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THE VOLTAGE DEPENDENCE OF THE CHLORIDE CONDUCTANCE OF FROG MUSCLE

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SUMMARY

1. The effect of extracellular pH changes on the voltage–current relation of frog muscle membrane has been studied using intracellular microelectrodes. To reduce the cation conductance of the membrane, potassium in Ringer solution was replaced by rubidium.

2. When the relatively impermeant methyl sulphate ion replaced extracellular chloride the membrane conductance was low, time independent and little influenced by extracellular pH changes. The voltage–current relation was linear at both high and low pH values.

3. In rubidium Ringer solution at pH 7·4 the membrane conductance fell as the inside of the fibre was made more negative, in a manner consistent with the predictions of the constant field theory.

4. At a high pH value (9·8) the resting conductance was high, but it fell steeply as the membrane potential was increased; for large hyperpolarizing voltages the membrane current tended to a limiting value. The voltage–current relation crossed those recorded at pH 7·4 and 5·0.

5. In acid Ringer solution (pH 5·0) the resting membrane conductance was low and remained constant until the membrane potential was hyperpolarized more than 30 mV beyond the resting value; the conductance then rose as the membrane potential was further increased. For large hyperpolarizations the membrane conductance was higher at pH 5·0 than at pH 9·8.

6. Experiments using two successive, identical, constant current pulses suggested that the membrane conductance altered during the passage of current across the membrane; in alkaline solution the conductance fell with time, in acid solution it rose.

7. Because no time or voltage dependence of the membrane conductance was seen in the absence of chloride ions it is inferred that the movements

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of chloride ions across the muscle membrane are responsible for both the time and voltage dependent alterations in membrane conductance seen at different pH values.

INTRODUCTION

The voltage dependence of the potassium and chloride conductances of frog muscle are known to be quite different. If potassium is the only ion available to carry current across the membrane the conductance *rises* as the internal potential of the muscle fibre is made more negative (Katz, 1949; Adrian & Freygang, 1962). When chloride ions also carry current the voltage–current relation is more nearly linear (Hutter & Noble, 1960) suggesting that the chloride conductance *falls* with hyperpolarization, in accord with the predictions of the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949). Moreover, the constant field theory has been used to describe the variations in chloride conductance with potential and concentration by Hodgkin & Horowicz (1959), who found the membrane permeability to chloride ions to change little over a wide range of membrane potentials and chloride concentrations.

More recently the passive permeability of frog muscle to chloride ions has been recognized to be highly sensitive to alterations in the extracellular pH (Hutter & Warner, 1967*a*, *b*) and one can ask: how far does the constant field theory provide an adequate description of the behaviour of the chloride conductance away from neutrality, when the anion permeability has been greatly altered? In the present work this question has been taken up by studying the voltage dependence of the chloride conductance at different extracellular pH values, in the hope that information on the shape of the voltage–current relation might help to characterize the mechanism of anion permeation through the membrane (Eisenman, Sandblom & Walker, 1967) and reveal whether potentials at the surface of the membrane play a part in regulating the transfer of chloride ions (cf. Frankenhauser, 1960; Hutter & Warner, 1967*a*, p. 241; Spurway, 1965).

As the anion conductance will be derived from measurements of the total membrane conductance in the presence and absence of chloride ions, experiments to establish the behaviour of the cation conductance under the appropriate experimental conditions are also included in this paper.

Some of these results have been briefly reported to a meeting of the Physiological Society (Hutter & Warner, 1969).

METHODS

Experiments were done at room temperature (20–22° C) on sartorius muscles of frogs, *Rana temporaria*, which were stored in the cold at 4° C until required for use. The muscles were pinned out over a Perspex strip in a bath holding 0·8 ml. through

which solutions flowed at between 6 and 12 ml./min; long-lasting impalements were generally required and faster fluid flows tended to dislodge the micro-electrodes.

The composition of the solutions is given in Table 1. Sodium and potassium methylsulphates were made in the laboratory as described previously (Hutter & Warner, 1967*a*).

The membrane resistance was measured using the method developed by Adrian & Freygang (1962) which allows the membrane current density to be recorded directly. Three micro-electrodes were inserted close to the pelvic end of a muscle fibre. The electrodes were placed in a line with the same distance between each electrode as between the first electrode and the tendon. The two electrodes nearest to the tendon recorded the membrane potential and the third was used to inject rectangular

TABLE 1. Composition of solutions (mM)

Solution	Na^+	K^+	Rb^+	Cl^-	$CH_3SO_4^-$	Ca^{2+}	Tris maleate	Acetyl glycine
A (Ringer)	116	2·5	—	120·5	—	3·0	2·0	2·0
B	116	—	2·5	120·5	—	3·0	2·0	2·0
C	116	2·5	—	6	114·5	3·0	2·0	2·0
D	116	—	2·5	6	114·5	3·0	2·0	2·0
E	116	100	—	218	—	3·0	2·0	2·0
F	116	—	100	218	—	3·0	2·0	2·0

These values apply to solutions at pH 7·4. pH adjusted electrometrically to required value with sodium hydroxide.

On occasion sodium in solutions *B*, *E* and *F* was replaced by choline. 10^{-5} % (w/v) tubocurarine chloride was also included.

current pulses. The membrane current density is proportional to the difference in voltage between the two end electrodes and is given by

$$I_m \simeq \frac{a(V_2 - V_1)}{2l^2 R_i},$$

where l is the distance between the electrodes, R_i the internal resistivity of the fibre and a the fibre radius (Adrian, Chandler & Hodgkin, 1970*a*; Stanfield, 1970). Taking R_i at 20° C to be 170 Ω.cm (Hodgkin & Nakajima, 1972), for an 80 μm fibre and an electrode separation of 500 μm, 1 mV difference in potential is equivalent to $3{\cdot}14 \times 10^{-6}$ A/cm². Provided l is less than twice the space constant, the error in this method is less than 5 % (Adrian, Chandler & Hodgkin, 1970*a*; Stanfield, 1970).

3 M-potassium chloride filled micro-electrodes with resistances between 10 and 50 MΩ and tip potentials of less than −5 mV were used for voltage recording. Electrodes filled with 0·8 or 2 M neutralized potassium citrate were used to inject current pulses. The voltage reference electrode made contact with the bath through an Agar–Ringer bridge.

Current and voltage were recorded on an oscilloscope and displayed simultaneously on a pen recorder. In all the experimental records in this and the following paper (Warner, 1972) inward current is shown as an *upward* deflexion of the oscilloscope trace. This convention allowed maximum usage of the oscilloscope face for photographic recording.

RESULTS

The cation voltage–current relation

When membrane current is carried by potassium ions alone both voltage and time dependent changes in ionic conductance are seen (Katz, 1949; Adrian & Freygang, 1962). These alterations in potassium current also take place in Ringer solution (Adrian & Freygang, 1962) and when studying the movement of chloride ions it is desirable to minimize complications arising from this source. In muscles permanently depolarized by soaking in isotonic potassium sulphate solution, Adrian (1964) found inward-going rectification to be abolished, making the cation voltage–current relation linear, when rubidium was used in place of potassium. His measurements on polarized muscles in rubidium-containing solution were made in the presence of chloride ions, but they suggested that under such conditions also the voltage and time dependent conductance changes due to potassium no longer occurred. To confirm this point a few experiments were done in which the input resistance and voltage–current relations in fibres of polarized muscles bathed in chloride free potassium or rubidium-containing solutions (solutions *C* and *D*, Table 1) at pH 7·4 were compared. These showed the membrane conductance to fall, the voltage–current relation to become linear, and time dependent conductance changes to be abolished by the presence of rubidium ions. Similar results have been obtained by Adrian, Chandler & Hodgkin (1970*b*). The behaviour of the cation conductance is thus greatly simplified when rubidium replaces potassium in the external solution.

The inward current carried by rubidium ions at different membrane potentials in acid and alkaline solutions is illustrated in Fig. 1. The final height of the electrotonic potential has been plotted against membrane current density as measured directly by the three micro-electrode technique of Adrian & Freygang (1962). At pH 5·0 the membrane conductance was slightly greater than in the alkaline solution, but the voltage–current relation remained linear at both pH values. Like the potassium conductance (Hutter & Warner, 1967*a*) the resting permeability to rubidium is evidently little influenced by changes in the extracellular pH.

In the course of these experiments several measurements of the absolute value of the cation conductance in methyl sulphate solutions containing 2·5 mM rubidium were made. For calculation of specific membrane conductances the fibre diameter was taken to be 80 μm, as accurate measurement of fibre diameter in a whole muscle preparation is difficult, particularly close to the tendon, and fibres close to 80 μm in diameter were generally chosen. In rubidium-containing solution at pH 7·4 the membrane conductance came to 42 ± 6 (mean $\pm$ S.E.) μmho/cm^2. The potassium

conductance of muscle fibres resting in 2·5 mM potassium is about 80 μmho/cm^2 (Hodgkin & Horowicz, 1959; Adrian & Freygang, 1962) so that replacement of extracellular potassium by rubidium halved the cation conductance of the muscle membrane. Potassium normally contributes about one-third of the total resting membrane conductance (Hodgkin & Horowicz, 1959; Hutter & Noble, 1960; Adrian & Freygang, 1962; Hubbard, 1963); in rubidium-containing Ringer solution, therefore, the cation contribution falls to about one-sixth of the total membrane conductance at pH 7·4. This means that in the presence of extracellular rubidium ions the

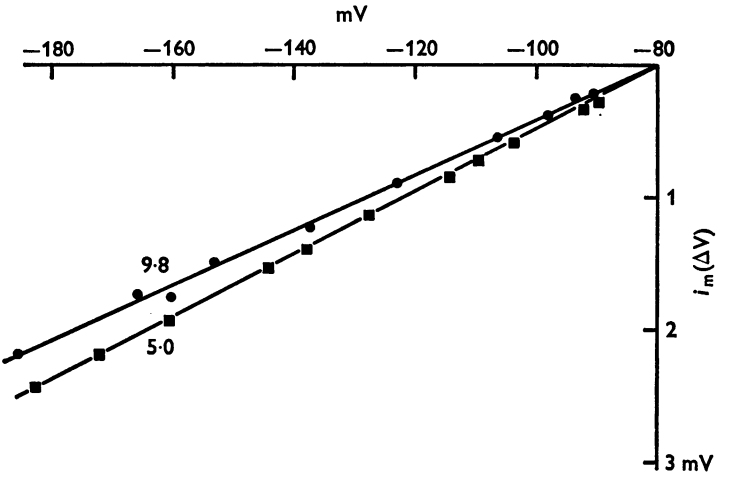

Fig. 1. Effect of extracellular pH on the voltage–current relation in a polarized muscle fibre in rubidium methyl sulphate-containing solution (solution *B*, Table 1). Abscissa: membrane potential in mV. Ordinate: membrane current density as ΔV. $l = 500\ \mu$m, $\Delta V = 1$ m$V \equiv 3{\cdot}14 \times 10^{-6}$ A/cm^2. ● pH 9·8, ■ pH 5·0, Temperature = 21° C.

membrane is overwhelmingly chloride permeable in alkaline solution and the chloride contribution is appreciable even in acid solution when the anion permeability is low. The cation conductance remaining in the presence of rubidium behaves as a linear component. Provided the properties of the cation conductance channel do not change when chloride ions also carry current across the membrane, the alterations in the shape of the voltage–current relation with pH to be described in chloride-containing solutions represent changes in the voltage dependence of the anion conductance.

The anion voltage–current relation

Results obtained from a muscle in rubidium-containing sodium chloride solution (solution *B*, Table 1) at pH 5·0, 7·4 and 9·8 are shown in Fig. 2. The final voltage displacement from the resting potential produced by a 1 sec

current pulse is plotted against membrane current density. Hyperpolarizing currents only were passed as with depolarizing currents the threshold for the action potential was quickly reached, particularly in alkaline solution, where the voltage threshold for impulse propagation is low (Brooks & Hutter, 1963). The electrodes were inserted while the muscle was superfused with solution at pH 7·4 and after insertion of the

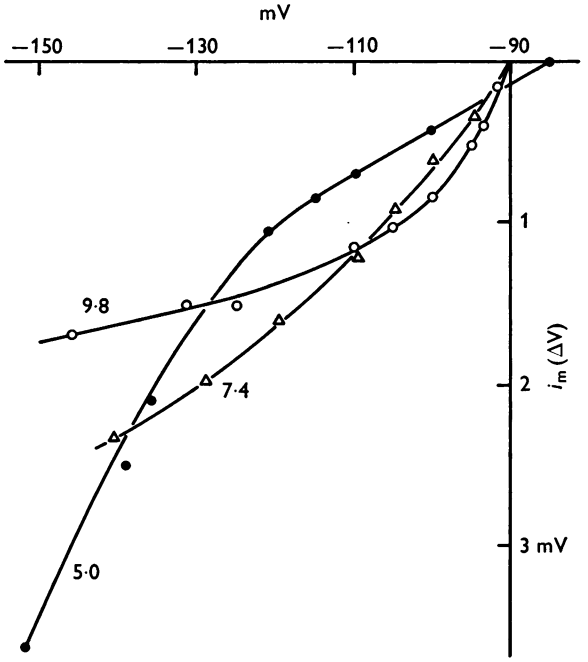

Fig. 2. Voltage–current relations in chloride-containing solution (solution *A*, Table 1) at different extracellular pH values. Abscissa: membrane potential. Ordinate: membrane current density as ΔV. $l = 625\,\mu$m; $\Delta V = 1$ mV $\equiv 2 \times 10^{-16}$ A/cm^2. ○ pH 9·8; Δ pH 7·4; ● pH 5·0; 21° C. Different fibre from Fig. 1.

third electrode the membrane potential was −90 mV. The voltage–current relation obtained at this pH value showed a gradual fall in the slope conductance as the membrane potential increased; 45 mV away from the resting potential the slope conductance was half the resting value. Raising the extracellular pH to 9·8 caused no change in resting potential and the input conductance for small voltage displacements rose 1·8 times. As the internal potential was made more negative the slope conductance rapidly dropped away, until, for hyperpolarizations greater than 50 mV, it had fallen to about one tenth of the resting value. Consequently the alkaline voltage–current relation crossed that obtained at pH 7·4 about 20 mV away

from the resting potential. This was a consistent feature of the results, although the potential at which the alkaline and neutral voltage–current relations crossed varied from fibre to fibre. When the increase in conductance on passing from neutral to alkaline solution was smaller than shown here the cross-over potential lay closer to the resting potential. Conversely it sometimes lay at a more negative potential than in the fibre illustrated.

On acidification of the external solution the membrane potential fell by a few millivolts, as observed by Hutter & Warner (1967*a*). The resting potential settled at −86 mV, by which time the input conductance for small voltage deflexions had fallen to about one fifth. The voltage–current relation was sensibly linear until the membrane had been hyperpolarized by about 40 mV. Over the next 20 mV the slope conductance increased greatly and eventually the pattern of resistance changes was completely reversed: for hyperpolarizations of about 60 mV the conductance, measured at the end of the current pulse, was about twice as high in the acid solution as in the alkaline solution.

In Fig. 5*A* the results of Fig. 2 have been replotted after subtraction of the voltage independent component of the membrane conductance attributable to the current carried by rubidium ions. The rubidium contribution was set at one sixth of the total membrane conductance at pH 7·4. This procedure emphasized the essential features of the alterations in voltage dependence which occur as the pH is varied either side of neutrality; it further suggested that in alkaline solution the chloride current reaches a limiting value for large negative voltage deflexions. Ideally the voltage–current relation in rubidium methyl sulphate solution should have been determined in the same fibre. But without voltage control of the membrane potential the changes in resting potential, which accompany extracellular pH variations (Hutter & Warner, 1967*a*), often led to progressive depolarization of the muscle fibre, particularly in the rubidium-containing methyl sulphate solution, where the input resistance was extremely high. An example of such an experiment done using a voltage clamp technique is shown in the following paper (Warner, 1972; Fig. 6), which confirms the suggestion that the chloride current can come to a limiting value.

In the experiment of Fig. 2 the input conductance fell only about 5 times on passing from the alkaline to the acid solution, but a tenfold fall in membrane conductance on lowering the pH from 9·8 to 5·0 was often observed in rubidium-containing solutions and in one fibre the conductance fell 22 times. Such effects are larger than those seen in experiments on muscles bathed in Ringer solution (Hutter & Warner, 1967*a*: Fig. 6), in accord with a low cation contribution to the membrane conductance in the presence of rubidium.

To bring out the difference between the steady-state conductance given

by small and large voltage deflexions, electrotonic potentials and membrane current records are illustrated in Fig. 3. *a*, *b* and *c* are examples of small electrotonic potentials recorded at pH 9·8, 7·4 and 5·0 in the experiment of Fig. 2. At all three pH values the voltage deflexion became steady after about 250 msec, the electrotonic potentials were fairly symmetrical and the membrane conductances, measured as the ratio $\Delta V/V$, at the end of the pulse at pH 5·0, 7·4 and 9·8 were in the ratios 1·0:2·7:5·3. Larger

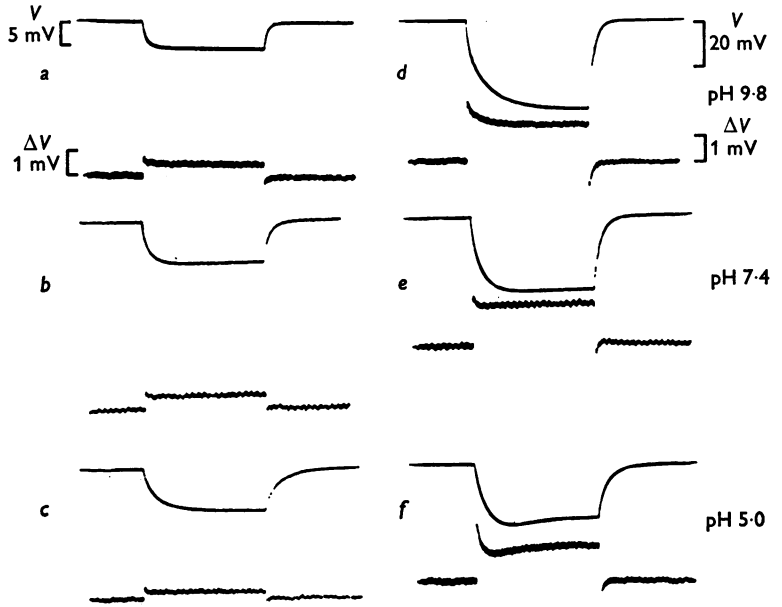

Fig. 3. Hyperpolarizing electrotonic potentials at pH 9·8, 7·4 and 5·0. Same experiment as Fig. 2. *a*, *b*, *c* small voltage deflexions; *d*, *e*, *f* large voltage deflexions. Note change in voltage gain between *c* and *d*. Inward current is given as the difference in potential between the two recording electrodes and is displayed as an *upward* deflexion of the trace.

electrotonic voltages, which gave points falling at the bottom of the voltage–current relation, are shown in *d*, *e* and *f*. Close to neutrality the rising and falling phases of the electrotonic potential again had a similar time course. However at pH 9·8 the voltage displacement gradually increased throughout the current pulse and an asymmetry in the make and break of the voltage record was noticeable. The membrane current also decreased during the passage of current. By contrast at pH 5·0 the voltage deflexion grew rapidly over the first 150 msec but then fell away so that by the end of the current pulse the conductance had apparently risen. Concomitantly the membrane current record showed first a decrease in current followed by a rapid increase to a steady-state level. Consequently the mem-

brane conductances at pH 5·0, 7·4 and 9·8 measured at the end of the pulse were in the ratios 1·0:0·8:0·55.

A delayed fall in the voltage deflexion during the current pulse was also seen at neutral and alkaline pH values if strong hyperpolarizing currents were used. The voltage level at which this occurred varied, but in most fibres it was necessary to hyperpolarize by more than 60 mV at pH 7·4 and 80 mV at pH 9·8. On occasion the voltage deflexion fell during a 1 sec current pulse at lower internal potentials, even in neutral and alkaline solutions; the increase in conductance with time normally observed in acid solutions then appeared correspondingly earlier. The fall in the electrotonic potential with time observed by Adrian (1964) in rubidium-containing chloride solutions probably corresponds to this phenomenon. The present analysis at neutral and alkaline pH values has been confined to the behaviour of the chloride conductance at voltage deflexions which do not provoke a rise in conductance within the duration of a one second current pulse. Other experiments have suggested that any increase in conductance with time at pH 7·4 and 9·8 shares the general properties of the conductance changes observed in acid solution.

The slow creep of potential seen in the course of inward current flow in the alkaline chloride-containing solution is reminiscent of the fall in conductance during a hyperpolarizing current pulse which occurs in the presence of extracellular potassium (Katz, 1949; Adrian & Freygang, 1962). Time dependent alterations in cation conductance are unlikely to occur in rubidium-containing solutions (see p. 278; Adrian, Chandler & Hodgkin, 1970*b*) but it could be that a time dependent fall in chloride conductance occurs in alkaline solution. Alternatively the increase in voltage deflexion with time during the current pulse could stem from the precipitate drop in membrane current as the inside of the fibre is made more negative (Fig. 2). However, it cannot be argued that the rise in conductance seen during the passage of current in acid solution is simply the consequence of the shape of the voltage–current relationship; some truly time dependent process, probably the result of alterations in chloride conductance, must underly this observation. A fall in the electrotonic potential during the current pulse in acid solutions was observed by Gage & Eisenberg (1969) who attributed it to a change in potassium permeability consequent upon depletion of potassium in the T-tubular space.

An experiment which shows that the increase in conductance seen during the electrotonic potential in acid solution persists after the end of the current pulse is detailed in the upper part of Table 2. A second, identical current pulse was injected 160 msec after the first and the conductance measured at various times during the two electrotonic potentials. To ensure that the membrane capacity was largely filled the first measurement was not made until 200 msec after the beginning of the pulse. The membrane conductance (given as $\Delta V/V$ %) rose throughout the remainder of the first current pulse reaching a final value 25% higher than at 200 msec. The conductance was still close to this level 200 msec after the start

of the second pulse and it rose still further over the next 400 msec, suggesting that the time constant for this increase in conductance is very long. A similar experiment done in alkaline solution with a pulse separation of 120 msec is given in the lower part of Table 2. It can be seen that the conductance was lower early in the second pulse than at the beginning of the first, although the final conductance was the same for both voltage deflexions. Such experiments suggest that in alkaline solution also the membrane conductance shows time dependent variations during inward current

TABLE 2. Variations in membrane conductance during consecutive current pulses

pH 5·0

Time after beginning of pulse (msec)	200	220	260	320	420	520	620	1000
Conductance during pulse 1 ($\Delta V/V$ %)	3·00	3·06	3·32	3·43	3·56	3·64	3·68	3·89
Conductance during pulse 2 ($\Delta V/V$ %)	3·89	3·89	3·9	3·96	4·0	4·25	4·32	4·32

Pulse separation 120 msec

pH 9·8

Time after beginning of pulse (msec)	200	—	260	320	420	520	620	1000
Conductance during pulse 1 ($\Delta V/V$ %)	9·5	—	8·45	7·85	7·55	7·1	6·9	6·8
Conductance during pulse 2 ($\Delta V/V$ %)	7·85	—	7·6	7·35	7·1	7·0	—	6·85

Pulse separation 160 msec

flow. A more detailed study of the activation and inactivation of the chloride conductance in acid and alkaline solutions using a voltage clamp technique is described in the following paper (Warner, 1972).

Experiments on depolarized fibres. To explore the shape of the voltage–current relation over a wide range of membrane potential displacements a few experiments were done on muscles which had been permanently depolarized by soaking in a Ringer solution containing additional potassium chloride (100 m-mole/l., solution *E*, Table 1). The muscles were soaked in this solution for 4 hr, by which time the uptake of additional potassium chloride should have gone to completion (Adrian, 1960; Hutter & Warner, 1967*b*). They were then transferred to a similar solution in which rubidium

chloride replaced potassium chloride (solution *F*), rendering the cation voltage–current relation more or less linear and the cation conductance low (Adrian, 1964). Fig. 4 shows voltage–current relations obtained in a fibre from such a muscle bathed in high rubidium chloride solution (solution *F*, Table 1) at pH 9·8, 7·4 and 5·0. The resting potential of this

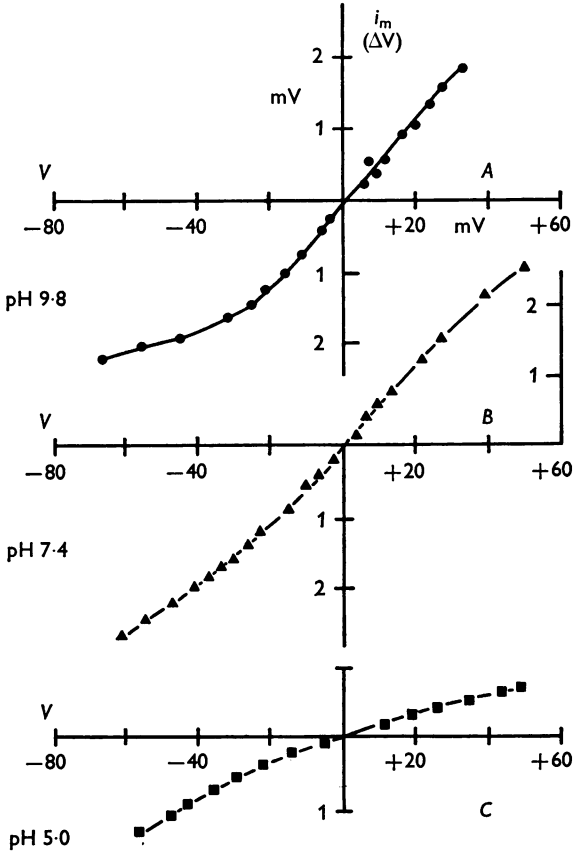

Fig. 4. Effect of extracellular pH on voltage–current relations in permanently depolarized muscle fibres. *A* pH 9·8; *B* pH 7·4; *C* pH 5·0. Abscissae: membrane potential mV. Ordinates: membrane current density as ΔV (mV). $l = 250\,\mu$m. $\Delta V = 1$ mV $\equiv 12{\cdot}5 \times 10^{-6}$ A/cm^2. Membrane potential $= -20$ mV. 21° C.

fibre was -20 mV. In the alkaline solution (Fig. 4*A*) the steady-state conductance was constant for voltage deflexions of ± 30 mV; for greater hyperpolarizations the slope conductance began to fall, as in polarized muscle fibres. Voltage deflexions which were sufficiently large to bring the potential close to the resting potential of polarized muscle sometimes caused localized contractile activation. At pH 5·0 (Fig. 4*C*) the slope con-

ductance, measured at the end of the current pulse, rose as the inside of the fibre was made more negative and an increase in conductance was seen during the current pulse, again as in muscles soaked in Ringer solution. In the depolarizing direction the slope conductance remained constant. Close to neutrality (Fig. 4*B*) the voltage–current relation was symmetrical about the resting potential. It was linear up to about 35 mV either side of the resting potential; the slope conductance then fell as the voltage deflexion was further increased, to the same degree in both hyperpolarizing and depolarizing directions.

DISCUSSION

The main conclusion of this paper is that variations in the pH of a solution bathing frog muscle affect the voltage dependence of the membrane conductance as well as greatly influencing the magnitude of the resting membrane permeability. Hutter & Warner (1967*a*, *b*) concluded that alterations in anion permeability alone were responsible for the pH-dependent changes in resting permeability, on the basis of conductance measurements made in the absence and presence of chloride ions and measurement of ^{36}Cl and ^{42}K effluxes in the same muscle. The present findings show that the pH dependent alterations in the voltage–current relation also only take place when chloride ions are available to carry current across the membrane. Provided the behaviour of the cation conductance does not alter in the presence of chloride ions these results mean that the voltage dependence of the chloride conductance, but not the cation conductance, is determined by the extracellular concentration of hydrogen ions.

Near to neutrality, the degree of rectification observed as the membrane potential is increased is close to that to be expected if the constant field assumptions (Goldman, 1943; Hodgkin & Katz, 1949) hold for the permeation of chloride ions (cf. Fig. 5*A*, *B* for pH 7·4). Similar observations have been made by others (Hutter & Noble, 1960; Adrian & Freygang, 1962; Hubbard, 1963). If a fall in the extracellular hydrogen ion concentration were to decrease the permeability coefficient for chloride ions, P_{Cl}, in the constant field equation (implying that the permeation mechanism does not change) then even if the resting anion permeability altered greatly there would be no difference in the degree or direction of rectification. Since the voltage dependence of the chloride conductance was found to change along with the resting anion permeability some other factor must be involved.

Fixed surface charges can impose a potential on a constant field membrane thereby altering its rectifying properties (cf. Frankenhaeuser, 1960; Adrian, 1969) and the extracellular pH value might determine the magni-

tude and sign of such a potential. To examine whether variations in a surface potential could provide an explanation for the voltage dependence observed in alkaline and acid solutions, voltage–current relations have been calculated from the constant field equation modified to include a surface potential term in the manner detailed by Frankenhaeuser (1960) and are plotted in Fig. 5*B*. The magnitude and sign of the surface potential was chosen to give the resting chloride conductance found experimentally

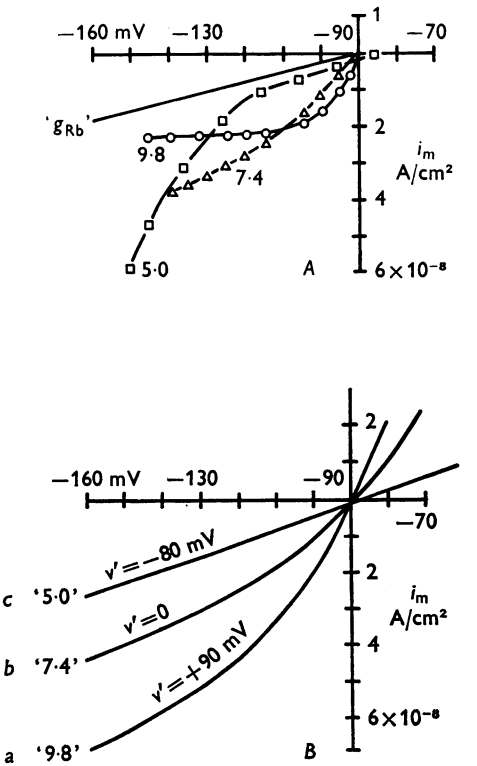

Fig. 5. Comparison of experimental voltage–current relations with those calculated on the constant field assumptions. *A*, experiment of Fig. 2. Results replotted after subtraction of a constant 'rubidium' conductance. 'g_{Rb}' set at 1/6 of total g_m at pH 7·4. ○ pH 9·8; Δ pH 7·4; □ pH 5·0. Abscissa: membrane potential (mV). Ordinate: membrane current density (A/cm²). *B*, voltage–current relations calculated according to the constant field equation modified to take account of surface potentials in the manner of Frankenhaeuser (1960):

$$I_{Cl} = P_{Cl}\frac{(V-V')F^2}{RT}\frac{[Cl]_o\exp(V'F/RT)-[Cl]_i\exp\{-(V-V')F/RT\}}{1-\exp\{-(V-V')F/RT\}}$$

for different values of the surface potential, V'. Abscissa: membrane potential. Ordinate: membrane current density (A/cm²).

at each pH value. As noted previously (Spurway, 1965; Hutter & Warner, 1967*a*) to account for the observed changes in resting conductance a *positive* surface potential would have to be operative in alkaline solution. The chloride conductance at pH 9·8 can be matched when the surface potential is set at +90 mV. Although the increase in resistance as the membrane potential rises is steeper than in the absence of a surface potential (compare curves *a* and *b*, Fig. 5*B*) the degree of rectification is much less than observed experimentally and at no point does curve *a* cross curve *b*. A more positive surface potential would further steepen the voltage–current relation, but at the same time the resting conductance would rise so that once again no crossing over would occur. For pH 5·0 the resting chloride conductance can be reproduced with a surface potential of −80 mV and the calculated (relation *c*, Fig. 5*B*) and experimental (pH 5·0, Fig. 5*A*) voltage–current relations run together until displacements of more than 40 mV away from the resting potential are reached. The slope conductance of the experimental relation then begins to rise and the divergence between calculated and experimental voltage–current relations becomes wider as polarization is increased.

These calculations confirm that close to neutrality the constant field equations provide a good description of the behaviour of the steady-state chloride conductance. However, it seems unlikely that variations in the magnitude of a surface potential alone could underly the changes in rectification seen in acid and alkaline solutions because although this modification of the constant field equation produces variations in rectification in the same direction as were observed in the experiments, it will not reproduce the crossing over of the voltage–current relations. The experiments with two consecutive, identical current pulses showed that time dependent changes in anion conductance, which are also pH dependent, take place during the passage of current through the membrane, suggesting that the behaviour of the chloride conductance is more complex than has hitherto been supposed. It therefore seems worth searching for a model which could incorporate both the alterations in voltage dependence and time dependence of the anion conductance. This will be deferred until the kinetics of the time dependent conductance changes have been described (Warner, 1972).

The experiments described in this paper happened to fall into two groups, one done during January, February and March, the other during July, August and September and the mean change in membrane resistance between pH 9·8 and pH 5·0 for the two groups was 4·3-fold (± 0·55 S.E. of mean) and 10·6-fold (± 2·2 S.E. of mean) respectively. The Student's *t* test for small samples showed the means to be different at the 2% level. This difference is most probably due to variations in the chloride contribution

to the membrane conductance (cf. Hutter & Warner, 1967*a*, *b*; Kao & Stanfield, 1968). The frogs used at the beginning of the year had been in cold store longer than the others and could have been in a different osmotic state. The osmotic strength of the fluid bathing frog muscle is known to influence the resting anion and cation conductances (Sperelakis & Schneider, 1968) and the pH-sensitivity of the chloride conductance may also be affected. We found the ^{36}Cl efflux from muscles taken from three visibly water-logged frogs to be rather insensitive to extracellular pH changes. Alterations in the chloride conductance of muscle accompanying disturbances in water balance also characterizes hereditary myotonia in goats (Lipiky & Bryant, 1966).

Although the cation conductance remains relatively insensitive to variations in the extracellular pH value in the presence of rubidium, a small increase in conductance was seen on passing from alkaline to acid solution. Rubidium is known to interfere with the movements of potassium (Sjodin, 1961; Adrian, 1964) and it seems possible that the degree of interference between the cations varies with pH. The interference between chloride and nitrate is known to be pH dependent (Hutter & Warner, 1968).

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