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THE POTASSIUM AND CHLORIDE CONDUCTANCE OF FROG MUSCLE MEMBRANE

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In 1949 Katz observed that in isotonic K_2SO_4 the conductance of the membrane of frog muscle for outward current was much less than the conductance for inward current (Katz, 1949). Since that time evidence has been accumulating that the passage of potassium across the muscle membrane is more complex than the passage of potassium across the membranes of nerves (Hodgkin & Horowicz, 1959*b*, 1960*a*; Falk & Landa, 1960*a*, *b*; Adrian, 1958, 1960; Hutter & Noble, 1960*a*). In solutions with a high potassium concentration it has been shown that the limiting conductance for an outward potassium current is a small fraction of the potassium conductance when the membrane potential is close to the potassium equilibrium potential. There is also evidence that the potassium conductance of mammalian heart muscle falls when the membrane is depolarized by an outward current (Weidmann, 1955; Hutter & Noble, 1960*b*; Carmeliet, 1961). A fall in the membrane conductance when the membrane current flows from the inside to the outside of the fibre, and rise in conductance when the current is in the opposite direction has been called *anomalous rectification*. These anomalous conductance changes are in the opposite direction to the conductance changes predicted for muscle membrane with constant potassium and chloride permeability coefficients by the constant field equations, and to *delayed rectification* (Hodgkin, Huxley & Katz, 1949) which is well known in nerve, and can be shown to occur in muscle under the appropriate conditions (Jenerick, 1959).

The object of the experiments described in this article was to separate and measure the potassium and chloride conductances of the membrane under a variety of conditions, and special attention has been given to the anomalous changes of potassium conductance.

A preliminary account of this work has appeared (Freygang & Adrian, 1961).

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METHODS

All the experiments were done on sartorius muscles of English frogs (*Rana temporaria*) at room temperature (15–20° C). The sartorius was left attached to the split pelvic bone, and all other muscles were removed to expose the ends of the fibres at their attachment to the pelvic tendon. As much as possible of the connective tissue on the deep surface of the pelvic end of the muscle was removed in order to be able to trace individual fibres for a length of 1 mm from their ends. The number of fibres for which this was possible varied considerably in individual muscles. Successful sets of measurements on more than eight fibres in a muscle were seldom achieved. The diameters of impaled fibres were measured with an eyepiece graticule, but the measurement is unreliable because adjacent fibres often overlap. Moreover, the ends of the fibres are seldom circular in cross-section.

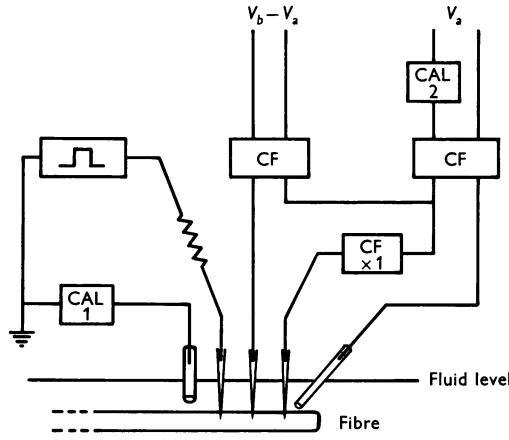

Fig. 1. Diagram of recording apparatus. The three micro-electrodes and the cathode followers connected to the recording micro-electrodes were mounted on manipulators. The positions of the electrodes were adjusted by using a graticule in the eyepiece of the microscope through which the impalements were observed.

The experimental arrangement and recording apparatus are shown diagramatically in Fig. 1. Three micro-electrodes of the Ling–Gerard type were inserted into the same fibre. The micro-electrodes were usually 445 μ apart and one of them was 445 μ from the end of the fibre. Constant current pulses of up to 1 μA were passed through the electrode farthest from the end of the fibre. This electrode was in series with either a 100 or a 1000 MΩ resistor. All three electrodes had resistances between 5 and 20 MΩ, and tip potentials of less than 5 mV. They were filled with 3 M-KCl. The two micro-electrodes which were used for recording potential were mounted on cathode-screened cathode follower probes. The two amplifiers of a Tektronix 502 double beam oscilloscope were connected to the electrodes in such a way that one amplifier recorded the potential across the membrane at the electrode nearest the end of the fibre (Trace 1) and the second amplifier recorded the difference in potential at the two recording electrodes (Trace 2). The in-phase rejection of the second amplifier and the cathode followers connected to it was tested for each experiment, and it was considered satisfactory if 100 mV, applied to both recording electrodes, produced a barely-detectable deflexion of the oscilloscope trace with the amplifier at maximum gain (200 μV/cm). The requirements for the rejection of in-phase signals were not so stringent for the channel which recorded the membrane potential, because the in-phase signal which

was produced by the current in the external fluid and in the earthing electrode was a small fraction of the electrotonic potential.

The impalement procedure was as follows: (1) both traces were coincided at a fixed vertical position on the tube face, and the amplifiers set at the same gain (10 or 20 mV/cm). (2) The three electrodes were manoeuvred into their proper positions just above a selected fibre. (3) The electrode nearest the end of the fibre was inserted. The potential difference across the membrane caused both traces to move in the same direction. Trace 1 was returned to its initial position by backing off the membrane potential with calibrator 2 (Fig. 1). The reading of this calibrator, whose output had been adjusted to take account of the amplification of the cathode followers gave the resting potential of the fibre. (4) The second recording electrode was inserted. Trace 2 then took up a position within 1 or 2 mV of trace 1. The insertion of the second electrode usually reduced the membrane potential by a few mV. The impalements were regarded as unsatisfactory if the reduction was greater than 5 mV. A further check on the quality of the first two impalements was provided by the change in note of a loudspeaker driven by an oscillator whose frequency was controlled by the potential difference between the two recording electrodes. (5) Repetitive current pulses were started and the third electrode was lowered towards the fibre. Its penetration into the fibre was signalled by the appearance of an electrotonic potential on trace 1. If all three electrodes had been correctly inserted into the same fibre, trace 1 showed a much larger potential change during a pulse of current than trace 2. The amplification of trace 2 was increased and the trace was moved to a convenient position on the tube face. Photographs were taken for a series of hyperpolarizing and depolarizing currents of varying sizes. The method is severely limited by the presence of thresholds for a twitch and for contracture, both of which dislodge the electrode and tear holes in the membrane (Fig. 4). Large depolarizations are therefore impossible under circumstances where the muscle is excitable, or if inexcitable, mechanically non-refractory (Hodgkin & Horowicz, 1960*b*).

Solutions

The solutions used in these experiments were based on the solutions described by Hodgkin & Horowicz (1959*b*). Choline and SO_4^{2-} were substituted for Na^+ and Cl^- either separately or simultaneously, in order to make the membrane electrically inexcitable and to separate the contributions of K^+ and Cl^- to the membrane conductance.

Solutions A–F, Table 1. Solution A is a Ringer's fluid of normal composition and solutions B–F are isotonic with A. B is a K-free choline chloride Ringer's fluid, and in solutions C–F Na^+ or Cl^- have been replaced by choline and SO_4^{2-}. Two variants of the choline sulphate Ringer's fluid are shown, one of which contains sucrose. Solutions C–F were made up with potassium phosphate rather than sodium phosphate as buffer. The concentrations of acid and alkaline phosphates were calculated to give a potassium concentration of 2·5 mM and a pH of 7·2. The pH of the solutions were checked with bromthymol blue.

Solutions G–I, Table 1. These solutions are derived from solutions C and E by the addition or omission of $CaCl_2$ or $CaSO_4$. Since the solutions containing SO_4^{2-} are nearly saturated with $CaSO_4$ in order to maintain the ionized Ca^{2+} close to the normal level (Hodgkin & Horowicz, 1959*b*), it is impossible to obtain a high-calcium solution when SO_4^{2-} replaces Cl^-.

Solutions J–L, Table 1. These solutions have a potassium concentration of 100 mM. Solution J can be considered as a choline chloride Ringer's fluid (solution C) to which 97·5 m-mole/l. of solid KCl has been added. Solutions K and L contain neither Na^+ nor Cl^-. K is isotonic with J, and L is isotonic with normal Ringer's fluid.

Preparation of choline sulphate. The properties of choline sulphate have been described by Renshaw (1910). A solution of choline was prepared by treating choline chloride in solution with an excess of moist AgO. After filtration the choline was neutralized with H_2SO_4 and the solution was evaporated almost to dryness over a water-bath at 50–60° C by applying suction with a water-pump. The residue was dissolved in absolute alcohol and

a white crystalline precipitate was obtained by adding petroleum ether and cooling. The highly deliquescent crystals were stored in a desiccator over H_2SO_4. Addition of $AgNO_3$ or NaCl in the presence of HNO_3 to a solution of the choline sulphate gave no detectable precipitate, but it seemed possible that there still might be sufficient silver ions present to poison the muscle. To test this possibility a physiological solution (E, Table 1) was made up with the choline sulphate, and two pairs of muscles were soaked in it for one hour. One of each pair was removed from this solution for Na^+ and K^+ analysis, and the remaining two muscles were soaked for a further hour in a normal Ringer's fluid before being analysed. The excitability of the two muscles returned within 1 min in Ringer's fluid, and the differences between the intracellular Na and K concentrations and the dry to wet weight ratios of each pair of muscles was not greater than would be expected from individual variation.

TABLE 1. Composition of solutions

	mg ion/l. solution									
Solution	K^+	Na^+	Choline$^+$	Ca^{2+}	Cl^-	SO_4^{2-}	HPO_4^{2-}	$H_2PO_4^-$	Sucrose mM	Rel tonicity
A	2·5	120	—	1·8	121	—	2·15	0·85	—	1
B	—	5	115	1·8	118·6	—	2·15	0·85	—	1
C	2·5	—	115	1·8	118·6	—	1·04	0·42	—	1
D	2·5	80	—	8·0	—	48	1·04	0·42	113	1
E	2·5	—	80	8·0	—	48	1·04	0·42	113	1
F	2·5	—	154	8·4	—	85·4	1·04	0·42	—	1
G	2·5	0·52	91	18	127	—	0·22	0·08	—	1
H	2·5	—	120	—	120	—	1·04	0·42	—	1
I	2·5	—	80	—	—	40	1·04	0·42	120	1
J	100	—	115	1·8	216	—	1·04	0·42	—	1·77
K	100	—	95	6·3	—	102·3	1·04	0·42	165	1·77
L	100	—	56·5	8·4	—	85·4	1·04	0·42	—	1

THEORY

The purpose of this section is to show how measurements of electrotonic potential at two places near the end of a fibre can be used to estimate the membrane conductance. The fibre is assumed to have a uniform cable-like structure with a conducting core surrounded by a membrane. The resistance of the membrane is not necessarily assumed to obey Ohm's Law. The end of the fibre is defined mathematically by saying that at the end the internal longitudinal current is zero. The fibre is assumed to be in a large volume of conducting fluid so that the potential of the outer surface of the membrane with respect to a distant point can be considered to be zero. The geometry of the theoretical system is shown in Fig. 2.

Definitions

x is the distance from the end of the fibre. The end of the fibre is at $x = 0$.

V_m is the change in potential across the membrane which results from the passage of current.

V_0 is the change in potential at the end of the fibre.

V_a is the change in potential at $x = a$.

V_b is the change in potential at $x = b$; $b = 2a$.

i_m is the membrane current in A cm^{-1}.
i_0 and i_b are the membrane currents produced by the voltages V_0 and V_b.
r_i is the resistance of the inside of the fibre in Ω cm^{-1}.
g_0 is the chord conductance of the membrane for the voltage V_0 in mho/cm; $g_0 = i_0/V_0$.
G is the chord conductance of the membrane in mho/cm^2.
$G_0 = g_0/2\pi\rho$.
ρ is the radius of the fibre.

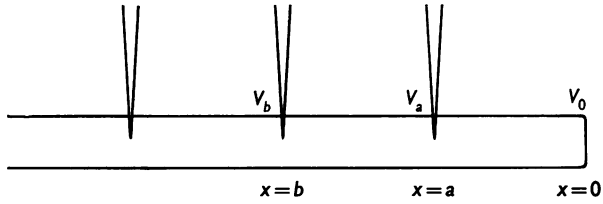

Fig. 2. Diagram of fibre with electrodes in position. Current is passed through the left-hand electrode and alters the membrane potential by V_a at $x = a$, and by V_b at $x = b$. V_a and the difference between V_b and V_a are the measured quantities.

Considering only the part of the fibre between $x = 0$ and $x = b$, if b is small, the difference between V_0 and V_b will be small. If g_s is the slope of the line joining the points (V_0, i_0) and (V_b, i_b) on the current–voltage relation of the membrane then in the limit when V_0 approaches V_b, g_s becomes the slope conductance of the membrane at V_0 in mho/cm. When $\partial V_m/\partial t = 0$,

$$\frac{1}{r_i}\frac{\mathrm{d}^2V_m}{\mathrm{d}x^2} = V_0 g_0 + (V_m - V_0)\, g_s. \tag{1}$$

The solution for equation (1) for the boundary conditions

$$V_m = V_0 \quad \text{and} \quad \frac{\mathrm{d}V_m}{\mathrm{d}x} = 0, \quad \text{at} \quad x = 0$$

is

$$V_m = \frac{V_0}{g_s}\{g_s - g_0(1 - \cosh x\sqrt{r_i g_s})\}. \tag{2}$$

When $g_s = g_0$, which corresponds to a constant chord conductance between V_0 and V_b,

$$V_m = V_0 \cosh x\sqrt{r_i g_0}. \tag{3}$$

If the change in membrane current is very small between V_0 and V_b, $x\sqrt{r_i g_s} \ll 1$

$$V_m = V_0(1 + \tfrac{1}{2} r_i g_0 x^2).$$

This relation can also be obtained from equation (1) by setting $g_s = 0$ and integrating twice with respect to x.

Let $\sqrt{(1/r_i g_0)} = \alpha$ and $\sqrt{(1/r_i g_s)} = \beta$. α and β have the dimension of length and are analogous to the length constant (λ) of a fibre determined by the exponential decline of the electrotonic potential in a region far from the end of the fibre.

The quantities measured experimentally are V_a and $(V_b - V_a)$. The ratio of these two quantities depends on the distances (a and b) of the two electrodes from the end of the fibre and the conductances:
from equation (1)

$$\frac{V_b - V_a}{V_a} = \frac{\cosh b/\beta - \cosh a/\beta}{(g_s/g_0) - 1 + \cosh a/\beta}, \tag{4}$$

when $g_s = g_0$

$$\frac{V_b - V_a}{V_a} = \frac{\cosh b/\alpha - \cosh a/\alpha}{\cosh a/\alpha}, \tag{5}$$

and as g_s approaches zero

$$\frac{V_b - V_a}{V_a} = \frac{b^2 - a^2}{2\alpha^2 + a^2}. \tag{6}$$

In Fig. 3 the values of $(V_b - V_a)/V_a$ from equations (4), (5), and (6) are plotted against $r_i g_0$. The values of a and b were .445 and 890μ, respectively. If b is less than α, and the membrane obeys Ohm's Law ($g_s = g_0$), $(V_b - V_a)/V_a$ % is very nearly directly proportional to $r_i g_0$. The three other curves of Fig. 3 show the relation between $r_i g_0$ and $(V_b - V_a)/V_a$ % for values of $r_i g_s$ equal to $+1$ mm^{-2}, 0 mm^{-2}, and -1 mm^{-2}. For small deviations of potential from the resting level the chord conductance will equal the slope conductance, and the assumption of a constant membrane conductance (equation (5)) will be valid. For large deviations of the potential from the resting level, if $(V_b - V_a)/V_a$ is less than 20 %, the chord conductance calculated by equation (5) will not differ from the true chord conductance by more than about 7 % so long as $r_i g_s$ is between 0 and $+1$ mm^{-2}. For values of $(V_b - V_a)/V_a$ less than 10 %, variation of $r_i g_s$ from 0 to -1 mm^{-2} makes the chord conductance calculated by equation (5) differ from the true value by up to 10 %.

Assuming that r_i is constant $(V_b - V_a)/V_a$ % gives a measure of the chord conductance at V_a. Strictly it measures the chord conductance at the value of V_0 corresponding to V_a, but in the majority of experiments a is much smaller than α so that V_a will not differ from V_0 by more than a few mV. Since $(V_b - V_a)/V_a$ is very nearly directly proportional to the chord conductance of the membrane, and relatively independent of the slope conductance of the membrane, the experimental results are presented in terms of $(V_b - V_a)/V_a$ %. Where conductances in absolute units are required, they have been calculated by equation (5) assuming a fibre diameter of 80μ and an internal specific resistance of 250 Ω cm (Katz, 1948).

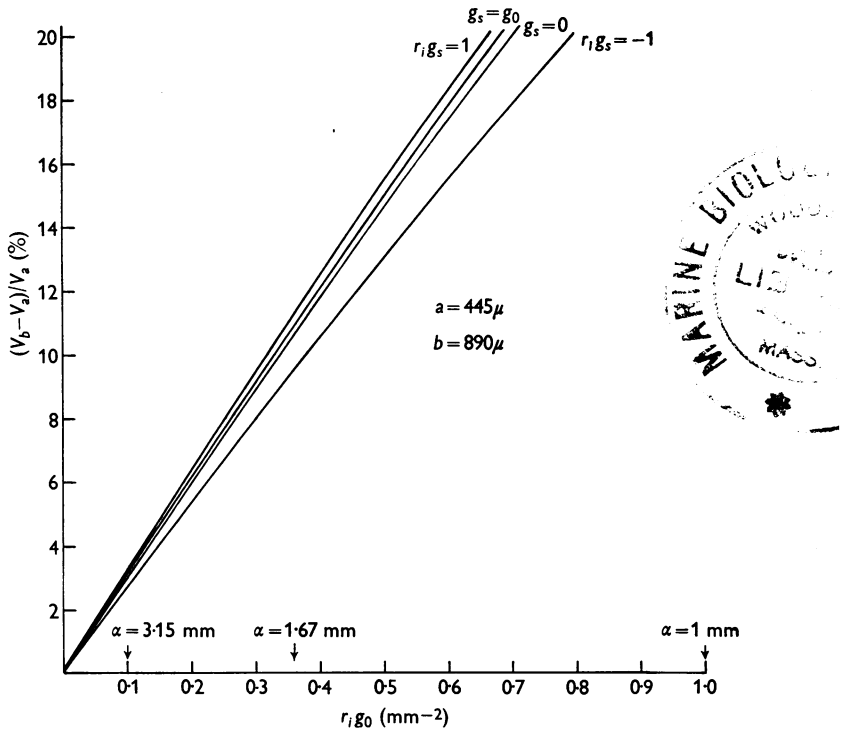

Fig. 3. Calculated values of $(V_b-V_a)/V_a$ % plotted against $r_i g_0 = (1/\alpha^2)$. The curves were calculated from equations (5), (6), and (7) making various assumptions about the value of g_s, the slope of the line joining the points (V_0, i_0) and (V_b, i_b) on the current–voltage relation of the membrane. $a = 445\,\mu$ and $b = 890\,\mu$.

RESULTS

The results will be presented in two groups: (1) for muscles in solutions with a normal potassium concentration (2·5 mM), and (2) for muscles in high potassium solutions (100 mM).

Solutions with normal potassium concentrations

Membrane potential. Table 2 gives the average membrane potential of sartorius muscle fibres in four different solutions with a potassium concentration of 2·5 mM. Replacement of sodium by choline and chloride by sulphate, either separately or simultaneously, makes no difference to the measured membrane potential. The readings of membrane potential which were averaged in Table 2 were taken after penetration with only one electrode, and the number of fibres is therefore greater than the number of successful measurements of conductance. If the difference between the

membrane potential and the potassium equilibrium potential is due to a small permeability to sodium, replacement of sodium by choline should produce a hyperpolarization if the membrane were strictly impermeable to choline. The results suggest that choline has approximately the same permeability as sodium in the resting membrane (Renkin, 1961). Alternatively the similarity of membrane potential may be fortuitous, because the effect of an imperfect seal round the tip of the electrode will be greater when the membrane resistance is large.

TABLE 2. Membrane potentials in solutions with normal potassium concentration (2·5 mM)

Major constituent of Ringer's fluid	Internal potential (mV)	Fibres	Muscles
NaCl (A)	$-89{\cdot}2 \pm 0{\cdot}8$	30	6
Na_2SO_4 (D)	$-89{\cdot}8 \pm 0{\cdot}6$	42	5
Choline chloride (C)	$-91{\cdot}1 \pm 0{\cdot}5$	41	6
Choline sulphate (E)	$-90{\cdot}0 \pm 0{\cdot}9$	31	5

± S.E. of mean.

Muscles had been in the solution for at least ½ hr before measurement of membrane potential.

Conductance changes with hyperpolarizing currents. When a hyperpolarizing current pulse is passed into a fibre near its end, the most obvious feature of the resulting electrotonic potential is that a steady level is only reached after about half a second. The same slow change in potential is seen when records are made in the middle of a fibre. At the start of the current there is a rapid rise in potential which corresponds to the charging of the membrane capacity, but thereafter there is a much slower increase in the electrotonic potential sometimes accompanied by a small decrease in the difference of potential at electrodes *a* and *b* ($V_b - V_a$). Examples of this type of hyperpolarizing record are shown in Figs. 4, 5 and 6. The slow potential rise occurs when sodium and chloride are replaced by choline and sulphate, either singly or together, but not in the absence of external potassium (Figs. 4, 5). When sulphate replaces chloride the slow change is more obvious and continues for longer. When the current stops the potential returns to its initial level more or less exponentially if the external solution contains chloride, but when sulphate replaces chloride there is a residual hyperpolarization of several mV which takes about 1 sec to disappear. If a second hyperpolarizing pulse of the same size follows the first pulse after a short interval, the potential rises rapidly to the level it had reached at the end of the first pulse. As the interval between the pulses increases, the initial potential produced by the second pulse decreases and a slow rise to the final level becomes more obvious. If the second pulse is smaller than the first pulse, then the potential during the

second pulse may either rise or fall to the same steady level depending on the interval between the pulses. When the interval is brief the potential during the second pulse falls, but it rises if the interval is increased. The records shown in Fig. 6, which illustrate this behaviour, were obtained from fibres in solution D (Table 1), a Ringer's fluid in which the chloride is replaced by sulphate.

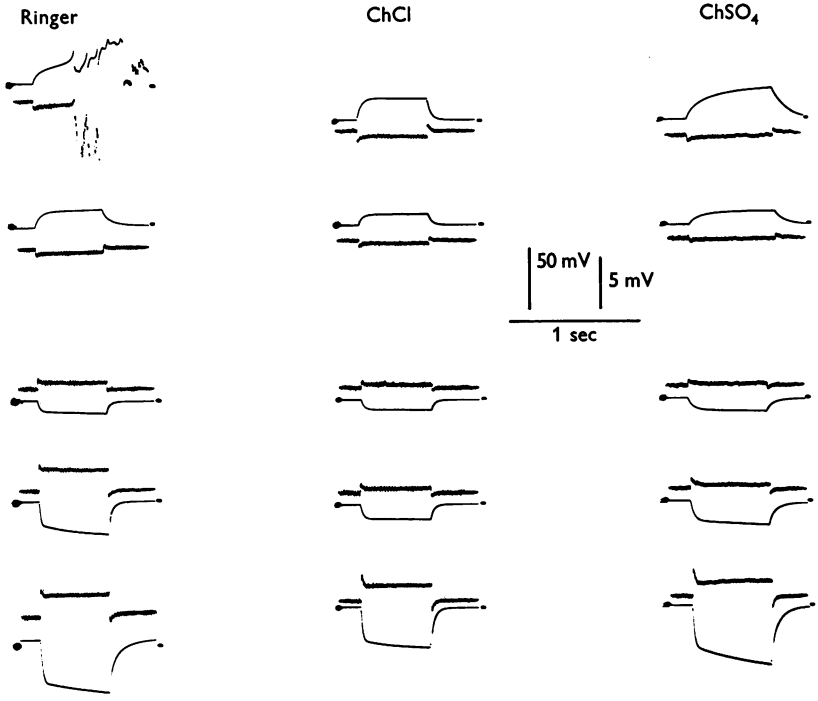

Fig. 4. Records of V_a and $(V_b - V_a)$ for depolarizations and hyperpolarizations. The three sets of records were from fibres of different muscles. The three solutions were Ringer's fluid, a solution without sodium, and a solution without either sodium or chloride. All three solutions (A, C and E, Table 1) had a potassium concentration of 2·5 mM. The top left-hand record is included to show the effect of a twitch, and was the last record obtained from that particular fibre.

The experimental records in this Fig. and in Figs. 5, 6, 12, and 13 are arranged so that a change in the membrane potential at $x = a$ moves both traces in the same direction. Since the potential change at $x = b$ is always greater than at $x = a$, the two traces, V_a and $(V_b - V_a)$, will move in opposite directions. For depolarizations V_a moves in an upward direction, and to avoid crossing, the $(V_b - V_a)$ traces, which are thickened by noise, are positioned below the V_a trace for depolarizations and above it for hyperpolarizations. When the current in the membrane capacity is negligible, the membrane resistance is very nearly directly proportional to the ratio of the deflexions of the two traces from their base lines. The voltage calibrations are shown by the two vertical lines above the 1 sec line. The larger amplification applies to the $(V_b - V_a)$ trace.

It was shown in the theoretical section that the fraction $(V_b - V_a)/V_a$ is constant if the membrane resistance and the internal longitudinal resistance are constant. It was also shown that under the conditions of these experiments $(V_b - V_a)/V_a$ is very nearly proportional to the chord conductance at V_a. The results will therefore be expressed as $(V_b - V_a)/V_a$ %, which will give a measure of the relative conductance of an individual fibre at different times or voltages. When $(V_b - V_a)/V_a$ % is plotted against time for experiments of the kind illustrated in Fig. 6, it becomes apparent that the conductance of the membrane depends on the length of the current pulse,

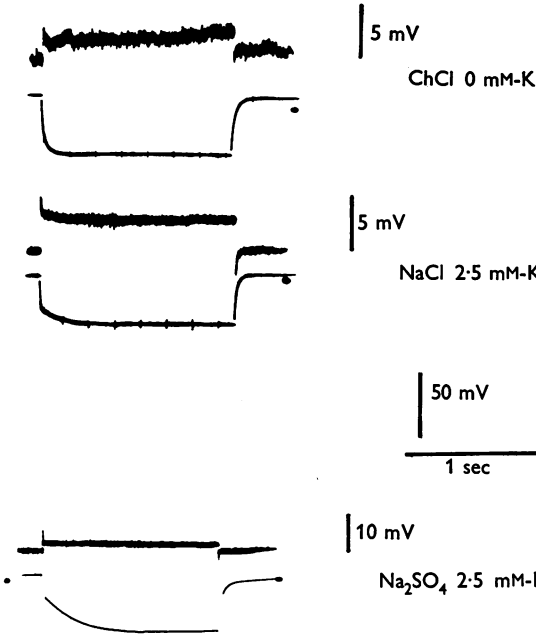

Fig. 5. Records to show that a slow increase of V_a does not take place in the absence of external potassium. In the presence of external chloride the slow change of V_a is diminished but not abolished.

on the time elapsed since a previous current pulse, and upon the membrane potential. Figure 7 shows, by means of a second pulse equal in size to the first, that the change underlying the conductance decrease during the passage of the first hyperpolarizing current persists for about half a second after that current has ceased. In Fig. 8, where the second current pulse is smaller than the first, although the final conductance levels for both pulses are nearly the same, the conductance at the beginning of the second pulse is altered and may either rise or fall during the second pulse depending on the interval between the pulses. (The experiment shown in

Figs. 6, 7 and 8 were from the same fibre.) In Fig. 9 the size of the first pulse was altered and the second pulse held constant. The size of the first pulse altered the way in which the conductance changed with time during the second pulse, but did not affect the final conductance level. At the beginning of the initial pulses the greater the current the larger was the conductance, but the final conductance levels reached a maximum and then declined as the current was increased. The relation of conductance

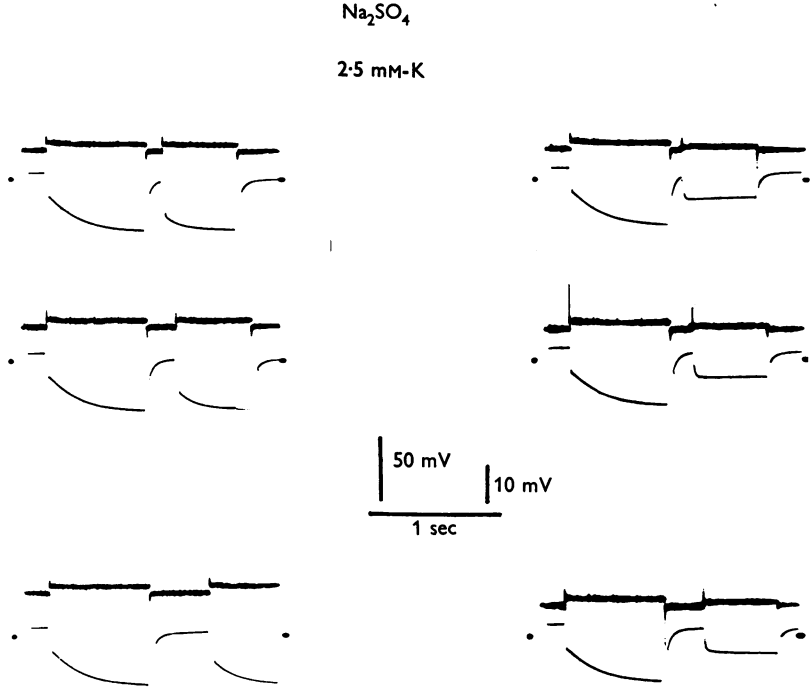

Fig. 6. Records of V_a and $(V_b - V_a)$ for hyperpolarizations by two consecutive pulses. The records are all from the same fibre which was in a chloride-free solution with a potassium concentration of 2·5 mM (solution D, Table 1). The size of the two current pulses was equal in the left-hand set of records. In the right-hand set of records the second pulse was smaller than the first.

to voltage at the beginning and end of a long hyperpolarizing pulse is more clearly shown in Fig. 10. The presence of a maximum in the final conductance levels is not invariable, but with this reservation Fig. 10 is typical of the results from a large number of fibres in Ringer's fluids made with either Na_2SO_4 or with choline sulphate (solutions D, E and F, Table 1).

These rather complex findings may be partially explained by supposing that the passage of a current alters some variable which affects the conductance. For each current strength this variable reaches a particular

value, but only after the current has been passed for a considerable time (0·5–1 sec); its return to the resting level when the current is stopped takes a comparable time. A more detailed hypothesis along these lines is given below (p. 85).

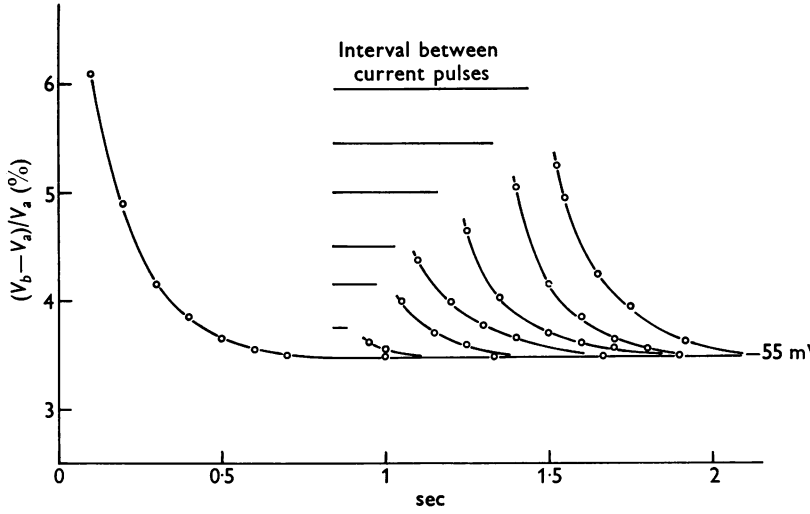

Fig. 7. The relation between $(V_b - V_a)/V_a$ % and time for the experiment which gave the records in the left-hand column of Fig. 6. For an uninterrupted current pulse of more than 2 sec duration the apparent conductance falls during the first 0·8 sec and then remains steady. If the pulse is ended when the steady level has been reached and then turned on again after increasing intervals, the curves show that the conductance, after falling during the first pulse, takes some time to recover to its initial level. Measurements of the experimental records were not made during the first 100 msec of each pulse because the current in the membrane capacity is large during that time. (Compare calculated curves in Fig. 18.)

In general, the photographic records were not measured during the initial 100 msec of any current pulse, in order to allow time for the charge on the membrane capacity to change. In some experiments, particularly for depolarization in solutions where the only ion capable of carrying current through the membrane was potassium, the membrane resistance was so large that the time constant was greater than 100 msec and measurements could only be made at 500 msec. For hyperpolarizations at times longer than 100 msec the membrane potential is changing slowly and there will be some displacement current across the membrane capacity. If this were large it would invalidate the calculation of relative membrane conductance which is based on the assumption of a steady membrane voltage. An approximate calculation of the relative magnitudes of the ionic and capacity currents can be made in the following way. If the potential produced by the current is 40 mV and the membrane capacity and resistance are 5μF/cm^2 and 10,000 Ω cm^2, then if the rate of change of potential is 20 mV/sec the capacity current will be 2·5 % of the total current, an amount not large enough to affect the estimate of membrane conductance seriously.

Figure 11 shows the results of two experiments on fibres in a normal Ringer's fluid. $(V_b - V_a)/V_a$ % is plotted against V_a and in both fibres at short

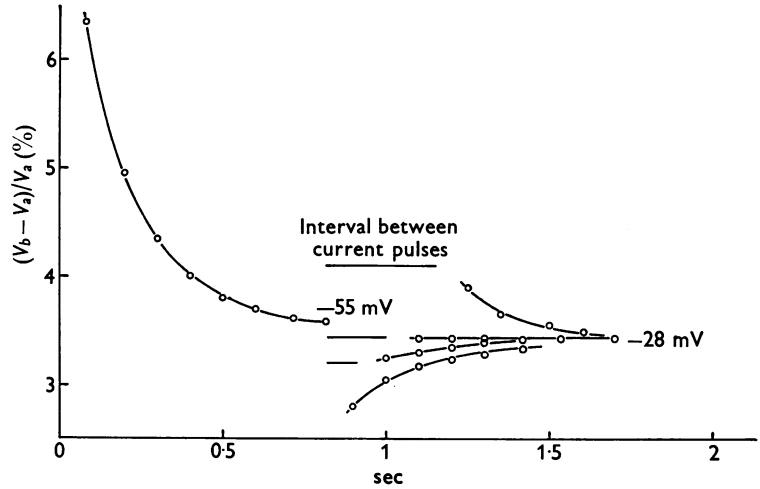

Fig. 8. The relation between $(V_b - V_a)/V_a$% and time for the experiment which gave the records in the right-hand column of Fig. 6. Two current pulses were chosen so that the final value of V_a for the second pulse was about half the final value of V_a for the first pulse. The interval between the pulses was varied between nothing and about 0·5 sec. The change in the apparent conductance during the first pulse was the same on each occasion, but the change during the second pulse depended on the time elapsed since the end of the first pulse, though the final conductance level at the end of the second pulse was always the same. (Compare calculated curves in Fig. 19.)

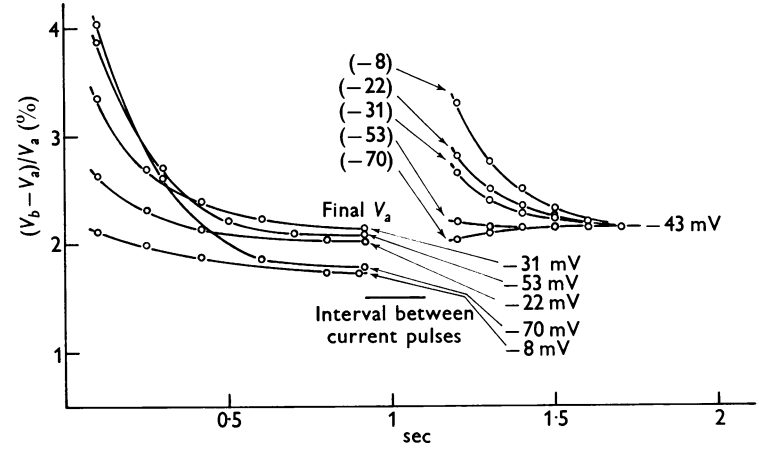

Fig. 9. The relation between $(V_b - V_a)/V_a$ % and time for an experiment where the first of two current pulses was altered in size. The second current pulse, which followed the first after a fixed interval was kept constant. The value of V_a at the end of each of the initial current pulses is shown against the appropriate curve. The figures in brackets against the right-hand set of curves indicate which of the curves during the second current pulse corresponds to each of the curves for the first pulse. (Compare the calculated curves in Fig. 20.)

times the conductance increased with increasing hyperpolarization, though for a particular value of the hyperpolarizing current the conductance fell with time. For one of the fibres two points for depolarization are included but the conductance is likely to be seriously underestimated because of subthreshold local responses. In one of the fibres the electrode spacing was half that normally used, but though $(V_b - V_a)/V_a$ % is smaller (as would be expected) it varies with V_a in the same way in both fibres. The values

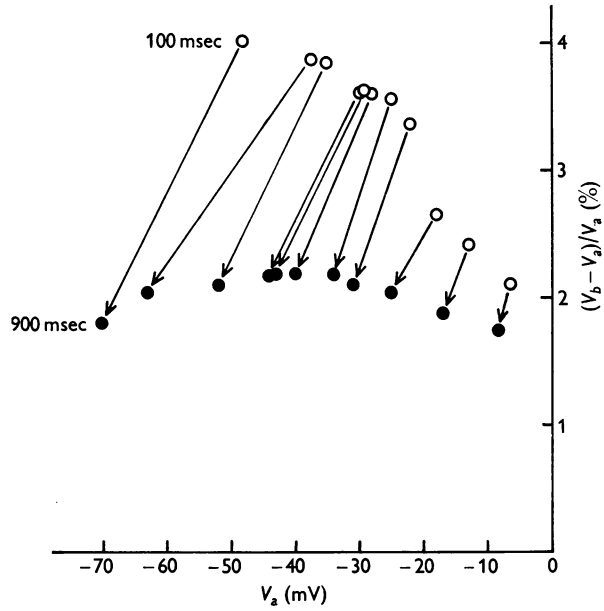

Fig. 10. The relation between $(V_b - V_a)/V_a$ % and V_a, at 100 and 900 msec from the beginning of constant current pulses. The lines join points obtained from the same record of a hyperpolarization with a current pulse of a particular strength. (The points in this figure are used in Fig. 17 for a comparison with curves calculated for a model system.) The fibre was in a chloride-free solution (solution D, Table 1), with a potassium concentration of 2·5 mM, and the electrode spacing was such that $b = 890\,\mu$.

of α $(1/\sqrt{r_i g_0})$ for a hyperpolarization of 10 mV at 100 msec are 1·7 mm for the fibre with half-normal electrode separation, and 2·0 mm for the other fibre. The mean value of α from six fibres in Ringer's fluid ($b = 890\mu$) for a 10 mV hyperpolarization at 100 msec was $2{\cdot}2 \pm 0{\cdot}15$ mm (S.E. of mean). This value compares well with values of λ obtained from the decline of the electrotonic potential in a region far from the end of a fibre. Using intracellular electrodes, Fatt & Katz (1951) obtained a mean value of 2·4 mm and Adrian (1960) reported a mean value of 2·0 mm. The mean value of α for a hyperpolarization of 10 mV at 100 msec in a Ringer's

fluid with the sodium replaced by choline was $2{\cdot}2 \pm 0{\cdot}11$ mm (twenty fibres, from five muscles in solution C, Table 1). Replacement of sodium by choline should not affect the membrane conductance if all the current through the resting membrane is carried by potassium and chloride.

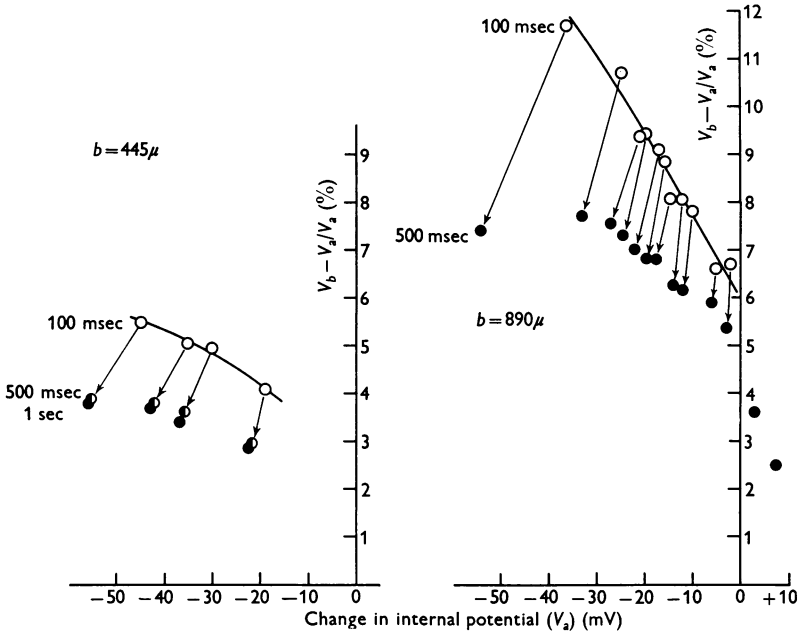

Fig. 11. The relation between $(V_b - V_a)/V_a$ % and V_a for two fibres in a normal Ringer's solution (A, Table 1). In one of the fibres the electrode spacing was halved ($b = 445\,\mu$). The changes of $(V_b - V_a)/V_a$ % with time and with V_a are similar to those shown in Fig. 10 for a fibre in a chloride-free solution with the same potassium concentration (2·5 mM). The values of $(V_b - V_a)/V_a$ % for the fibre in Ringer's solution and $b = 890\,\mu$ are larger than for the fibre in Fig. 10 which had the same electrode spacing.

In order to compare the results from different muscles in different solutions it was necessary to adopt a standard procedure for the extraction of information from the photographic records. The records were projected, traced on to graph paper and measured at different times against a calibration grid. The values of $(V_b - V_a)/V_a$ % were plotted against V_a and a smooth curve drawn through the points by eye as in Fig. 11. The deviation of the points from the line was sometimes rather large, particularly for small changes in membrane potential, because the signal-to-noise ratio of one of the traces was often uncomfortably small. Values of $(V_b - V_a)/V_a$ % for specified changes of membrane potential (V_a) were then read from the curve for each fibre. Table 3 gives the mean values from the fibres of each

muscle in four different solutions at up to five values of V_a. The solutions were Ringer's fluid, and three similar solutions, one without sodium, one without chloride, and one without either sodium or chloride (solutions A, C, D and E, Table 1). The mean values show considerable scatter for muscles in the same solution. Within each muscle the scatter for the values of individual fibres at a given voltage is less. Nevertheless, it is clear that in the absence of chloride the values are considerably reduced (see below).

TABLE 3. Mean values of $(V_b - V_a)/V_a$ % for muscles in various solutions

V_a (mV) ...	+30	+20	+10	−10	−40	No. of fibres
Ringer's solution (2·5 mM-K, 120 mM-Cl, 120 mM-Na)						
$b = 445\,\mu$	—	—	—	3·5	5·0	3
				1·7	3·1	4
$b = 890\,\mu$	—	—	—	7·9	13·2	3
				5·1	9·1	3*
Choline chloride Ringer's solution (2·5 mM-K, 120 mM-Cl, 0 mM-Na)						
$b = 890\,\mu$	—	7·5	9·6	13·3	15·6	7
	3·2	2·5	3·3	5·3	8·2	3
	3·2	2·4	2·4	3·1	4·6	3†
	—	5·7	5·1	6·3	8·8	3
	—	5·2	4·8	6·7	8·4	4
Na_2SO_4 Ringer's solution (2·5 mM-K, 0 mM-Cl, 80 mM-Na)						
$b = 890\,\mu$	—	—	—	2·5	5·0	4*
				2·2	3·9	1
Choline sulphate Ringer's solution (2·5 mM-K, 0 mM-Cl, 0 mM-Na)						
$b = 890\,\mu$	2·5	0·8	1·4	4·5	9·2	3
	1·6	0·7	1·2	3·8	7·4	4
	0·6	0·5	0·7	1·7	3·8	3†
	1·0	0·6	0·8	1·5	3·2	3‡

Hyperpolarizations measured at 100 msec. Depolarizations measured at 500 msec.

*† Fibres from same muscle.

‡ Fibres from this muscle were also used in a 100 mM-K solution (see p. 83).

In all four solutions the value of $(V_b - V_a)/V_a$ %, which can be considered to be proportional to the membrane conductance, increases with increasing hyperpolarization, and in the absence of chloride it is almost twice as large for a hyperpolarization of 40 mV as for a hyperpolarization of 10 mV. The figures for hyperpolarization were obtained from measurement at 100 msec from the beginning of the current pulse.

Conductance changes with depolarizing currents. In Table 3 no values for depolarizations in the two sodium-containing solutions are given, because subthreshold local responses would make them meaningless as measures of conductance. But in the absence of external sodium the membrane conductance is less for a depolarization of 10 mV than for a hyperpolarization of 10 mV. The lowest membrane conductance occurs at depolarizations of about 20 mV, and for greater depolarizations the conductance

rises. The values for depolarization were obtained from measurements at 500 msec in both the sodium-free solutions so that a direct comparison may be made. In the choline sulphate solution (E, Table 1) the conductance was so low that the time constant of the membrane was greater than 100 msec. In the two sodium-free solutions the muscle fibres are inexcitable, but the presence of a threshold depolarization for the production of contracture prevented the use of depolarizations much greater than 30 mV. This was unfortunate as we were unable to discover whether the rather small rise in conductance for depolarizations between 20 and 30 mV is continued for even greater depolarizations. The increase of conductance which occurred between the limits set by the potential for minimum conductance and the threshold for contracture might be large or small;

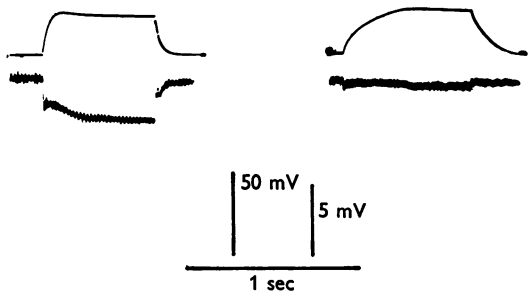

Fig. 12. Records from fibres in sodium-free solutions showing delayed rectification. The left-hand record was from a fibre in a Ringer's fluid made with choline chloride. The right-hand record was from a fibre in a similar solution made with choline sulphate (solutions C and E, Table 1).

in some fibres the conductance continued to fall slowly until the contracture threshold was reached. When the conductance rise was large the potential for minimum conductance was nearer the resting potential. These observations suggest that there is no fixed relation between the contracture threshold and the potential for minimum conductance. The rise in conductance which occurred with depolarizations greater than 20 mV could frequently be seen to take place some time after the onset of the current. Figure 12 shows examples where a delayed decrease in V_a is accompanied by an increase in $(V_b - V_a)$. The records of V_a are similar to those of Jenerick (1959) who suggested that the fall of the electrotonic potential was due to a rise in the potassium conductance, and observed that for depolarizations of less than 20 mV there was no evidence of increased conductance. For depolarizations which do not produce obvious delayed rectification, there does not seem to be a fall of conductance with time comparable with the fall for hyperpolarization. At least if there is such a fall it is considerably smaller for depolarization than for hyper-

polarization. It is difficult to be quite sure about this point for the following reasons. (1) The depolarizations are necessarily small; for hyperpolarization the temporal change is more obvious for large changes in voltage. (2) The membrane conductance is low and the time constant therefore long. (3) For hyperpolarization the temporal change of conductance is in the opposite direction to the change of conductance with voltage, whereas for depolarization, if a temporal fall of conductance occurs, it is in the same direction as the change of conductance produced by the voltage change.

Potassium is the only ion which is known to contribute substantially to the membrane conductance and which is common to all four solutions in Table 3. It is reasonable, therefore, to suggest that the changes in membrane conductance with voltage found in all four solutions represent a change in the potassium conductance of the membrane. In contrast, when there is no potassium in the external solution, and therefore no potassium to carry an inward current through the membrane, the membrane conductance falls with increasing hyperpolarization. The mean internal potential in these experiments was -113 mV (solution B, Table 1). If chloride had a constant permeability, the constant field equations would predict a fall in the chloride conductance with an inward current. However, because of the uncertainty of the internal chloride concentration in the potassium-free solution, it is difficult to say whether the experimental fall in conductance agrees quantitatively with the constant field predictions.

Chloride conductance. The replacement of external chloride by sulphate causes a fall in the total conductance both for depolarization and for hyperpolarization. Provided no current is carried through the membrane by sulphate, the difference between the conductances, at a given membrane potential, in the choline chloride and choline sulphate solutions (C and E) should represent the chloride conductance of the membrane at that potential. Table 4 gives the means of the values of $(V_b - V_a)/V_a$ % for the muscles in the two solutions and the difference between the means at four values of V_a. The difference appears to be nearly constant and equal to 4 %. However, the variation between muscles in the same solution is large enough to make the constancy of the difference not significant. Table 5 gives the means of the values of $(V_b - V_a)/V_a$ % for two groups of three fibres all from the same muscle which was put successively into the choline chloride and choline sulphate Ringer's solution. This particular muscle had a lower than average conductance in both solutions. The standard error of the difference between the means is still unfortunately large, but in this experiment there is a suggestion that the difference between the means is larger for depolarization than for hyperpolarization. In the bottom lines of Tables 4 and 5 the values of $(V_b - V_a)/V_a$ % have been calculated on the basis

of the constant field equations assuming a constant value for the chloride permeability (P_{Cl}). The agreement between the experimental and calculated values is sufficient to suggest that P_{Cl} remains constant, although the variability of the experimental results does not allow great confidence in this conclusion.

$(V_b - V_a)/V_a$ % was found by means of the following equation:

$$r_i g_{Cl} = \frac{2R_i P_{Cl} VF^2}{\rho RT} \frac{[Cl_o] - [Cl_i] \exp(-VF/RT)}{(1 - \exp(-VF/RT))(V - V_{Cl})}, \quad (7)$$

where V is the internal potential, V_{Cl} is the equilibrium potential for Cl, ρ is the radius of the fibre, and R_i the internal specific resistance. The diameter of the fibre was taken to be 80 μ, and R_i 250 Ω cm. The chloride current was assumed to be zero at -90 mV, which corresponds to an internal chloride concentration of 3·3 mM. The constant value for P_{Cl} was chosen to fit the conductance for small values of V_a. $r_i g_{Cl}$ was calculated for various values of V_a and the corresponding values of $(V_b - V_a)/V_a$ % read from Fig. 3.

TABLE 4. Comparison of $(V_b - V_a)/V_a$ % in choline chloride and choline sulphate Ringer's solutions (C and E)

V_a (mV) ...	+30	+20	+10	−10	−40
Average of means from muscles in ChCl	—	4·7	5·0	6·9	9·1
Average of means from muscles in Ch_2SO_4	1·5	0·7	1·0	2·9	5·9
Difference ± S.E. of difference	—	4·0 ± 1·0	4·0 ± 1·3	4·0 ± 1·8	3·2 ± 2·0
Calculated $(V_b - V_a)/V_a$ %	—	4·6	4·4	3·7	2·7

$P_{Cl} = 2{\cdot}4 \times 10^{-6}$ cm/sec.

TABLE 5. Comparison of $(V_b - V_a)/V_a$ % in choline chloride and choline sulphate Ringer's solution for fibres from the same muscle (C and E)

V_a (mV) ...	+30	+20	+10	−10	−40
Average of 3 fibres in choline chloride	3·2	2·4	2·4	3·1	4·6
Average of 3 fibres in choline sulphate	0·6	0·5	0·7	1·7	3·8
Difference ± S.E. of difference	2·6 ± 0·45	1·9 ± 0·41	1·7 ± 0·19	1·4 ± 0·47	0·8 ± 1·1
Calculated $(V_b - V_a)/V_a$ %	2·2	2·0	1·8	1·3	1·1

$P_{Cl} = 0{\cdot}9 \times 10^{-6}$ cm/sec.

The effect on the membrane conductance of adding 0·1 % cocaine to the choline sulphate and choline chloride Ringer's solutions gives some more information about the chloride permeability. Addition of cocaine to the choline chloride Ringer's solution had no detectable effect on the resting potential. The average potential in the presence of cocaine was -88 mV. The addition of cocaine to the choline sulphate solution, however, altered the average membrane potential from -90 to -51 mV. This effect of cocaine was readily reversible, judged by the rapid return of excitability when the muscle was put into a normal Ringer's fluid. The fall in the membrane potential in the absence of chloride, and the lack of an effect

in the presence of chloride, suggest that the action of cocaine is principally on the potassium permeability. This action on the potassium permeability is confirmed by the fact that in choline sulphate with cocaine the membrane conductance is approximately equal to the minimum conductance in the same solution without cocaine. In the presence of cocaine the conductance is constant ($(V_b - V_a)/V_a = 1\%$) for values of the membrane potential between -50 and -110 mV. Variation of the membrane conductance with potential in the choline chloride solution with 0·1 % cocaine should therefore reflect changes in the chloride conductance. The first line of Table 6 gives the average values of $(V_b - V_a)/V_a$ % for three fibres in this solution at five values of V_a. The effect of cocaine is to reverse the direction of the conductance change; depolarization causes an increase in conductance. If 1 % is subtracted from each of the figures in the first row of

TABLE 6. The effect of 0·1 % cocaine on $(V_b - V_a)/V_a$ % in choline chloride Ringer's solution (C)

V_a (mV) ...	+20	+10	−10	−20	−40
$(V_b - V_a)/V_a$ % ± S.E. of mean	5·1 ± 0·21	4·5 ± 0·20	3·2 ± 0·07	3·1 ± 0·1	3·1 ± 0·13
Less 1 % for K conductance	4·1	3·5	2·2	2·1	2·1
Calculated $(V_b - V_a)/V_a$ %	3·9	3·5	2·8	2·6	2·2

$P_{Cl} = 1{\cdot}9 \times 10^{-6}$ cm/sec. Number of fibres = 3.

Table 6 to allow for the residual potassium conductance and for the conductance of any other ions which may be capable of carrying small amounts of current through the membrane, the figures in the second line should be proportional to the chloride conductance of the membrane. The third line of Table 6 gives the calculated values assuming a constant chloride permeability of $1{\cdot}9 \times 10^{-6}$ cm/sec. It appears that cocaine has little or no effect on the chloride permeability although it causes a pronounced reduction in the potassium permeability.

In these experiments the contribution of chloride to the total membrane conductance both in the presence and absence of cocaine is consistent with a constant chloride permeability. Hodgkin & Horowicz (1959*b*) concluded that the chloride permeability of isolated muscle fibres is constant for a wide variety of membrane potentials and chloride concentration ratios. Their mean value for P_{Cl} was 4×10^{-6} cm/sec which is somewhat higher than the average value in these experiments. Their values, however, ranged from 1·9 to $6{\cdot}6 \times 10^{-6}$ cm/sec so that the difference is probably not significant. The present experiments also confirm their finding that chloride carries about two thirds of the current through the membrane for small changes of potential in normal Ringer's solution. Hutter & Noble (1960*a*) have also concluded that the permeability of the membrane to chloride in sartorius fibres is constant.

External calcium concentration. Alteration of the external calcium concentration had no significant effect on the resting potential of fibres in choline chloride Ringer's fluid (Table 7). Jenerick & Gerard (1953) found that increasing the calcium in a normal Ringer's fluid increased the resting potential. The absence of an increase in choline chloride suggests that the effect of calcium is mainly on the sodium permeability. Although the marked variability of the conductance from muscle to muscle would obscure a small effect, there seemed to be no obvious difference between the conductance and its changes on hyperpolarization in a choline chloride Ringer's solution with 0, 1·8, and 18 mM calcium (Table 8).

TABLE 7. The membrane potential in choline chloride Ringer's solution with altered calcium concentration (solutions H, C, and G)

External calcium concentration ...	0 mM	1·8 mM	18 mM
Internal potential (mV)	−93·5 ± 1·5 (13)	—	—
	−93·9 ± 0·8 (9)	—	—
	−88·2 ± 1·9 (4)	−90·0 ± 2·6 (5)	—
	—	−92·0 ± 0·7 (6)	−91·8 ± 2·0 (6)
	—	—	−97·4 ± 0·7 (17)

± S.E. of mean; figures in brackets are the number of fibres. Large brackets indicate groups of fibres from the same muscle. Calcium-free solutions were made by omitting $CaCl_2$ from the solution.

TABLE 8. Mean values of $(V_b - V_a)/V_a$ % for muscles in choline chloride Ringer's solution with varying calcium concentration (solutions H, C, and G)

V_a (mV) ...	+40	+30	+20	+10	−10	−40	No. of fibres
Calcium = 0 mM	—	—	—	3·2	4·3	6·3	3
= 0 mM	—	—	—	9·3	11·4	13·7	4
Calcium = 1·8 mM (average of muscles in Table 4)	—	—	4·7	5·0	6·9	9·1	
Calcium = 18 mM	4·1	3·9	3·8	3·9	5·9	8·7	4
= 18 mM	3·6	2·9	2·9	3·3	4·4	6·7	3

Omission of calcium from the choline sulphate Ringer's solution produced no obvious effect on the conductance. The calcium concentration did, however, affect the membrane potential at which contracture was initiated in these solutions. A rough estimate of the contracture threshold was given by the size of a depolarization which just produced movement artifacts in the record of $(V_b - V_a)$. In the calcium-free solutions contracture occurred with depolarizations greater than about 10 mV; in solutions with a normal calcium concentration depolarizations of between 20 and 30 mV were needed to produce contracture; with 10 times the normal calcium concentration depolarizations of between 40 and 50 mV were possible before movement artifacts appeared. In the calcium-free solutions no evidence of a delayed rise of conductance was seen, presumably because

the threshold for contracture was reached before the threshold for delayed rectification. In solutions with 18 mM calcium a rise in conductance occurred between 30 and 40 mV. The most striking effect of calcium is on the contracture threshold. Calcium does not have large effects on the relation between conductance and voltage, but it is possible that it needs a greater depolarization to produce the delayed rise in potassium conductance when calcium is present in high concentration.

Solutions with a potassium concentration of 100 mM

The membrane conductance was measured in three different solutions with a potassium concentration of 100 mM: a choline chloride Ringer's solution to which an extra 97·5 m-mole/l. KCl had been added (solution J); a solution isotonic with J but containing potassium and choline sulphate (solution K); and a solution containing potassium and choline sulphate but isotonic with normal Ringer's solution (solution L). The average internal potential in the first two solutions was -21 mV, and in the third solution it was -9 mV. In all three solutions there was a large rise in membrane conductance when the membrane current was inward (hyperpolarizing), and a large fall when the membrane current was outward (depolarizing). Records from a fibre in the high-K solution isotonic with Ringer's fluid (L) are shown in Fig. 13. The alteration of the time constant with inward and outward current is very conspicuous, but in contrast to fibres in 2·5 mM potassium the change of conductance during a hyperpolarizing current pulse is much smaller, and in Fig. 13 it is barely visible. It is not complete in one second.

In the solution containing extra KCl (J) the value for the space constant for a small hyperpolarization was as low as 0·5 mm. With an electrode separation of 445μ ($b = 890\mu$) the difference between V_b and V_a was often greater than 20 % of V_a, so that direct proportionality between $(V_b - V_a)/V_a$ % and the membrane conductance was no longer a valid approximation. The electrode separation was therefore reduced to 222μ ($b = 445\mu$) or even 180μ ($b = 360\mu$). It is not possible to compare directly the values of $(V_b - V_a)/V_a$ % from fibres where the electrode separation differs, unless these values are converted to values of $r_i g_0$ by using equation (5). Even with the smaller electrode separations the conductance increase with inward membrane current is so large that the precise values of $r_i g_0$ must be treated with reserve since it is no longer reasonable to treat the membrane conductance as constant between $x = 0$ and $x = b$.

Figure 14 shows the mean values of $r_i g_0$ for fibres in the three solutions plotted against the membrane potential. The arrows indicate the membrane potential in the absence of current. In the 100 mM-K choline sulphate solution isotonic with Ringer's fluid (L), the change in $r_i g_0$ with membrane

potential is less than in the other two solutions. It seems likely that the fibres of the muscle on which these measurements were made (five fibres) had lower than average permeabilities to potassium. Measurements of $(V_b - V_a)/V_a$ % on three other fibres of the same muscle in a choline sulphate solution containing 2·5 mM potassium gave lower than average values (see Table 3). It is interesting to compare the values of $r_i g_0$ for a

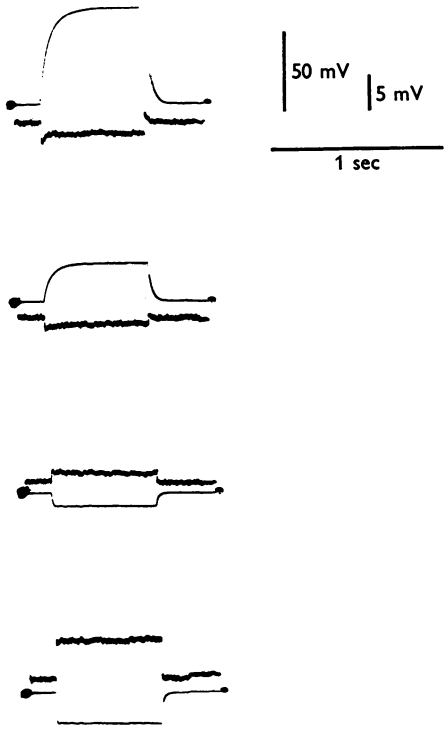

Fig. 13. Records of hyperpolarization (downward) and depolarizations from a fibre in a solution with a potassium concentration of 100 mM, and made with choline sulphate (solution L, Table 1).

hyperpolarization of 10 mV for the two groups of fibres from the same muscle. In the choline sulphate solution containing 2·5 mM potassium, $r_i g_0$ was 0·5 mm^{-2}. In the choline sulphate solution containing 100 mM potassium $r_i g_0$ was 0·15 mm^{-2}. Since these two solutions were isotonic, r_i should have been the same in both solutions, so that increasing the external potassium concentration from 2·5 to 100 mM only increases the membrane potassium conductance three times (see discussion).

Though the variation between the fibres was large the mean values of $r_i g_0$ in the high-KCl solution (J) were greater at all membrane potentials than in the 100 mM-K choline sulphate solution isotonic with J (solution

K). However, for inward currents the differences are not significant. In the chloride solution the fibres have almost the same volume at equilibrium as they have in Ringer's solution, but the internal concentrations of potassium and chloride have each risen by about 100 mM (Boyle & Conway, 1941). In the sulphate solution the fibres shrink so that the concentrations of potassium and the impermeant anion rise. At equilibrium the internal potassium concentrations in the two solutions are about equal (Adrian, 1960). If the mobilities of chloride and the internal anion were the same the internal specific resistances in the two

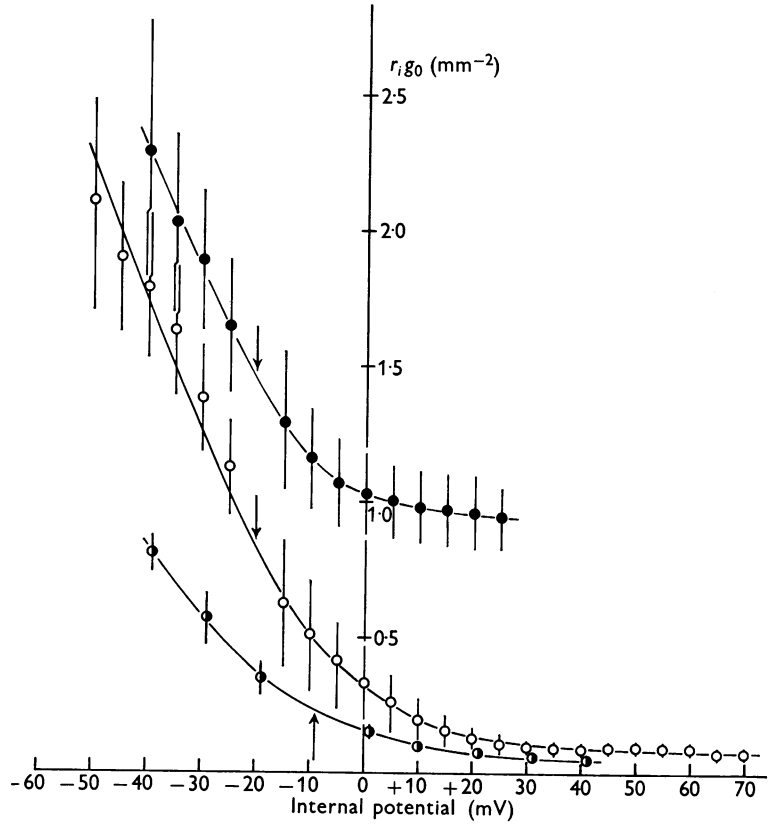

Fig. 14. The relation between $r_i g_0$ and the internal potential in solutions with a potassium concentration of 100 mM. Mean values are plotted ± S.E. of mean. Filled circles: five fibres from two muscles in solution J (220 mM-Cl, 100 mM-K). Open circles: eight fibres from one muscle in solution K (0 mM-Cl, 100 mM-K, isotonic with J). Half-filled circles: five fibres from one muscle in solution L (0 mM-Cl, 100 mM-K, isotonic with Ringer's fluid). Arrows indicate the internal potential in each solution in the absence of current. Records of hyperpolarizations and of depolarization in solution J were measured at 100 msec; depolarizations in solutions K and L were measured at 500 msec.

solutions would be nearly the same, and the internal longitudinal resistance (r_i), which would be greater in the sulphate solution, could be calculated from the diameter in each solution. Since the mobility of the internal anion and the measurements of fibre diameter are so uncertain, an attempt to assess the constancy or the chloride permeability in the same way as was done for the low potassium solutions would be too uncertain to be worth while. Nevertheless, it is obvious that roughly the same conductance change takes place whether chloride is present or not, especially if it is remembered that the effect of a larger r_i in the sulphate solution will be to diminish the difference between $r_i g_0$ in the two solutions when g_0 is large.

An estimate of the chloride permeability may be made from Fig. 14 if the difference between $r_i g_0$ in the two solutions is taken at a membrane potential where the potassium conductance is small. At an internal potential of $+25$ mV $r_i g_0$ in the chloride solution is 1 mm^{-2}, and in the sulphate solution it is 0·1 mm^{-2}. Since the latter value is small, one can say that in the chloride solution $r_i g_{Cl}$ is approximately equal to 0·9 mm^{-2}. Provided the internal specific resistance is known, P_{Cl} can be calculated by equation (7). In the high-KCl solution (J) the internal concentrations of potassium and chloride have both risen by 100 m-mole/l., so that a reasonable estimate of the internal specific resistance in this solution would be half that in normal Ringer's solution. If a value of 125 Ω cm is taken for R_i, and 80 μ for the diameter, then P_{Cl} would be $2{\cdot}2 \times 10^{-6}$ cm/sec. The agreement with the value for P_{Cl} in a choline chloride solution containing 2·5 mM potassium (Table 4) is to some extent fortuitous as the estimate in the high-KCl solution depends directly on the value chosen for the internal specific resistance.

Theoretical model

The purpose of this section is to describe a model which will account for a number of the experimental findings presented in this paper. The implications of the model and its application to the actual situation in muscle will be postponed until the discussion. The simplest way of accounting for the dependence of conductance on potential and time for hyperpolarizations in solutions containing sodium or choline sulphate is to suppose that in these solutions all the current that flows between the inside and the outside of the fibre must pass across two membranes in series, which are separated by a volume small in comparison with the fibre volume. The membrane between the intermediate space and the extracellular fluid is supposed to have constant permeabilities to sodium and potassium, but the inner membrane between the space and the sarcoplasm is permeable only to potassium, and its permeability depends only

on the potential across that membrane. When a constant current is passed, initially more potassium will cross the inner membrane than crosses the outer membrane and the concentrations of both potassium and sodium in the space will alter. The conductance at the beginning of a constant current pulse will depend largely on the potential across the inner of the two membranes. During the course of the pulse the conductance will change as the concentration of potassium in the space changes towards a final level which will depend on the strength of the current. Some further assumptions must be made to keep the mathematical treatment of such a system relatively simple. (1) Within the intermediate space the concentrations are everywhere the same at any moment. (2) The electrical resistance of the contents of space 2 is negligible compared with the resistance of the membranes bounding it. (3) The capacities of both the membranes are ignored. (4) The volume of the space remains constant. If the walls of the space were rigid or impermeable to water, the total quantity of ions within the space could alter without a change in volume. Alternatively, if the walls of the intermediate space were impermeable to anions, the total quantity of ions within the space would be constant and no volume change would occur. The assumption of anion impermeability has been adopted as the less unlikely of these two possibilities; it implies that the current pathway through the space must be in parallel with a chloride pathway between the extracellular fluid and the sarcoplasm. Indirect evidence for this separation of chloride and potassium pathways has been given by Hodgkin & Horowicz (1960*a*). In chloride-free solutions the model may be diagrammatically represented by Fig. 15.

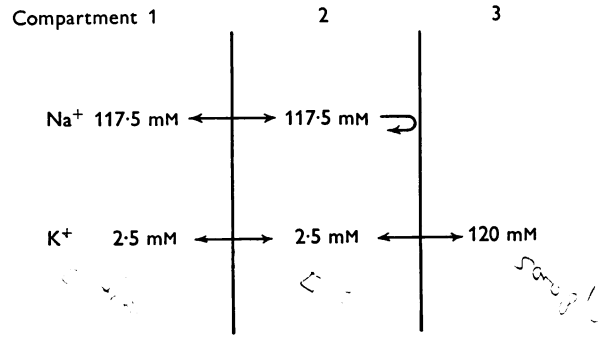

Fig. 15. Diagram of the two-membrane model showing the cation concentrations in the absence of a current.

The total concentration C is the same in all three compartments and is equal to 120 mM.

The volumes of spaces 1 and 3, representing the extracellular fluid and the sarcoplasm are effectively infinite.

v is the volume of space 2 in cm^3/cm^2 fibre surface.

V_{12} is the potential of 2 with respect to 1 in mV; $\theta_1 = V_{12}F/RT$.

V_{23} is the potential of 3 with respect to 2 in mV; $\theta_2 = V_{23}F/RT$.

V_{13} is the potential of 3 with respect to 1 in mV.

I is the total current in A/cm^2 fibre surface; I is positive when the current flows from 1 to 2 to 3.

I_{12}^{K}, I_{12}^{Na}, I_{23}^{K} are the currents carried by sodium and potassium across the membranes in A/cm^2 fibre surface.

p_{12}^{K}, p_{12}^{Na}, p_{23}^{K} are the permeabilities of the two membranes to sodium and potassium in cm/sec. p_{12}^{K} and p_{12}^{Na} are constant; p_{23}^{K} is a function of V_{23}.

A_{12} and A_{23} are the areas of the two membranes in cm^2/cm^2 fibre surface.

Let

$$A_{12}p_{12}^{K} = P'_{K},$$
$$A_{12}p_{12}^{Na} = P'_{Na},$$
$$A_{23}p_{23}^{K} = P_{K}.$$

From the constant field assumptions,

$$I_{12}^{K} = P'_{K}F\theta_1 \frac{[K_1] - [K_2]\exp(\theta_1)}{\exp(\theta_1) - 1}, \tag{8}$$

$$I_{12}^{Na} = P'_{Na}F\theta_1 \frac{[Na_1] - [Na_2]\exp(\theta_1)}{\exp(\theta_1) - 1}, \tag{9}$$

$$I_{23}^{K} = P_{K}F\theta_2 \frac{[K_2] - [K_3]\exp(\theta_2)}{\exp(\theta_2) - 1}. \tag{10}$$

If it is assumed that there are no metabolic movements of ions across the membrane between spaces 1 and 2, then in the absence of current $[K_1]$ will equal $[K_2]$ and $[Na_1]$ will equal $[Na_2]$.

Consider the passage of a constant current; at $t < 0$, $I = 0$; at $t \geqslant 0$, $I = I$.

At all times,

$$I = I_{23}^{K} = I_{12}^{K} + I_{12}^{Na}.$$

Therefore at $t = 0$, before the current has had time to alter $[K_2]$ and $[Na_2]$,

$$\theta_1 = \frac{-I}{F(P'_{K}[K_1] + P'_{Na}[Na_1])}. \tag{11}$$

After the current has been passing for a sufficiently long time ($t = \infty$), no current will be carried across the outer membrane by sodium

$$\theta_1 = \ln \frac{[Na_1]}{[Na_2]}.$$

But since $[Na_1] = C - [K_1]$

and $[Na_2] = C - [K_2]$,

$$\theta_1 = \ln \frac{C-[K_1]}{C-[K_2]}.$$

At $t = \infty$ all the current across the outer membrane will be carried by potassium,

$$I = -CP'_K F \ln \frac{C-[K_1]}{C-[K_2]},$$

$$[K_2] = C - (C-[K_1]) \exp (I/CP'_K F).$$

Since at all times $I = I_{23}^K$ from equation (10),

$$I = P_K F \theta_2 \frac{C-(C-[K_1]) \exp (I/CP'_K F) - [K_3] \exp (\theta_2)}{\exp (\theta_2) - 1}. \qquad (12)$$

Provided P_K is known for each value of θ_2, equation (12) may be solved graphically to give the relation between I and V_{23} at $t = \infty$. Since at that time V_{12} is less than 1 mV, this relation can be taken as the current voltage relation of the two membranes in series at $t = \infty$. (If P_K were assumed to be constant the model would rectify, but in the opposite direction to that found experimentally.)

In order to obtain a relation between $[K_2]$ and t the following approximations to the constant field equations (8) and (9) may be used. If V_{12} is between ± 10 mV and $[K_2]/[K_1]$ between 4 and 0·25, the error introduced by the approximation is less than 2 %.

$$I_{12}^K = P'_K F\{([K_1]-[K_2]) - \tfrac{1}{2}\theta_1([K_1]+[K_2])\},$$

$$I_{12}^{Na} = P'_{Na} F\{([Na_1]-[Na_2]) - \tfrac{1}{2}\theta_1([Na_1]+[Na_2])\}.$$

Since $I = I_{12}^K + I_{12}^{Na}$

$$\frac{\theta_1}{2} = \frac{(P'_K - P'_{Na})([K_1]-[K_2]) - I/F}{(P'_K - P'_{Na})([K_1]+[K_2]) + 2CP'_{Na}}, \qquad (13)$$

$$I_{12}^K = P'_K F \frac{2CP'_{Na}([K_1]-[K_2]) + I([K_1]+[K_2])/F}{(P'_{Na} - P'_K)([K_1]+[K_2]) + 2CP'_{Na}},$$

$$\frac{d[K_2]}{dt} = (I_{12}^K - I)/vF,$$

$$\frac{t}{v} = A \int_{[K_2]_{t=t}}^{[K_2]_{t=0}} \frac{[K_2]+B}{[K_2]+D} d[K_2]$$

$$\left[\frac{t}{v}\right]_{[K_2]_{t=t}}^{[K_2]_{t=0}} = A\Big[[K_2] + (B-D)\ln|[K_2]+D|\Big]_{[K_2]_{t=t}}^{[K_2]_{t=0}}, \qquad (14)$$

where

$$A = \frac{(P'_K - P'_{Na})}{P'_{Na}(I/F - 2CP'_K)},$$

$$B = [K_1] + 2CP'_{Na}/(P'_K - P'_{Na}),$$

$$D = \frac{[K_1](I/F + 2CP'_K) - 2CI/F}{(I/F - 2CP'_K)}.$$

If P'_K and P'_{Na} are known t/v can be calculated as a function of $[K_2]$ for various values of the current (I). V_{12} can be calculated by equation (13), and so long as P_K is known as a function of V_{23} equation (10) can be used to obtain V_{23}.

We are now in a position to see how well the model fits the experimental results. Because of the amount of computation involved only the experiment shown in Figs. 9 and 10 has been treated at all fully. The problem is to find P_K as a function of V_{23} and constant values of P'_K and P'_{Na} which will reproduce the experimental variations of apparent conductance with both time and voltage. The value of v will affect only the time scale of the predicted changes. The experimental values of $(V_b - V_a)/V_a$ % in Fig. 10 were converted to mho/cm^2 fibre surface, assuming a fibre diameter of 80 μ and an internal specific resistance of 250 Ω cm. It was assumed that, at least for the smaller values of current, $[K_2]$ could be regarded as unchanged during the first 100 msec of the current. From an experimental value of the conductance at 100 msec for a particular value of V_a the current in A/cm^2 fibre surface was found. Choosing an arbitrary pair of values for P'_K and P'_{Na}, V_{12} was calculated by equation (11). V_{23} was taken to be the difference between the experimental potential and the calculated value of V_{12}. Equation (10) then gave the value of P_K at that value of V_{23}, and by repeating the process for a number of experimental points a curve of P_K against V_{23} was built up. Equation (12) was then used to calculate the conductance at long times, and these values were compared with the experimental points at 900 msec. When the current was large $[K_2]$ could not be considered to be unaltered during the first 100 msec, so that the values of P_K at large values of V_{23} were obtained by extrapolation of the curve at low values of V_{23}. After a number of trials a reasonable fit with the experimental points was achieved with P'_K equal to 18×10^{-6} cm/sec, P'_{Na} equal to $0{\cdot}9 \times 10^{-6}$ cm/sec, and the relation between P_K and V_{23} shown in Fig. 16. The value of P_K for zero current is about the same as that found by Hodgkin & Horowicz (1959*b*) though their results show a somewhat steeper rise of P_K with potential. Their values of P_K may be compared directly with the present values since they were both calculated on the basis of 1 cm^2 of fibre surface. Evidence will be given in a subsequent paper (Adrian & Freygang, 1962) that the changes in permeability under-

lying anomalous rectification do not lag behind the changes in membrane potential by more than a few msec. Since the time scale of the conductance changes is long compared to a delay of a few msec, the assumption made in these calculations that P_K is a function of V_{23} only should not be seriously wrong.

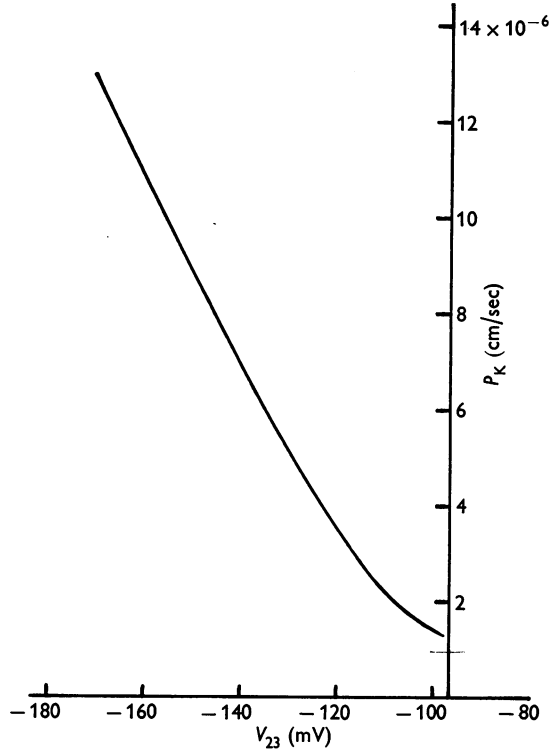

Fig. 16. The relation between P_K and V_{23} which was found to give the best fit for the experimental points in Fig. 10. For the method of estimation of P_K and V_{23} see text.

Figure 17 compares the experimental and calculated conductances for hyperpolarizations at short and long times. Figures 18, 19 and 20 show the conductance changes calculated by means of equations (10), (13) and (14) and plotted against time, for the three experimental procedures of Figs. 7, 8 and 9. The calculated variation of the potassium concentration in the intermediate space ($[K_2]$) is also shown for each set of conditions. The volume of the intermediate space was taken to be $3{\cdot}5 \times 10^{-6}$ cm^3/cm^2 of fibre surface, or about 1/575 of the fibre volume. All three sets of calculated curves utilized the permeability coefficients found to fit the data of Fig. 9. Figures 7 and 18, and Figs. 8 and 19 are therefore not quantitatively comparable because the experimental results for these two com-

parisons were not from the same fibre as the results in Fig. 9. However, all the main qualitative features of the experimental results are reproduced in the calculated curves.

If P_K falls for outward (negative) currents the model predicts that the alteration of apparent conductance with time should be less conspicuous for depolarizations than it is for hyperpolarizations. In order to calculate

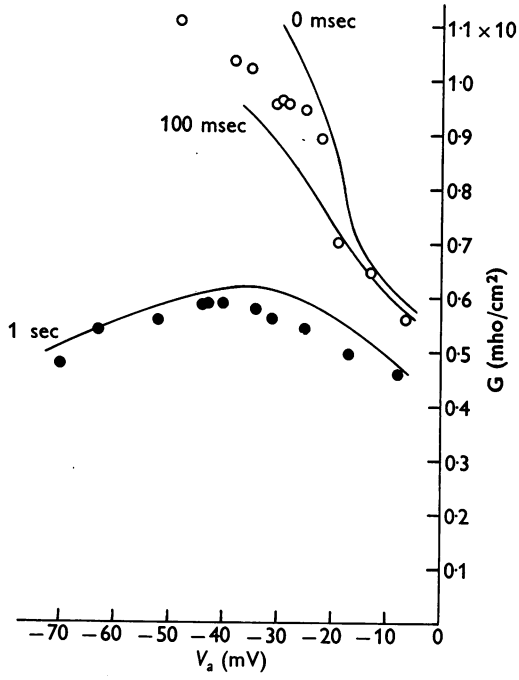

Fig. 17. Comparison of the experimental points from Fig. 10 (converted to mho/cm²) and the apparent conductance calculated at the beginning of a current pulse and at two times after the beginning of the pulse (100 msec, 1 sec). The experimental points and the calculated curves are plotted against V_a. $P'_K = 18 \times 10^{-6}$ cm/sec, $P'_{Na} = 0{\cdot}9 \times 10^{-6}$ cm/sec; P_K was related to V_{23} by the curve given in Fig. 16.

V_{13} and $[K_2]$ as functions of time for depolarizations, the average values of $(V_b - V_a)/V_a$ % in choline sulphate at +10 and +20 mV (Table 4) were used to obtain P_K as a function of V_{23}. The rise in conductance at +30 mV, thought to be due to delayed rectification was ignored; it was assumed that the conductance did not alter between +20 and +35 mV. P'_K, P'_{Na} and v had the same values as in the calculations for hyperpolarizations. Figure 21 shows the calculated change with time of V_{13} and $[K_2]$ for two currents, producing equal hyperpolarization and depolarization.

A greater membrane current density is required to change the membrane potential by equal amounts when the external potassium concentration ($[K_1]$) is 100 mM rather than 2·5 mM. However, if P'_K and P'_{Na} are unaltered by increasing the potassium concentration, the model predicts that for currents producing equal changes in the membrane potential the time required for $[K_2]$ to reach its final value is about ten times longer when

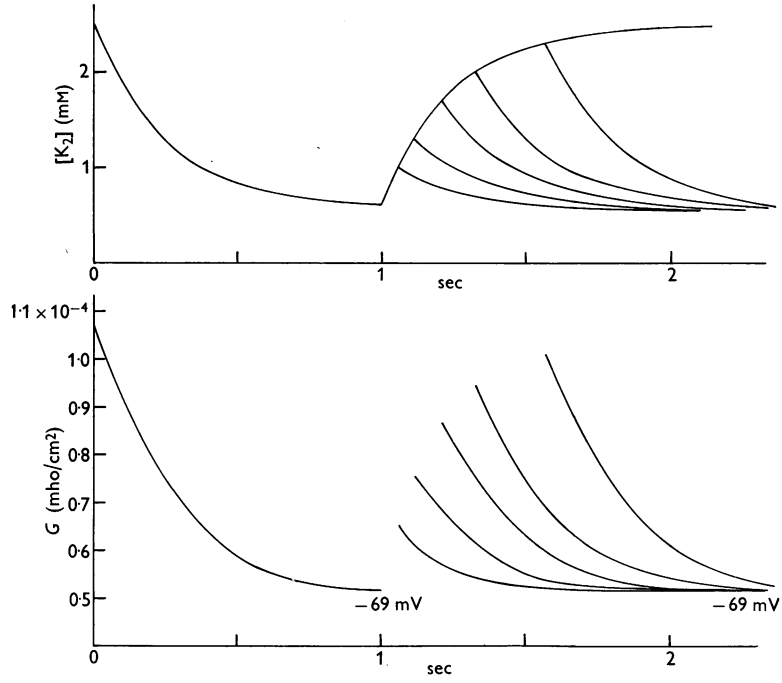

Fig. 18. The variation with time of $[K_2]$ and the apparent conductance calculated on the basis of the proposed model for a double current pulse experiment in which two current pulses of equal size are separated by increasing intervals. The rise of $[K_2]$ after the end of the initial 1 sec pulse in the absence of a second pulse is also shown. Compare the experimental curves in Fig. 7. The volume of the intermediate compartment was $3{\cdot}5 \times 10^{-6}$ cm³/cm² fibre surface, and the permeability coefficients were the same as in Fig. 17.

$[K_1]$ is 100 mM than when it is 2·5 mM. Furthermore, though the change in $[K_2]$ is larger when $[K_1]$ is 100 mM, the ratio $[K_3]/[K_2]$ is altered less. The model is consistent with the experimental finding that the apparent conductance alters very slowly with time when the fibre is hyperpolarized in solutions with a potassium concentration of 100 mM.

It is worth considering how far the agreement between the calculated and experimental curves constitutes evidence for the proposed model,

because several of the constants can be arbitrarily chosen. The time scale depends only on the volume of space 2. P'_K, P'_{Na} and the relation between P_K and V_{23} are derived by fitting the conductance data at the beginning and end of the initial current pulse. No further arbitrary constants are available to fit the conductance changes during the second pulse, the points of crossing of the family of curves in Fig. 20, and the much reduced size of the slow potential changes in 100 mM-K; it seems unlikely that all this rather complex behaviour would be predicted if the proposed model

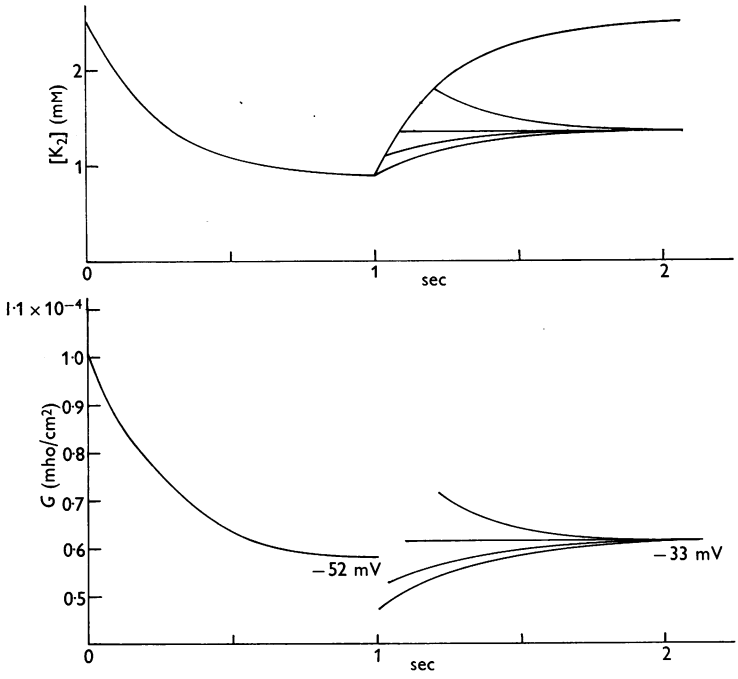

Fig. 19. The variation of $[K_2]$ and apparent conductance calculated on the basis of the proposed model for a double current pulse experiment in which the second of two pulses was smaller than the first and the pulses were separated by varying intervals. In one case there was no interval between the pulses. The rise of $[K_2]$ at the end of the initial 1-sec pulse in the absence of a second pulse is also shown. Compare the experimental curves in Fig. 8. The permeability coefficients and the space volume were the same as in Figs. 17 and 18.

bore no relation to the actual state of affairs. There are, however, a number of observations for which the model cannot account. In the model, the fall of $[K_2]$ at the beginning of an inward current pulse and the rise of $[K_2]$ after the end of the pulse are symmetrical. Agreement between experiment and calculation would be better if the calculated rise of $[K_2]$ was somewhat faster than the fall. The assumption of complete anion

impermeability, which was made to facilitate the calculation, seems inherently improbable, and it was observed that in solutions containing chloride but without potassium, or one to which cocaine had been added, a small rise in conductance takes place with prolonged inward current. The rise is, however, much smaller than the fall which takes place in the presence of potassium. If the walls of the postulated channel are chloride permeable they are probably only sparingly so.

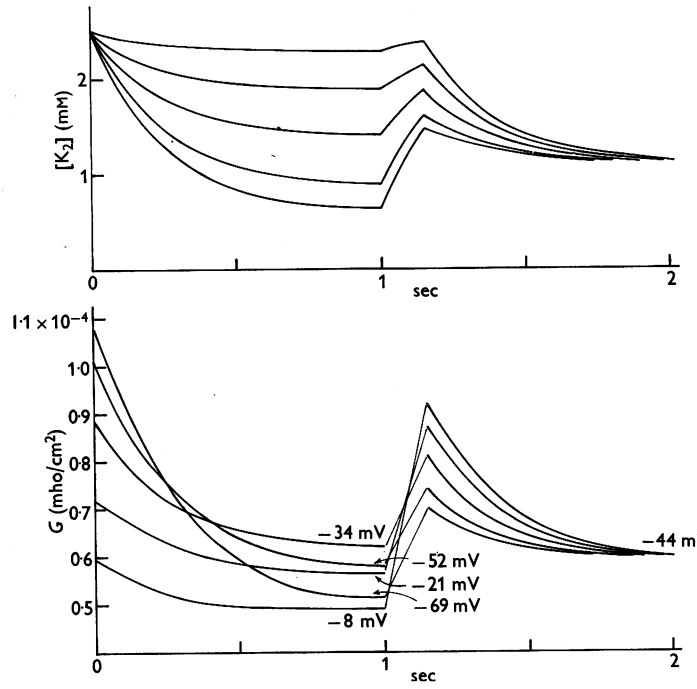

Fig. 20. The variation with time of $[K_2]$ and the apparent conductance calculated on the basis of the proposed model for a double current pulse experiment in which the initial pulse varies in size and is followed at a fixed interval by a second pulse of constant size. The thin lines in the lower figure show which of the curves for the second pulse follows each of the curves for the first pulse. Compare the experimental curves in Fig. 9. The permeability coefficients and space volume were the same as in Figs. 17 and 18.

It is difficult to predict quantitatively the behaviour of any extension of the model which includes a finite conductance in a membrane directly separating compartments 1 and 3. Such a current pathway would be in parallel with the double membrane system that has been treated in isolation in this section. When a parallel conductance exists across a 1–3 membrane, the current flowing via space 2, across the 1–2 membrane and the

2–3 membrane in series, varies with time because it is not a constant fraction of a constant total current. An essential assumption of the calculations in this section is that the current across space 2 is constant.

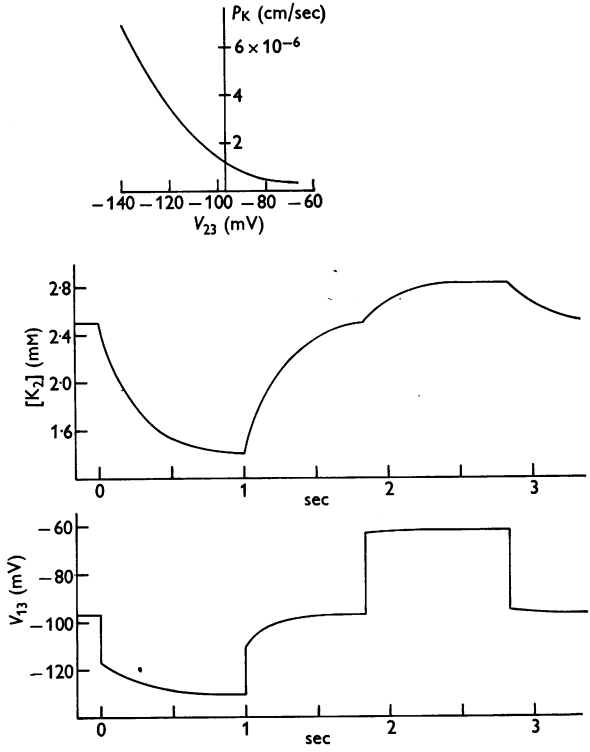

Fig. 21. The variation with time of $[K_2]$ and V_{13} calculated on the basis of the proposed model for two current pulses which hyperpolarize and depolarize by amounts which are equal at the end of each pulse. The relation between P_K and V_{23} used in this calculation is shown at the top of the figure. P'_K, P'_{Na} and v had the same values as in Figs. 17 and 18. The change of V_{13} during the course of the current pulse is much less for depolarization than for hyperpolarization.

DISCUSSION

The conclusion from these experiments is that the ionic currents can be best described in terms of a single channel for chloride with a constant permeability, and two channels for potassium in parallel, which rectify in opposite directions. The results also suggest that, at least in some circumstances, potassium must pass across two membranes in series separated by a small space, in order to get from the sarcoplasm to the extracellular fluid and vice versa. The existence of such a space, restricting the access of potassium, but not of chloride, to the membrane or to parts of the

membrane has already been postulated by Hodgkin & Horowicz (1960*a*) as a partial explanation of the rapid changes in membrane potential produced by sudden alterations of the external concentration of either potassium or chloride. They suggested that chloride influenced the measured membrane potential by acting at the surface of the fibre, but that the action of potassium was on the potential across the wall of some part of the endoplasmic reticulum which was in electrical continuity with the extracellular fluid. When the external potassium concentration was altered, the potassium concentration within the tubules of the endoplasmic reticulum would alter with a delay imposed by diffusion along the tubule. They also suggested that the region in which potassium was temporarily retained might correspond with the tubular system postulated by Huxley & Taylor (1958) to account for the inward spread of contraction of single sarcomeres when small areas of the fibre surface were depolarized in the region of the *Z* line. Despite the paucity of convincing evidence that the triad structures of the endoplasmic reticulum (Porter & Palade, 1957; Anderson-Cedergren, 1959; Fawcett & Revel, 1961) are in contact with the surface membrane, or in electrical continuity with the external fluid, there is strong circumstantial evidence that the triads or some part of them are concerned with the inward spread of contraction (Huxley, 1959).

A number of the observations reported in the present paper can be explained in terms of a space hypothesis, and it seems worth while considering how this model can be related to the suggestions of Huxley, Taylor, Hodgkin and Horowicz. It is obviously tempting to equate 'space 2' with the lumen of a tubular component of the triads. The membrane separating the tubular lumen from the sarcoplasm would then be equivalent to the 2–3 membrane in the model, so the tubular walls would be the site of the anomalous changes in potassium conductance. The 1–2 membrane might correspond to the postulated contacts between the triads and the surface of the fibre and therefore to Huxley & Taylor's sensitive spots. If this identification is correct, the fact that the 1–2 membrane is twenty times more permeable to potassium than to sodium suggests that the tubules are separated from the external solution by a membrane rather than opening directly to the outside.

One very obvious deficiency of the model as a representation of what might occur in a system of reticular tubules, is that it assumes that potential and concentration gradients within space 2 are negligible. If space 2 is to be equated with a tubular system passing into the fibre from the surface, account should be taken of the cable characteristics of the tubule and diffusion along the tubule, when attempting to predict the response either to sudden alterations of external potassium concentration or to the passage of a current. A complete mathematical treatment of

diffusion and current flow in a tubular system whose walls rectified in the way suggested would be difficult, but it is possible that the proposed non-linear current–voltage relation of the tubular walls will account for the asymmetry of the rapid potential changes when the external potassium concentration is raised or lowered (Hodgkin & Horowicz, 1960*a*).

If the membrane at the surface of the muscle fibre is permeable to chloride, then the channel for chloride will be in parallel with the pathway for potassium through the walls of the tubular system, and the high chloride permeability of muscle fibres does not conflict with the assumption of impermeability to anions made for the two membranes of the model. With a membrane of high chloride conductance between the external fluid and the sarcoplasm, in parallel with the tubular membrane, the total membrane resistance and the change in potential which occurs during the passage of a constant inward current would be reduced by the presence of chloride in the external solution. It seems unlikely that the membrane at the surface of the fibre is permeable only to chloride. In a later paper (Adrian & Freygang, 1962) we shall suggest that the surface membrane has a small potassium permeability. It is probably also slightly permeable to sodium. Likewise the assumption that the 2–3 membrane is permeable only to potassium is probably not valid. It could be very sparingly permeable to sodium.

If the cation concentrations within the tubular lumen are supposed to be the same as in the extracellular fluid, an action potential might possibly be propagated along the tubule. Huxley & Taylor (1958) have pointed out that when a sensitive spot is depolarized the contraction of a single sarcomere is graded, and that if the tubular system were capable of producing an action potential a contraction of an all-or-none type would be expected. Therefore it seems likely that the change in sodium conductance responsible for the rising phase of the action potential takes place only on the surface of the fibre. It would be attractive to suppose that the change in potassium conductance responsible for the repolarization of the action potential (delayed rectification) takes place also only at the surface of the fibre. If this were the case delayed and anomalous rectification would be the properties of two spatially separate membranes. However, the present experiments do not exclude the possibility that the walls of the tubular system might become much more permeable to potassium during the repolarization of the action potential. If the potassium loss during an action potential occurred via the tubular system, the potassium concentrations within the tubule would rise. Hodgkin & Horowicz (1960*a*) estimate the size of the tubular system to be between 1/200 and 1/500 of the fibre volume, which is in reasonable agreement with the estimate from the one fibre in this paper (1/575). They also estimate the potassium loss

from frog muscle fibres to be 9·6 p-mole/cm²/impulse (Hodgkin & Horowicz 1959*a*), so that the change of concentration in the tubule, if all this potassium was liberated into its lumen, would be 1 mM for a volume of 1/200 and 2·4 mM for a volume of 1/500. (The change in concentration would be rather less than these values because some of the current necessary to recharge the surface membrane would be carried by potassium across the hypothetical membrane at the mouth of the tubule.) The potassium equilibrium potential across the tubular wall, immediately following an action potential, would be altered by between +8·5 mV and about +17 mV. The size of the recorded after-potential (measured from the resting potential) would be less than half these figures because of the presence of a membrane more permeable to potassium at the mouth of the tubule and the effect of the chloride potential across the fibre surface. The negative after-potential is often as large as +20 mV which is considerably larger than could be accounted for by a potassium accumulation mechanism. Moreover, the negative after-potential in muscle after two impulses separated by an interval of 4–6 msec is hardly greater than the after-potential after a single impulse (Buchthal & Sten-Knudsen, 1959). If each impulse raised the tubular potassium concentration by the same amount (1–2·4 mM/impulse) the after-potential following two impulses should be increased by more than 50%.

Hodgkin & Horowicz (1960*a*) have shown that the membrane repolarizes with a time constant of about 1 sec when the external potassium concentration is suddenly altered from 10 to 2·5 mM in the presence of 120 mM chloride. On the basis of the present model and the supposition that a raised tubular potassium concentration accounts for the whole of the negative after-potential, one would expect the time constant of repolarization of the negative after-potential to have the same value. However, the membrane repolarizes almost completely within about 40 msec. Freygang (unpublished observations) has shown that following a brief train of impulses at a high frequency there is a residual depolarization which disappears with a time constant of about 1 sec. In a typical experiment of this kind on an isolated muscle fibre in a hypertonic Ringer's fluid, 16 impulses at 64/sec left the membrane depolarized by 10 mV and the potential returned to the resting level with a time constant of 0·75 sec. It seems possible that this residual depolarization, which builds up during a train of impulses, is due to potassium accumulation in the tubular system, but if this is the case the loss per impulse across the tubular wall can only be a small fraction of the total potassium loss from the fibre per impulse. The most plausible explanation of the greater part of the negative after-potential is that the increase of potassium conductance in muscle does not last longer than the spike of the action potential as it does in the

squid giant axon, and by the time the membrane has repolarized to about −70 mV the potassium conductance has already reached a low value. The time constant of the after-potential would then be approximately the same as the time constant of the resting membrane.

The conductance of the membrane in sulphate solutions for small (less than 5 mV) deviations of the membrane potential from its value in the absence of current, is only increased three times when the external potassium concentration is raised from 2·5 to 100 mM. On the basis of the constant field assumptions, with a constant potassium permeability, this increase of external potassium concentration should increase the potassium conductance by about twelve times. It appears that, in the present experiments, the potassium permeability of fibres which have been depolarized for some time is less than the permeability in Ringer's solution. Hodgkin & Horowicz (1959*b*) concluded that the potassium permeability, for small deviations of the membrane potential from its resting level, was not much altered by high external potassium concentration. The apparent reduction of potassium permeability found in these experiments could easily be accounted for by variation of diameter and permeability between individual fibres. It is, however, reasonably clear that the potassium permeability is not permanently increased in high potassium solutions (Hodgkin & Horowicz, 1959*a*).

It has been suggested that the anomalous changes in potassium permeability are related to the magnitude and direction of the driving force on the potassium ion ($V-V_K$), rather than to the absolute value of the membrane potential (V). Though it is clear that the potassium permeability depends on the external potassium concentration (Hodgkin & Horowicz, 1959*b*; Adrian, 1958, 1960) it is not yet established that the identical relation between P_K and ($V-V_K$) holds for solutions of different potassium concentration. There is no evidence on which to decide whether P_K depends on the internal potassium concentration as well as upon the external concentration.

If the proposed tubular system is in electrical continuity with the extracellular fluid, the capacity of the tubular walls will be in parallel with the capacity of the membrane at the surface of the muscle fibre. The area of the tubular membrane in 1 cm of fibre could well be several times as great as the area of the surface of the same length of fibre. The large membrane capacity of muscle fibres, when measured with constant current pulses (5–9 μF/cm^2), could be the combined capacity of the surface and tubular membranes, each of which had a capacity more nearly equal to the membrane capacity of nerve or other cells (1–2 μF/cm^2). If the resistance of the mouth of the tube or its internal longitudinal resistance was appreciable, the membrane impedance could not be interpreted in

terms of an equivalent circuit with a single capacity in parallel with a resistance (Noble, 1962). Fatt (1961) has measured the transverse impedance of muscle. He concludes that high frequency alternating current passes through the myoplasm and also has to traverse a capacity of about 2 μF/cm^2 of fibre surface. He has detected an alternative pathway for low frequency alternating current which does not involve the myoplasm and which appears to have a greater capacity and resistance than the pathway for higher frequencies.

Tasaki & Hagiwara (1957) concluded that the muscle membrane behaved as if it were a resistance in parallel with a capacity. They state that alternating current measurements gave the same large value for the capacity as measurements with constant current pulses. They used alternating current of between 25 and 1500 c/s, and made their measurements on muscle of the toad (*Bufo marinus*). They also obtained an estimate of the membrane capacity from the velocity of the action potential and the time constant of its initial exponential rise. Used on the same fibre these two methods gave the same result, and the capacity appeared to be constant between 25 and 1500 c/s. The maximum amplitude of the sinusoidal potential change diminishes exponentially with distance from the microelectrode passing alternating current into the fibre. For a given frequency f (greater than 30 c/s) the length constant is λ/m, where $m = \sqrt{\pi f \tau_m}$ and λ is the length constant measured with a constant current ($\sqrt{[r_m/r_i]}$). Since $\tau_m = r_m c_m$, τ_m/λ^2 should be equal to $r_i c_m$ (r_i, r_m and c_m are the internal resistance, the membrane resistance, and the membrane capacity of 1 cm of fibre). With constant current pulses Tasaki & Hagiwara obtained values of 30 msec and 3 mm for τ_m and λ. τ_m/λ^2 was therefore equal to 0·33 sec/cm^2. The mean value of $r_i c_m$ determined on five fibres by both alternating current and by the rate of rise of the action potential was $0{\cdot}15 \pm 0{\cdot}02$ sec/cm^2 (S.E. of mean). Thus although the membrane capacity seemed to be constant within the range employed by Tasaki & Hagiwara, it appears from their results that the estimate of the capacity with constant current pulses is larger than with alternating currents. It is not possible to obtain an estimate of the membrane capacity without assuming either the fibre diameter or the internal specific resistance. Assuming the internal specific resistance to be 250 Ω cm, the a.c. measurements of Tasaki & Hagiwara give a membrane capacity of 2·4 μF/cm^2. Using constant current pulses and the same value for the internal specific resistance, Fatt & Katz (1951) and Adrian (1960) obtained values on frog muscle of 8·2 and 5·7 μF/cm^2.

Adrian (1960) observed spontaneous oscillations of membrane potential which occurred when KCl was being lost from the muscle fibres into a chloride-free solution with a high potassium concentration. These oscillations were explained in terms of a membrane whose permeability to potassium depended on time as well as on the membrane potential. The experiments described in the present paper seem adequately explained by postulating that the potassium permeability of the inner of two membranes depends only on the potential across that membrane, and it is suggested that changes of conductance with time depend on alterations of the potassium concentration in the region between the two membranes. The present experiments do not exclude time-dependent permeability changes, but it is at least possible that the oscillations could be explained in terms of the present hypothesis, without involving time-dependent permeability changes.

SUMMARY

1. When a constant current is passed across the membrane of a muscle fibre in a solution with a normal potassium concentration, the membrane conductance falls to a steady level in about 1 sec, and returns to its initial level in a comparable time after the end of the current. In the absence of external potassium the slow conductance change is not seen, and it is very much reduced when the external potassium concentration is 100 mM.

2. The conductance at the beginning of a pulse of constant current increases with increasing hyperpolarization, and decreases with increasing depolarization up to depolarizations of about 20 mV (anomalous rectification). For larger depolarizations the membrane conductance rises. No information was obtained for depolarizations which exceeded the contracture threshold.

3. The *change* in conductance with membrane potential is little affected by the presence or absence of chloride, but the absolute value of the conductance at each voltage is reduced when there is no chloride in the external solution.

4. In solutions with 100 mM potassium, in the presence or absence of chloride, the conductance increases as the internal potential is made more negative, and falls to a low and constant level when the internal potential is made less negative or positive. The value of the conductance at any membrane potential is greater when chloride is present, though the *change* in conductance with membrane potential is not much altered by the presence of chloride.

5. For small deviations of potential from the resting level, about two thirds of the membrane current is carried by chloride.

6. Cocaine (0·1 %) reduces the potassium conductance to a low value, but has no detectable effect on the chloride conductance.

7. The experimental variations of the chloride conductance are adequately described by the constant field equations with a constant chloride permeability (P_{Cl}).

8. A tentative hypothesis is described which can reproduce many of the experimental variations of potassium conductance. The main suggestions are: (1) that the surface of a muscle fibre at rest has a very small potassium permeability, but a large and constant chloride permeability; (2) that the sodium and probably the potassium currents underlying the action potential pass across the membrane at the surface of the muscle fibre; (3) that the greater part of the resting potassium permeability is in the walls of a part of the endoplasmic reticulum which is in contact with the extracellular fluid; (4) that the membrane between the lumen of this

reticular component and the sarcoplasm is the site of the potassium permeability changes which underly anomalous rectification.

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