
<td>71.4</td>
<td>17.6</td>
<td>7.3</td>
</tr>
<tr>
<td>12.7</td>
<td>96</td>
<td>72.4</td>
<td>23.6</td>
<td>7.5</td>
</tr>
<tr>
<td>14.1</td>
<td>105</td>
<td>91</td>
<td>14</td>
<td>7.6</td>
</tr>
<tr>
<td>14.2</td>
<td>102</td>
<td>93</td>
<td>9</td>
<td>7.7</td>
</tr>
<tr>
<td>14.4</td>
<td>103</td>
<td>93</td>
<td>10</td>
<td>7.7</td>
</tr>
<tr>
<td>Mean ± s.e.</td>
<td>95.4 ± 2.2</td>
<td>84.6 ± 2.8</td>
<td>10.8 ± 1.8</td>
<td>72.5 ± 1.3</td>
</tr>
<tr>
<td>V (calculated)</td>
<td>96.4</td>
<td>84.6</td>
<td>11.8</td>
<td>68.1 ± 2.0</td>
</tr>
</tbody>
</table>

**10 mm-K**

<table>
<thead>
<tr>
<th>Fibre no.</th>
<th>$V_R$ (mV)</th>
<th>$V'_K$ (mV)</th>
<th>$V'_K - V_R$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>72</td>
<td>66.5</td>
<td>5.5</td>
</tr>
<tr>
<td>6.3</td>
<td>74</td>
<td>69</td>
<td>5</td>
</tr>
<tr>
<td>6.4</td>
<td>69.5</td>
<td>65</td>
<td>4.5</td>
</tr>
<tr>
<td>7.1</td>
<td>76</td>
<td>70</td>
<td>6</td>
</tr>
<tr>
<td>7.5</td>
<td>65</td>
<td>54.5</td>
<td>10.5</td>
</tr>
<tr>
<td>7.7</td>
<td>74</td>
<td>73</td>
<td>1</td>
</tr>
<tr>
<td>7.9</td>
<td>73</td>
<td>71</td>
<td>2</td>
</tr>
<tr>
<td>7.7</td>
<td>70</td>
<td>69</td>
<td>1</td>
</tr>
<tr>
<td>7.9</td>
<td>79</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>Mean ± s.e.</td>
<td>72.5 ± 1.3</td>
<td>68.1 ± 2.0</td>
<td>4.4 ± 1.0</td>
</tr>
<tr>
<td>V (calculated)</td>
<td>70.4</td>
<td>66.1</td>
<td>4.3</td>
</tr>
</tbody>
</table>

**20 mm-K**

<table>
<thead>
<tr>
<th>Fibre no.</th>
<th>$V_R$ (mV)</th>
<th>$V'_K$ (mV)</th>
<th>$V'_K - V_R$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.2</td>
<td>48</td>
<td>47.5</td>
<td>0.5</td>
</tr>
<tr>
<td>9.3</td>
<td>58.5</td>
<td>54</td>
<td>4.5</td>
</tr>
<tr>
<td>9.4</td>
<td>58</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>9.5</td>
<td>60</td>
<td>53</td>
<td>7</td>
</tr>
<tr>
<td>Mean ± s.e.</td>
<td>56.1 ± 2.7</td>
<td>52.6 ± 1.8</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>V (calculated)</td>
<td>55.4</td>
<td>53.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

**B. Experiments at 19°C**

<table>
<thead>
<tr>
<th>Fibre no.</th>
<th>$V_R$ (mV)</th>
<th>$V'_K$ (mV)</th>
<th>$V'_K - V_R$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mm-K</td>
<td>78</td>
<td>63.3</td>
<td>14.7</td>
</tr>
<tr>
<td>78</td>
<td>97</td>
<td>81.0</td>
<td>16</td>
</tr>
<tr>
<td>Mean</td>
<td>88</td>
<td>72</td>
<td>15</td>
</tr>
</tbody>
</table>

All experiments were carried out with chloride Ringer fluid to which 350 mm sucrose had been added. Calculated values of $V$ were obtained from eqn. (22) using $b = 0.01$ for resting fibres and $b = 0.03$ for the delayed channel (equilibrium potential $V'_K$). $[K]_b$ was taken as 220 mm. The holding potential was close to $-100$ mV in A and equal to the resting potential in B. Tetrodotoxin ($10^{-6}$ g/ml.) was present in all cases except muscles 11 and 12. The values of $V'_K$ are those observed at the beginning of the experiment.
discriminates less well against sodium than the resting membrane. We have no evidence that sodium is the ion mainly responsible for the imperfect selectivity of the potassium channel but it seems the most likely candidate. The value of 220 mm for the internal potassium concentration in fibres in solutions with a tonicity 2·4 times that of Ringer is about two-thirds of that expected. The low value may indicate either a very low activity coefficient or a loss of potassium from these fibres.

The lower selectivity of the delayed rectifier provides a straightforward explanation of the characteristic after-depolarization in muscle. During the spike the potassium conductance increases and this rapidly brings the potential back to $V'_K$. The return to the resting level depends partly on the time for the potassium conductance to decline and partly on the membrane time constant. The explanation is essentially the same as that for the after-hyperpolarization of isolated squid axons. In both tissues the potential at the peak of the after-potential is close to the equilibrium potential of the delayed rectifier, but in squid axons the resting selectivity is less than that of the delayed rectifier, whereas in frog muscle it is greater. A similar explanation of the after-potential of muscle has been proposed by Persson (1963).

The potential at the beginning of the after-depolarization was consistent with this explanation. In muscle 11 in Table 4 two fibres studied with the voltage clamp gave $V'_K = -85$ and $-80$ mV. The potential at the beginning of the after-potential ($V^*$) varied between $-78$ and $-85$ mV with a mean of $-83$ mV on six fibres in the same muscle. In muscle 12 two fibres gave $V'_K = -71$ and $-72$ mV and two other fibres gave $V^* = -73$ and $-76$ mV.

The absolute value of $V'_K$ was not altered by changing the holding potential from $-110$ to $-80$ mV nor did it vary with the amplitude of the depolarizing pulse provided the duration of the pulse was less than 30 msec.

The effect of the pulse duration on $V'_K$

In squid axons the apparent potassium equilibrium potential becomes less negative as the duration of the depolarizing pulse increases (Frankenhaeuser & Hodgkin, 1956). The explanation probably is that potassium ions which move outwards during the depolarization accumulate in the space between Schwann cell and axolemma. If delayed rectification were located in the transverse tubules of muscle one might expect a similar effect to that seen in the squid axon. On the other hand if the delayed rectifying channels are mainly on the surface there should be little change in equilibrium potential, since muscle fibres have no layer of Schwann cells. It was therefore important to see whether prolonged outward currents affected the equilibrium potential.

Fig. 11 shows that changing the pulse duration from 15 to 30 msec had
no detectable effect on $V'_K$. Table 5 shows that depolarizing pulses lasting 400–500 msec did cause a clear shift in the expected direction, though the effect was not fully reversible. Column 6 indicates the change in external potassium concentration which would cause a shift equal to that observed. Column 7 gives the change in concentration expected if all the potassium ions which moved out during the depolarization were dissolved in a volume equal to that of the fibre. From eqn. (1) it follows that in a cylindrical fibre the membrane current per unit volume of muscle is

$$\frac{i_m}{\pi a^2} = \frac{2(V_2 - V_1)}{3l^2 R_1},$$  \hspace{1cm} (24)
The rise in potassium concentration in a volume equal to the fibre volume is then

\[ \Delta [K] = \frac{Q}{(F \pi a^2)} \]  

\[ = \frac{2}{3l^2 R F} \int_0^r (V_2 - V_1) \, dt. \]  

It can be seen from Table 5 that the values in column 7 for the long depolarizing pulses are \( \frac{1}{6} \) to \( \frac{1}{3} \) of those in column 6. Since the volume of the transverse tubular system is roughly \( \frac{1}{300} \) of that of the fibre (Peachey, 1965) it seems unlikely that much of the delayed current can flow through the transverse tubular membrane. Accumulation of potassium in the space between muscle fibres or in an unstirred layer would seem to be the simplest explanation of the small shifts in \( V_K' \) seen in Table 5.

### Table 5. Effect of long depolarizing pulses on equilibrium potential of delayed current, \( V_K' \)

<table>
<thead>
<tr>
<th>Fibre ref.</th>
<th>( V_R )</th>
<th>( V_p )</th>
<th>Duration</th>
<th>( V_K' )</th>
<th>( \Delta [K]_0 )</th>
<th>( Q/\pi a^2 F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>14·4</td>
<td>-103</td>
<td>-39</td>
<td>125</td>
<td>-78</td>
<td>0</td>
<td>0·33</td>
</tr>
<tr>
<td></td>
<td>-39</td>
<td>50</td>
<td>-78</td>
<td>0</td>
<td>0·09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-39</td>
<td>400</td>
<td>-68</td>
<td>4·5</td>
<td>0·75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-39</td>
<td>50</td>
<td>-73</td>
<td>2</td>
<td>0·04</td>
<td></td>
</tr>
<tr>
<td>14·7</td>
<td>-102</td>
<td>-30</td>
<td>43</td>
<td>-101</td>
<td>0</td>
<td>0·07</td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>99</td>
<td>-94</td>
<td>1·5</td>
<td>0·25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>33</td>
<td>-99</td>
<td>0·2</td>
<td>0·04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>93</td>
<td>-93</td>
<td>1·7</td>
<td>0·25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>95</td>
<td>-94</td>
<td>1·5</td>
<td>0·25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>480</td>
<td>-87</td>
<td>3·5</td>
<td>1·40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>35</td>
<td>-94</td>
<td>1·5</td>
<td>0·03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>480</td>
<td>-87</td>
<td>3·5</td>
<td>1·00</td>
<td></td>
</tr>
</tbody>
</table>

\( V_R \) is the initial resting potential which is equal to the holding potential. \( V_p \) is the potential during the depolarizing pulse. \( V_K' \) is the apparent equilibrium potential after the pulse. \( \Delta [K]_0 \) in column 6 is the increase in \([K]_0\) calculated from the change in \( V_K' \) by eqn. (23) with \( b = 0·05 \) in fibre 14·4 and 0·015 in fibre 14·7. These values of \( b \) imply \([K]_0 + b[Na]_0 = 226 \text{ mM} \) in fibre 14·4 and 300 \text{ mM} \) in fibre 14·7. \( Q/\pi a^2 F \) is the rise in \([K]_0\) expected if all the \( K^+ \) which move out during the pulse were dissolved in the fibre volume (see text); \( R \) was taken as 400 \( \Omega \) cm. Temperature 3·5° C; fibres in Ringer fluid + 350 mM sucrose with 2·5 mM-K and 121 mM-Na; tetrodotoxin, \( 10^{-6} \) g/ml. The measurements on fibre 14·4 were done some time after those in Table 4.

### Instantaneous current–voltage relation in the potassium channel

The method for measuring the instantaneous relation between current and voltage in the potassium channel was the same as that used in determining
the equilibrium potential. A family of experimental curves is shown on the left in Fig. 12 and agrees closely with the theoretical curves calculated by equations similar to those used by Hodgkin & Huxley (1952d). Tetrodotoxin was not used in this experiment and there was an inward current at the beginning of the record. Tetrodotoxin abolished this component but had no obvious effect on the currents at longer times. Measurement of an instantaneous current–voltage relation depends on extrapolating the tail current to the beginning of the second pulse. Extrapolation probably gave little error for $V > -80 \text{ mV}$, but for more negative potentials the conductance declined so rapidly that the initial current was uncertain. As can be seen from Fig. 13, the instantaneous current–voltage relation was roughly linear over the range where it could be measured satisfactorily. When the external potassium concentration was

![Fig. 12. A. Tracings of membrane potential, above, and membrane current density, below, from muscle fibre in hypertonic Ringer fluid (Ringer plus 350 mM sucrose) at 3°C. The holding potential was $-100 \text{ mV}$ and the initial resting potential was $-89 \text{ mV}$; fibre diameter 50 \( \mu \), $r_i = 10 \text{ M\Omega/cm}$; 1 mA/cm$^2 \equiv 36.4 \text{ mV}$ on the $\Delta V$ records.](image-url)

B. Ionic currents calculated for the voltage steps used experimentally by equations similar to those of Hodgkin & Huxley (1952d). The equations are given on p. 632 and in Table 6 with $\bar{g}_L = 1.47 \text{ mmho/cm}^2$, $V_L = -100 \text{ mV}$, $\bar{g}_{Na} = 60 \text{ mmho/cm}^2$, $V_{Na} = +22 \text{ mV}$, $\bar{g}_K = 19.7 \text{ mmho/cm}^2$, $V_K = -84 \text{ mV}$, $\alpha_n = 0.0044 \text{ msec}^{-1}$, $\beta_n = 0.0185 \text{ msec}^{-1}$, $V_n = -40 \text{ mV}$, $\alpha_m$, $\beta_m$, $\alpha_h$, $\beta_h$ as in Fig. 5. The relatively high $\bar{g}_L$ is consistent with the presence of some slowly declining $g_K$ (Adrian et al. 1970).
Fig. 13. A. (○) Instantaneous current–voltage relation at end of 25 msec pulse to $-3 \text{ mV}$. B. (●) ‘Steady-state’ current–voltage relation scaled by a factor $\alpha$, where $\alpha = 0.88$ and is the ratio of the current at 25 msec to the ‘steady’ current at 100 msec with $V = -3 \text{ mV}$. Experimental details as in Fig. 12. The points at $-90$ and $-100 \text{ mV}$ are uncertain.

Fig. 14. Rate constant for decline of potassium conductance on repolarizing fibre to different membrane potentials. Open circles, from the experiment of Fig. 12 with 2.5 mM K hypertonic Ringer fluid, at 3–4° C. Filled circles, from a similar experiment with 20 mM K hypertonic Ringer fluid at 1.5° C, tetrodotoxin present.
increased from 2.5 to 20 mm the instantaneous current–voltage curve was similar but shifted to the right. With 2.5 mm-K the intersection of instantaneous and steady-state curves was at about $-85$ mV and with 20 mm-K at about $-52$ mV.

**Variation of rate constant with membrane potential**

The double step experiment also gave information about the rate at which potassium conductance declines when the fibre is repolarized to different levels. Typical results for fibres at 1.5–3°C are given in Fig. 14. This curve is similar to that described for Hodgkin & Huxley (1952b) in the squid axon except that the absolute values of the rate constant in muscle are about half those in the squid axon and that the rate constant varies more steeply with potential in muscle. The rate constant at 19°C was about 5 times that at 3°C.

**Inactivation of potassium currents**

A striking difference between frog muscle and squid nerve is that the delayed current in frog muscle inactivates fairly rapidly and completely during a prolonged depolarization (Nakajima, Iwasaki & Obata, 1962; Heistracher & Hunt, 1969; Stanfield, 1970). Fig. 15 illustrates the

![Figure 15](image-url)
phenomenon at 20°C and shows that inactivation followed an exponential course with a time constant of 0.6 sec at -24 mV or 1 sec at -40 mV. Other experiments with hypertonic Ringer solutions gave values between 0.4 and 1.2 sec at +10 mV and 20°C or 1.5-3.0 sec at 3°C. Stanfield (1970) found 0.27 sec at 10 mV and 20°C in one fibre. Measurements with the two electrode method (Costantin, 1968) gave about 0.4 sec at -18 mV or 0.16 sec at +2 mV in contracting fibres in isotonic sulphate Ringer at 20°C. Fibres in isotonic 80 mM-K sulphate Ringer which were repolarized for 1-5 sec to remove some inactivation gave shorter time constants, e.g. 0.4 sec at +11 mV and 2°C or 0.2 to 0.3 at +75 mV and 2°C or 0.05 to 0.1 sec at 20°C and +60 mV. Increasing the duration of the repriming pulse sometimes increased the time constant of inactivation.

Fig. 16. Steady-state relation for inactivation of potassium current. The abscissa V is the membrane potential during the first step. The ordinate is the peak outward current during the second step relative to the same quantity when V = -100 mV. The curve is drawn according to eqn. (28) in the text.

O, fibre diameter 50 μ, 19°C; duration of first step 2.9 sec; second step to 0 mV. ● △, both on same fibre, diameter 45 μ, 21°C; duration of first step 1.9 sec, second step to -10 mV (●) or to +4 mV (△). Hypertonic Ringer plus tetrodotoxin, 10^-6 g/ml. Holding potential -100 mV.

Quantitative interpretation of records like those in Fig. 15 is complicated by observations described in a later paper (Adrian, Chandler & Hodgkin, 1970) which show that inactivation of the ordinary delayed current is masked by the development of a slow component. When this is allowed for the rate of inactivation of the fast component is found to be increased by about 70%.
An experiment with two pulses indicated that potassium inactivation is removed exponentially with a time constant of 0.7 sec at 20°C and −100 mV. When fibres equilibrated in 80 mM-K sulphate were hyperpolarized the ability to give large outward potassium currents recovered along an S-shaped curve as in Heistracher & Hunt’s (1969) experiments. It seems that recovery is exponential after a short depolarization but along an S-shaped curve if the fibre is depolarized for a long period.

The steady-state relation between membrane potential and the variable \( h_K \) which determines the fraction of the potassium system that is readily available can be determined in exactly the same ways as for the sodium current. The relation is similar to that seen with sodium currents, but is less steep and shifted about 20 mV to more positive internal potentials. The smooth curve which is a reasonable fit to the experimental points in Fig. 16 is

\[
h_K(\infty) = \frac{1}{1 + \exp\left(\frac{V + 40}{10}\right)}.
\]

(28)

Analysis in terms of \( m, h \) and \( n \)

Although the results are incomplete and subject to error at times less than 1 msec, they provide some basis for calculating the rate constants used by Hodgkin & Huxley (1952d) in their description of the membrane currents in the squid axon. As can be seen from Fig. 5, the early and late currents do not overlap to any great extent, and it was not difficult to draw curves through the two phases of the ionic current. Better estimates of the potassium current could be obtained from records at the sodium equilibrium potential, or in fibres treated with tetrodotoxin. The experimental basis for \( \tau_m \) was slender and to obtain an \( m_\infty \) curve we had to combine the data for small currents, obtained on fibres in isotonic Ringer fluid, with information near \( V'_Na \) obtained in hypertonic Ringer fluid. Since there was no basis for calculating \( \alpha_m \) and \( \beta_m \) over more than a limited range we assumed that the voltage dependence of these rate constants was as in squid axons. The data for the \( h \) and \( n \) variables are more complete and except for the limiting value of \( \beta_n \) at positive internal potentials should be reasonably free from error.

The points in Figs. 17, 18 and 19 show the relation between membrane potential and \( y_\infty \) (above) or \( r y^{-1} \) (below) where \( y \) stands for \( m, h \) or \( n \). They were obtained by assuming

\[
I_{Na} = \bar{g}_{Na} m^3 h(V - V'Na),
\]

(29)

\[
I_K = \bar{g}_K n^4 (V - V'K),
\]

(30)

\[
I_L = g_L (V - V_L)
\]

(31)
with the usual first order equation for \( m, h \) or \( n \), i.e.
\[
\dot{y} = \alpha_y (1 - y) - \beta_y (y).
\]  
(32)

Potassium inactivation and the slow changes in conductance (Adrian et al. 1970) were neglected in fitting curves to the delayed current. The curves in Figs. 17, 18 and 19 were calculated from
\[
y_\infty = \frac{\alpha_y}{\alpha_y + \beta_y},
\]  
(33)
\[
\tau_y^{-1} = \alpha_y + \beta_y
\]  
(34)

with the \( \alpha \)'s and \( \beta \)'s as given in Table 6. This table also illustrates the similarities and differences between the muscle equations and those for squid nerve. In muscle, the 'steady-state' relations for \( m \) and \( h \) are shifted 5-10 mV in the negative direction whereas the relation for \( n \) is shifted about 10 mV positive; the curves for \( h \) and \( n \) are somewhat steeper than in nerve. The absolute value of the rate constants for \( h \) and \( n \) are less than in the squid and the voltage dependence of \( \beta_n \) is greater. In general, however, the similarity between the two systems is more striking than these quantitative differences. The limiting conductances \( \bar{g}_{Na} \) and \( \bar{g}_K \) are roughly half those in squid, but permeabilities would be somewhat greater (Na) or nearly equal (K), since the ionic concentrations are lower in a frog muscle in Ringer plus 350 mm sucrose than in a squid axon in sea water.

In applying Table 6 to normal muscle fibres some adjustment must be made for the direct action of tonicity (p. 617) and for any deleterious effect associated with prolonged exposure to hypertonic solutions. A plausible guess as to likely values of \( V'_{Na} \) and \( V'_K \) in normal fibres is \( V'_{Na} = +50 \) mV and \( V'_K = -70 \) mV. The effect of temperature is uncertain but the data on potassium currents indicates that the \( Q_{10} \) of the potassium rate constants is about 2-7.

**RECONSTRUCTION OF ACTION POTENTIALS**

Column 3 in Table 6B gives the values used for reconstructing the action potential at 2° C. The values chosen are within the experimental range and in most cases are close to those in a fibre for which the analysis was most complete. For other temperatures the \( Q_{10} \) of the rate constants was assumed to be 2·5, and for \( \bar{g}_{Na} \) and \( \bar{g}_K \) it was taken as 1·5. Both temperature coefficients are somewhat arbitrary but the results of Moore (1958) and unpublished experiments of Chandler & Meves are consistent with a \( Q_{10} \) of 1·5 for the conductances in squid axons. In choosing a \( Q_{10} \) of 2·5 for the rate constants we were influenced by the fact that the effect of temperature on spike duration seems to be rather less in muscle than in squid (cf. Nastuk & Hodgkin, 1950; Hodgkin & Katz, 1949) and by the comparison
Fig. 17. Relation between membrane potential and $m_\infty$ (above) or $\tau_m^{-1}$ (below) obtained by fitting $m^3h$ to early currents. ○, Δ, fibres in hypertonic Ringer ($1^\circ$ C); ▽, mean value from three fibres in isotonic Ringer using method illustrated in Fig. 8. The smooth curves are

$$m_\infty = \frac{\alpha_m}{\alpha_m + \beta_m} \quad \text{and} \quad \tau_m^{-1} = \alpha_m + \beta_m$$

with $\alpha_m$ and $\beta_m$ as shown in Table 6. $V_m = -48$ mV and $\beta_m/\alpha_m = 10:0$ in all three sets of points; ○, $\alpha_m = 0.016$ msec$^{-1}$ and Δ, $\alpha_m = 0.042$ msec$^{-1}$. 
Fig. 18. Above, steady-state relation between $h_n$ and membrane potential in two fibres in hypertonic Ringer at 2° C. The abscissa is membrane potential, $V$, and the ordinate is the ratio of the inward current with prepulse to $V$ to the inward current with $V = -100$ mV. Holding potential = resting potential = -102 mV; duration of prepulse, 126 msec, test pulse to -21 mV. Holding potential = -76 mV, holding potential = -100 mV, duration of prepulse 280 msec, test pulse to -25 mV. Below, relation between rate constant of inactivation and membrane potential at 2° C. Same fibre as above with variable duration prepulse to give $\tau_h^{-1}$ at -65 mV or with two pulse experiment to give $\tau_h^{-1}$ at -102 mV. ○, Two pulse experiment. ○, $\triangle$, Estimates of $\tau_h^{-1}$ obtained by fitting $m^3h$ to $I_{Na}$. The smooth curves are

$$h_\infty = \frac{\alpha_h}{\alpha_h + \beta_h} \quad \text{and} \quad \tau_h^{-1} = \alpha_h + \beta_h$$

with $\alpha_h$ and $\beta_h$ as shown in Table 6. The interrupted curves give $\alpha_h$ and $\beta_h$. 
of the results of Rougier et al. (1968) with our own. In myelinated axons Frankenhaeuser & Moore (1963) found temperature coefficients ranging from $Q_{10} = 1.7$ to 3.2.

Fig. 19. For legend see facing page.
Calculations with $V_m = -44$ mV at $20^\circ$ C showed that there was a stable resting potential at $-53$ mV as well as $-95$ mV, although the potential returned to $-95$ mV after a membrane action potential. With $V_m = -42$ mV, $-95$ mV is the only stable potential.

The equivalent circuit was that proposed by Falk & Fatt (1964) and is shown in Fig. 4C. The values chosen were $C'_M$ (surface capacity) = $1 \mu F/cm^2$, $C_T$ (effective tubular capacity) = $4 \mu F/cm^2$, $R_s = 150 \Omega cm^2$. These give a total capacity ($C_M$) of $5 \mu F/cm^2$ and a time constant $\tau_n$ of 0.6 msec which are consistent with Table 3 and with the tentative values calculated by Adrian et al. (1969).

Table 6.4. Equations for rate constants in sartorius muscles and squid giant axon. Membrane potentials are in mV and are given in the sense internal potential minus external potential

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Squid giant axon</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_m$</td>
<td>$\alpha_m \frac{(V - V_m)}{1 - \exp - (V - V_m)}$</td>
<td>$\alpha_m \frac{(V - V_m)}{1 - \exp - (V - V_m)}$</td>
</tr>
<tr>
<td>$\beta_m$</td>
<td>$\beta_m \exp - (V - V_m)$</td>
<td>$\beta_m \exp - (V - V_m)$</td>
</tr>
<tr>
<td>$\alpha_n$</td>
<td>$\alpha_n \exp - \frac{(V - V_n)}{14.7}$</td>
<td>$\alpha_n \exp - \frac{(V - V_n)}{20}$</td>
</tr>
<tr>
<td>$\beta_n$</td>
<td>$\beta_n \left[1 + \exp - \frac{(V - V_n)}{7.6}\right]^{-1}$</td>
<td>$\beta_n \left[1 + \exp - \frac{(V - V_n)}{10}\right]^{-1}$</td>
</tr>
<tr>
<td>$\alpha_n$</td>
<td>$\alpha_n \frac{(V - V_n)}{1 - \exp - (V - V_n)}$</td>
<td>$\alpha_n \frac{(V - V_n)}{1 - \exp - (V - V_n)}$</td>
</tr>
<tr>
<td>$\beta_n$</td>
<td>$\beta_n \exp - \frac{(V - V_n)}{40}$</td>
<td>$\beta_n \exp - \frac{(V - V_n)}{80}$</td>
</tr>
</tbody>
</table>

Fig. 19. Above, relation between $n_\infty$ (from 'steady-state' potassium conductance) and membrane potential in one fibre at $3^\circ$ C. The experimental points (○) are from the final currents in Fig. 12; the curve was calculated.

Below, relation between rate constant $\tau_n^{-1}$ and membrane potential in same fibre. ○, Rate constants obtained by fitting eqn. (32) to the 'on' curve at different membrane potentials, e.g. $-3$ mV as shown in Fig. 12 or similar curves at other potentials. ●, Rate constants obtained by fitting eqn. (32) to the 'off' curves in Fig. 12.

The continuous curves are

$n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}$ and $\tau_n^{-1} = \alpha_n + \beta_n$

with $\alpha_n$ and $\beta_n$ as shown in Table 6 and $V'_n = -40$ mV, $\alpha_n = 0.0044$ msec$^{-1}$, $\beta_n = 0.0185$ msec$^{-1}$. The interrupted lines give $\alpha_n$ and $\beta_n$. 

TABLE 6.4. Equations for rate constants in sartorius muscles and squid giant axon. Membrane potentials are in mV and are given in the sense internal potential minus external potential
TABLE 6B. Values of constants derived from voltage clamp analysis in the frog sartorius muscle at 1-3°C and in the squid giant axon at 6.3°C. Membrane potentials are in mV and are given in the sense internal potential minus external potential. The squid constants were calculated from Hodgkin & Huxley's (1952a, d) values, on the basis that the resting potential, after correction for junction potential was -62 mV. \( \bar{\alpha}_m, \beta_m, V_m \) etc. are defined in Table 6A. Muscle constants are based on experiments in Ringer solution plus 350 mM sucrose.

<table>
<thead>
<tr>
<th></th>
<th>Muscle 1–3°C</th>
<th>Squid 6.3°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{\alpha}_m )</td>
<td>0.016, 0.042 msec(^{-1} )</td>
<td>0.04</td>
</tr>
<tr>
<td>( \beta_m )</td>
<td>0.16, 0.42 msec(^{-1} )</td>
<td>0.4</td>
</tr>
<tr>
<td>( V_m )</td>
<td>-40 to -48 mV</td>
<td>-42</td>
</tr>
<tr>
<td>( g_{Na} )</td>
<td>55 to 70 mmho/cm(^2 )</td>
<td>70</td>
</tr>
<tr>
<td>( V'_{Na} )</td>
<td>+15 to +22 mV</td>
<td>38 (50)</td>
</tr>
<tr>
<td>( V_A )</td>
<td>+16 to +35 mV</td>
<td>—</td>
</tr>
<tr>
<td>( \alpha_h )</td>
<td>0.003 msec(^{-1} )</td>
<td>0.003</td>
</tr>
<tr>
<td>( \beta_h )</td>
<td>0.65 msec(^{-1} )</td>
<td>0.65</td>
</tr>
<tr>
<td>( V_h )</td>
<td>-41 mV</td>
<td>-41</td>
</tr>
<tr>
<td>( \alpha_n )</td>
<td>0.0021 to 0.0044 msec(^{-1} )</td>
<td>0.0044</td>
</tr>
<tr>
<td>( \beta_n )</td>
<td>0.009 to 0.0185 msec(^{-1} )</td>
<td>0.0185</td>
</tr>
<tr>
<td>( V_n )</td>
<td>-40 to -45 mV</td>
<td>-40</td>
</tr>
<tr>
<td>( g_K )</td>
<td>8 to 20 mmho/cm(^2 )</td>
<td>20</td>
</tr>
<tr>
<td>( V'_{K} )</td>
<td>-70 to -85 mV</td>
<td>-78 (-70)</td>
</tr>
<tr>
<td>( g_L )</td>
<td>0.5 to 1.0 mmho/cm(^2 )</td>
<td>0.3</td>
</tr>
<tr>
<td>( V_L )</td>
<td>-95 mV</td>
<td>-95</td>
</tr>
</tbody>
</table>

Notes. \( V_A \) gives the value of the potential at the crest of the spike obtained on eight fibres (three muscles) in Ringer plus 350 mM sucrose at 3°C. The values in column 3 are those used for computing theoretical action potentials at 2°C; estimates of \( V'_{Na} \) and \( V'_{K} \) for isotonic Ringer fluid are in parentheses. The value of \( V_L \) was close to -95 but was adjusted so that the resting potential was exactly -95 mV.

The mathematical procedure was essentially similar to that used by Hodgkin & Huxley (1952d) except for the complications introduced by the tubular resistance and capacity. It was assumed that all the ionic current was carried across the surface membrane, i.e. through the element labelled \( R_m \) in Fig. 4C.

For an action potential propagating at constant velocity \( \theta \), the cable equation

\[
\frac{\partial^2 V}{\partial x^2} = r_1 i_m
\]

becomes

\[
\frac{d^2 V}{dt^2} = \theta^2 r_1 i_m,
\]

where the symbols are similar to those used on page 610. In addition, there is the network equation

\[
R_s C_T \frac{dV_T}{dt} = V - V_T
\]
where $V_T$ is the potential across $C_T$ in Fig. 4C. By defining $w$ as follows, the equations can be rearranged to give
\[
\frac{dV}{dt} = w \tag{35.1}
\]
\[
\frac{dw}{dt} = K \left[w + \frac{I_i}{C'_M} + \frac{V - V_T}{R_s C'_M}\right], \tag{35.2}
\]
\[
\frac{dV_T}{dt} = \frac{V - V_T}{R_s C_T}. \tag{35.3}
\]

The propagation constant $K$ is similar to that used by Hodgkin & Huxley (1952d) and is given by
\[
K = \frac{4R_i g_0 C'_M}{D}. \tag{36}
\]

$R_i$ is the internal resistivity and $D$ is the diameter. $I_i$ is the ionic current per cm$^2$ of surface membrane which is given by
\[
I_i = I_{Na} + I_K + I_L
\]
as defined in eqns. (29) to (34).

The digital computations, which were carried out on the computer (IBM 7094–7040 direct couple operating system) at Yale University, involved solving eqns. (35) and three equations for $m$, $h$ and $n$ of the form of eqn. (32) using a modified fourth order Runge–Kutta method as described by Fitzhugh (1966). In the initial phase of the action potential $V$ and $V_T$ rise exponentially, so that the equation for $V$ takes the form $V_R + \Delta V \exp(\mu t)$ and the equation for $V_T$ is $V_R + \Delta V_T \exp(\mu t)$. Equations (35) reduce to a cubic equation from which $\mu$ can be obtained,
\[
\mu^3 + \left(\frac{1}{R_s C_T} - K \right) \mu^2 - \left(\frac{g_0}{C'_M} + \frac{1}{R_s C'_M} + \frac{1}{R_s C_T}\right) K \mu - \frac{g_0 K}{R_s C'_M C_T} = 0 \tag{38}
\]
where $g_0$ is the resting membrane conductance. In addition, the ratio $\Delta V_T/\Delta V$ is determined by
\[
\frac{\Delta V_T}{\Delta V} = \frac{1}{1 + R_s C_T \mu}. \tag{39}
\]

In the calculations eqn. (38) has one positive and two negative real roots. The positive root gives the rate constant for the foot of the action potential whereas the smaller negative root gives the rate constant for the exponential fall at the end of the action potential. $\Delta V$ was taken as 0.1 mV and $\Delta V_T$ was calculated from eqn. (39).

As is well known in this situation the solution for $V$ goes to $+\infty$ if $K$ is chosen too high and $-\infty$ if it is too low. The programme was arranged so that a single calculation was terminated if $V > V_{Na}$ or $V < V_R$; $K$ was
interpolated between values which gave solutions diverging in opposite directions until $K$ was determined to within 2 parts in $10^6$. This procedure usually takes the action potential through about two thirds of the falling phase. For the remaining part of the action potential successive iterations using the interpolation procedure described by Fitzhugh & Antosiewicz (1959) were used.

A comparison of experimental and theoretical action potentials for a muscle at $2^\circ$ C in a hypertonic Ringer fluid is given in Fig. 20. Fig. 21 is for isotonic Ringer solution in the cold and Figs. 22 and 23 are for isotonic
Ringer at 20° C. Table 7 gives details of the computed action potentials and Table 8 compares experimental and theoretical values such as the conduction velocity and the time constant of the foot ($\tau_f$). It will be seen that

Fig. 22. A. Action potential from sartorius muscle fibre in Ringer at 21.8° C (S. Nakajima, unpublished). B. Computed action potential for isotonic Ringer at 20° C. Continuous line is $V$, dashed line is $V_T$. In this and Fig. 23B the rate constants were assumed to have a $Q_{10} = 2.5$ and $\bar{g}_{Na}$, $\bar{g}_{K}$ a $Q_{10} = 1.5$.

Fig. 23. A. Action potentials from sartorius muscle fibres in Ringer at about 20° C (Persson, 1963). B. Action potential computed as in Fig. 22B.

the theoretical curves reproduce the main features of the muscle action potential fairly successfully and in particular that the theoretical curves give a negative after-potential of approximately the right duration. The final return of the potential to the resting level is exponential with a time constant roughly equal to that of the resting membrane. The value of 17.3 msec at 20° C is in good agreement with the average value of 19.8 msec found by Persson (1963).
Table 7. Values from computations of propagated action potentials

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>V_A (mV)</th>
<th>V_max (V/sec)</th>
<th>V_min (V/sec)</th>
<th>Duration (msec)</th>
<th>K (msec⁻¹)</th>
<th>τ_i (msec)</th>
<th>C_M (foot) (µF/cm²)</th>
<th>θ (cm/sec)</th>
<th>R_i (Ω cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2*</td>
<td>21</td>
<td>42</td>
<td>-19</td>
<td>5-66</td>
<td>0-1482</td>
<td>1-551</td>
<td>3-88</td>
<td>30-4</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>56</td>
<td>-22</td>
<td>5-70</td>
<td>0-1958</td>
<td>1-252</td>
<td>3-70</td>
<td>40-2</td>
<td>303</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>75</td>
<td>-29</td>
<td>4-11</td>
<td>0-2908</td>
<td>0-928</td>
<td>3-43</td>
<td>52-2</td>
<td>267</td>
</tr>
<tr>
<td>17</td>
<td>22</td>
<td>167</td>
<td>-62</td>
<td>1-605</td>
<td>0-9205</td>
<td>0-400</td>
<td>2-60</td>
<td>110-9</td>
<td>187</td>
</tr>
<tr>
<td>20</td>
<td>21</td>
<td>209</td>
<td>-79</td>
<td>1-215</td>
<td>0-316</td>
<td>2-38</td>
<td>136-9</td>
<td>171</td>
<td></td>
</tr>
</tbody>
</table>

* Hypertonic.

The duration of the action potential in this and the next Table was taken as the period of time V > -30 mV. C_M (foot) was evaluated from the equation

\[
C_M (\text{foot}) = \frac{D}{(4R_i g_0 \tau_i)} - g_0 \tau_i,
\]

where \(g_0\) is the resting membrane conductance, \(g_0 \div \overline{g}_L\). The conduction velocity \(\theta\) was calculated from eqn. (36) using \(D\) (diameter) = 100 µ and \(R_i\) as given in the last column. These values are based on unpublished observations of S. Nakajima.

Table 8. Comparison of experimental and theoretical values for propagated action potentials

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>V_A (mV)</th>
<th>V_max (V/sec)</th>
<th>V_min (V/sec)</th>
<th>Duration (msec)</th>
<th>τ_i (msec)</th>
<th>C_M (foot) (µF/cm²)</th>
<th>D (µ)</th>
<th>R_i (Ω cm)</th>
<th>θ (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt.</td>
<td>3-5</td>
<td>38</td>
<td>81</td>
<td>-17-6</td>
<td>7-9</td>
<td>0-823</td>
<td>3-73</td>
<td>88</td>
<td>289</td>
</tr>
<tr>
<td>Theoret.</td>
<td>3-5</td>
<td>33</td>
<td>63</td>
<td>-24-1</td>
<td>5-0</td>
<td>1-119</td>
<td>3-60</td>
<td>88</td>
<td>289</td>
</tr>
<tr>
<td>Expt.</td>
<td>21-5</td>
<td>36</td>
<td>495</td>
<td>-158</td>
<td>0-92</td>
<td>0-127</td>
<td>2-56</td>
<td>94</td>
<td>163</td>
</tr>
<tr>
<td>Theoret.</td>
<td>21-5</td>
<td>20</td>
<td>232</td>
<td>-90</td>
<td>1-05</td>
<td>0-280</td>
<td>2-27</td>
<td>94</td>
<td>163</td>
</tr>
<tr>
<td>Expt. (detub.)</td>
<td>20-9</td>
<td>40</td>
<td>406</td>
<td>-180</td>
<td>0-89</td>
<td>0-131</td>
<td>0-90</td>
<td>90</td>
<td>166</td>
</tr>
<tr>
<td>Theoret. ((R_s = \infty))</td>
<td>20-9</td>
<td>43</td>
<td>441</td>
<td>-152</td>
<td>0-92</td>
<td>0-170</td>
<td>1-00</td>
<td>90</td>
<td>166</td>
</tr>
</tbody>
</table>

The experimental values and estimates of \(R_i\) are from unpublished results of S. Nakajima. The theoretical values are from computations for the same conditions. The last set of experimental values are from fibres detubulated by glycerol treatment, and the corresponding theoretical calculation was carried out with the same parameters as used previously except \(R_s = \infty\). At 20 °C the calculated ionic movements were 7-6 p.mole/cm² for Na and 5-2 p.mole/cm² for K.
The following discrepancies between the theoretical and experimental curves may be important: (1) the conduction velocity and maximum rate of rise of the theoretical action potentials is smaller than that observed experimentally; (2) the experimental action potential at 2°C C has a flatter maximum than the theoretical one; (3) the falling phase of the theoretical action potential merges rather smoothly into the negative after-potential and does not give the dip which is often seen in records from muscle. It is not clear whether these defects arise from some relatively trivial cause such as the deleterious action of hypertonic solutions or from the assumption that the ionic currents are confined to the surface membrane. Distributing the leak conductance between the tubules and the surface membrane would probably make little difference to the form of the calculated action potentials but the analysis might be seriously in error if sodium current, or some other type of active current, is present in the tubules.

We are grateful to Dr S. Nakajima for information about internal resistivity, maximum rates of rise and fall of action potential and for permission to use the unpublished records in Figs. 21 and 22. We are also indebted to Dr R. Fitzhugh for helpful discussion over the computation of action potentials.

Part of the work was supported by the United States Public Health Service Grant NB 07474 and by the National Science Foundation.

REFERENCES


22-2
R. H. ADRIAN AND OTHERS


