

THE DECLINE OF POTASSIUM
PERMEABILITY DURING EXTREME HYPERPOLARIZATION
IN FROG SKELETAL MUSCLE

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SUMMARY

1. The voltage-clamp technique was used to separate the effects of K depletion in the T-system from the decline in K permeability during hyperpolarization, and to characterize the time- and voltage-dependence of the latter.

2. K permeability due to the inward rectifier can be described as being proportional to a parameter which diminishes when the membrane is hyperpolarized beyond -120 mV. The parameter obeys first-order kinetics. At 24° C, it can change with a time constant of 49 msec at -150 mV and 25 msec at -65 mV. At -200 mV the fall in membrane conductance due to the permeability change is to 30% of its initial value. The Q_{10} for the rate of conductance change at that potential is about 2.8.

3. It is estimated that K inward current can lower the average K concentration in the T-system by more than 50%, and that, on the average, the space enclosed by the T-system should be less than 0.8% of the fibre volume. Assuming the T-system space to be 0.3% of the fibre volume, it is calculated that on the average, and during hyperpolarization to about -150 mV, no more than 20% of the initial current should flow across the surface membrane.

INTRODUCTION

Adrian & Freygang (1962*a*) have found that when one hyperpolarizes a muscle fibre by passing inward current, the K conductance declines with time. They explained their observation by assuming that inward current diminished the K-concentration in the T-system. In a previous paper

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(Almers, 1972) it was shown that this is indeed the case, and that at membrane potentials less negative than -120 mV, potassium depletion can account for the slow hyperpolarization described by Adrian & Freygang and for the decline of inward current observed under voltage-clamp conditions (Adrian, Chandler & Hodgkin, 1970*a*).

There is evidence that at more negative potentials, the K permeability diminishes too (Adrian *et al.* 1970*a*; Almers, 1972). In that potential range, the decline of K conductance appears to be due to a change of K equilibrium potential (V_K) in the T-system as well as to a permeability decrease, both of which seem to occur with similar rates at potentials where the decline can conveniently be observed.

Situations where V_K and K permeability both vary have been encountered previously, most notably in voltage-clamp experiments on the squid giant axon (Frankenhaeuser & Hodgkin, 1956). In this preparation, the instantaneous current-voltage relation for K is very nearly linear and V_K changes uniformly. This allows one to distinguish clearly between the two effects.

In skeletal muscle, one is not so fortunate. The instantaneous current-voltage relation is strikingly non-linear, and changes in V_K during current flow are clearly non-uniform, as they involve only the T-system and not, for instance, the surface membrane. The distinction between depletion and permeability change therefore has to be made indirectly. In the present study, this was done by investigating the kinetics of recovery from the effects of hyperpolarization.

METHODS

All experimental procedures were the same as those described previously (Almers, 1972). As before, the voltage-clamp technique employed three micro-electrodes inserted into the pelvic end of a frog sartorius muscle fibre. Two of these, located at distances a and b or l and $2l$ from the end of the fibre, served to measure the potentials V_1 and V_2 . V_1 was controlled by injecting current through the third electrode. Membrane current was measured as the difference ($V_2 - V_1$) and was calculated by the approximate equations

$$\left. \begin{aligned} I_m &\doteq \frac{\phi}{2R_1(b^2 - a^2)} (V_2 - V_1), \\ I_m &\doteq \frac{\phi}{6l^2R_1} (V_2 - V_1) \end{aligned} \right\} \quad (1)$$

or, if referred to a unit fibre volume, by

$$\left. \begin{aligned} j_m &\doteq \frac{2}{R_1(b^2 - a^2)} (V_2 - V_1), \\ j_m &\doteq \frac{2}{3l^2R_1} (V_2 - V_1), \end{aligned} \right\} \quad (2)$$

where ϕ is the fibre diameter and R_i the internal resistivity of the sarcoplasm. R_i was calculated from Nakajima's values (303 Ω cm at 2° C and 180 Ω cm at 18.5° C, cited in Adrian *et al.* 1970b) by assuming a linear dependence between $\log R_i$ and $1/T$ (° K). Note that the quantity j_m is independent of the fibre diameter.

All external media employed in this study were poisoned with tetrodotoxin and were free of permeant anions. Under these conditions, only potassium ions can carry appreciable current across the membrane. The composition of the solutions is given in Table 1. Unless indicated otherwise, experiments were carried out in the solution containing 10 mM-K (solution A).

TABLE 1. Composition of solutions

Solution	K ⁺ (mM)	Na ⁺ (mM)	SO ₄ (mM)	Glucuro- nate (mM)	CaSO ₄ (mM)	Ca ²⁺ glucuro- nate (mM)	Sucrose (mM)
A	10	70	40	—	7.9	—	113
B	40	40	40	—	7.9	—	113
C	117	—	—	117	—	1.8	—
D	190	—	95	—	7.9	—	—

All solutions were buffered with 1 mM-Tris (hydroxymethyl) aminomethane maleate buffer to give a pH between 7.0 and 7.4; tetrodotoxin was added to all solutions to give a concentration of 10^{-7} M.

RESULTS

Recovery of K conductance from hyperpolarization beyond -120 mV

The recovery can conveniently be investigated by two-pulse experiments of the kind schematized on the left-hand side of Fig. 1. The conductance is first diminished with a hyperpolarizing conditioning pulse, then a test pulse is applied t (sec) after the end of the conditioning pulse. Initial currents during the pulses are the measured parameters. The effects of the conditioning pulse are completely reversed within 10 sec. Recovery could therefore be described by the ratio $I_2(t)/I_2(10 \text{ sec})$ of the initial currents $I_2(t)$ and $I_2(10 \text{ sec})$ during test pulses after t (sec) and 10 sec intervals, respectively. In practice, however, it was often necessary to introduce a correction for small conductance changes which occurred during the course of an experiment but were not caused by the conditioning pulses. Such changes were indicated by the slight variations of initial currents during conditioning pulses. It was therefore considered preferable to describe recovery by the variable $R(t)$,

$$R(t) = \frac{I_2(t) I_{1, 10 \text{ sec}}}{I_2(10 \text{ sec}) I_{1, t}}, \quad (3)$$

where $I_{1, t}$ and $I_{1, 10 \text{ sec}}$ are the initial currents during the conditioning pulses belonging to the pulse pairs with intervals of t (sec) and 10 sec, respec-

tively. The correction factor $I_{1, 10 \text{ sec}}/I_{1, t}$ never differed from unity by more than 10%.

On the right-hand side of Fig. 1, the magnitude $(1 - R(t))$ is plotted on a logarithmic ordinate scale against the interpulse interval. The Figure illustrates the recovery of conductance after a long pulse to -111 mV (curve A) and after pulses to -161 mV of varying duration (curves B-E). After the pulses to -161 mV , recovery shows a rapid and slower phase,

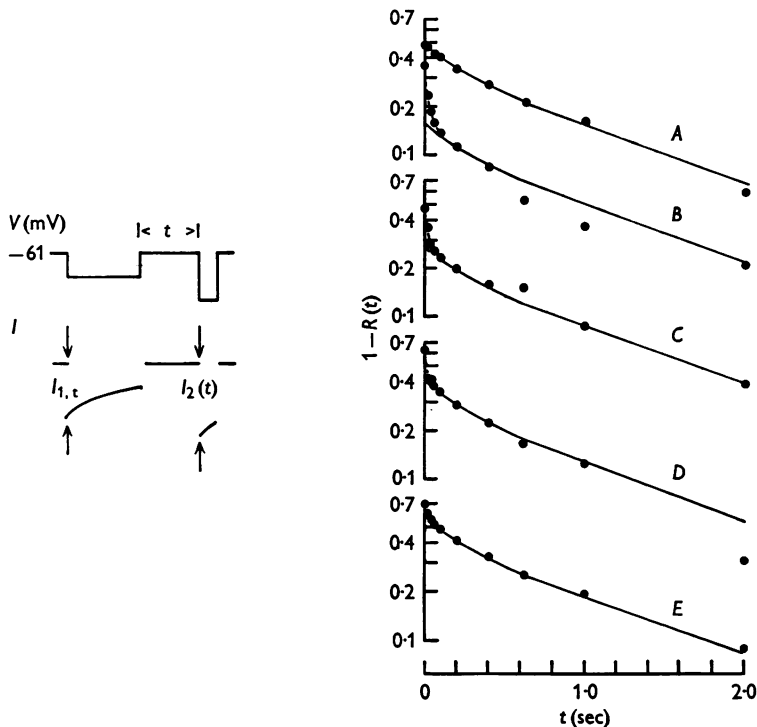


Fig. 1. Left: schematic drawing illustrating the pulse sequence and defining the variables of the experiment. Right: kinetics of recovery from a 3 sec conditioning pulse to -111 mV (curve A) and others to -161 mV (curves B-E) of varying duration. The variable on the ordinate represents the conductance deficit found t (sec) after the conditioning pulse and is defined in the text; interval between conditioning and test pulse is given on the abscissa. Note logarithmic ordinate scale. Durations of conditioning pulses were 0.05 sec in curve B, 0.08 sec in C, 0.2 sec in D and 0.5 sec in E. Continuous line in curve A fitted by eye; in all other curves, the continuous line in curve A was scaled to fit the points at $t = 0.2 \text{ sec}$. Test pulses were to -161 mV . Initial currents were estimated by extrapolation along a straight line which intersected the current trace at 10 and 20 msec after onset of pulse. Interval between successive pulse pairs 15-25 sec. Resting and holding potential -61 mV ; temperature 22.5° C ; $l = 0.5 \text{ mm}$; fibre 67-4.3.

while only a slow phase is present after the pulse to -111 mV. It was shown in a previous paper (Almers, 1972) that the fast phase represents the reversal of a permeability decline which occurred during the conditioning pulse, while the slower phase represents the re-filling of the T-system with K ions which had been removed during the conditioning pulse. One would not necessarily expect the latter process to be exponential or to proceed with the same time course regardless of duration and amplitude of the preceding conditioning pulse. From Fig. 1, the latter nevertheless seems to be approximately true for $t > 0.2$ sec, when the fast phase is complete and permeability has reached its resting value. In order to compare the slow phases in curves *A-E*, a line was drawn by eye through the points obtained after the long pulse to -111 mV (curve *A*). For each of the other curves, the continuous line was then obtained by scaling curve *A* to fit the points at $t = 0.2$ sec. There is some indication that the slow phase of recovery was somewhat accelerated when the conditioning pulse was short and depletion only slight, e.g. in curve *B*. Apart from this rather small effect, however, the lines give a good fit to the experimental points under conditions where the degree of depletion produced by the various conditioning pulses must have varied by more than a factor of three. The experiment suggests that one can determine the extent of depletion and permeability change produced by a pulse by measuring to what extent its effects are reversed via slow and fast phase. First, however, one needs a formalism for describing currents through the inward rectifier.

A model for currents through the inward rectifier

The dependence of ionic currents on the membrane potential can be described generally in terms of a kinetic and an instantaneous factor (Noble & Tsien, 1968, 1969). The kinetic factor in the present system will be called $P(V, t)$ and assumed to depend only on the membrane potential and time. It will represent the permeability changes which occur for instance, during the fast recovery phase. In the steady state at the resting potential, P is assigned the value 1. The variable diminishes upon hyperpolarization, but is assumed not to change instantaneously when the membrane potential V is varied.

Fig. 2 shows the equivalent circuit on which the present analysis is based; it is a modified version of the circuit proposed by Noble & Tsien (1968) for a similar system in sheep Purkinje fibres. The two branches represent the current paths through T-system and surface, and the two batteries $V_{K,S}$ and $V_{K,T}$ the K-equilibrium potentials there. The diodes represent the instantaneous rectifier property and the elements labelled $P(V, t)$ the time-dependence. If the K concentration in the T-system remained constant and the same as that on the surface, one could attempt to

describe K currents (I) through the inward rectifier at a potential (V) by an expression of the type

$$I = P(V, t)f(V, V_{K,S})$$

where the function $f(V, V_{K,S})$ represents the instantaneous current-voltage relation which is that of an inward rectifier. This equation is incomplete, however, since time-dependent conductance changes can result

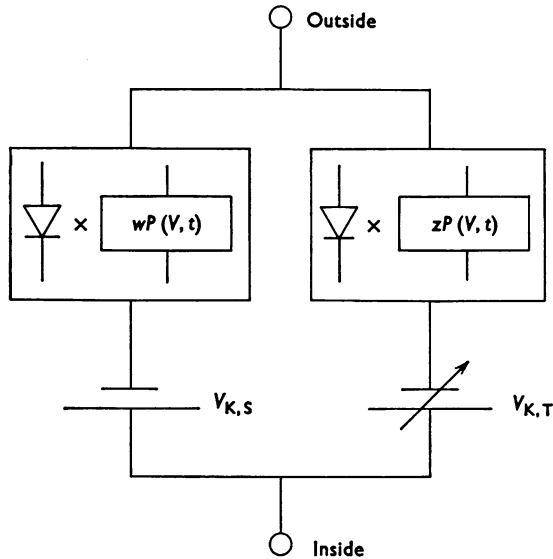


Fig. 2. Equivalent circuit of the inward rectifier in frog skeletal muscle. $V_{K,S}$ and $V_{K,T}$ are the K-equilibrium potentials at the surface and in the T-system, respectively; the possibilities of accumulation and depletion of K ions in the T-system have been depicted by drawing $V_{K,T}$ as a variable battery. The diodes represent the rectifier property; $P(V, t)$ is the permeability variable, and w and z are the relative contributions of surface and T-system to potassium permeability in the hyperpolarized fibre.

not only from variations in $P(V, t)$ but also from alterations of the K concentration in the T-system. Assuming that P affects K-permeable sites in the tubules and on the surface to the same extent, one can write the more complete equation,

$$I = PQf(V, V_{K,S}), \quad (4)$$

where Q is defined as that fraction to which the conductance has been reduced on account of K concentration changes in the T-system. $Q = 1$ at rest, when tubular and external K concentrations are the same. The relation between the value of Q and the tubular K-concentration may otherwise be complicated and need not be specified at this point. In terms of the

variable $R(t)$ defined in eqn. (3) one has for the recovery from a hyperpolarizing conditioning pulse

$$R(t) = P(t)Q(t) \quad (5)$$

The correction factor $I_{1, 10 \text{ sec}}/I_{1, t}$ is assumed to be unity, since only conductance changes due to the conditioning pulse are considered here. For interpulse intervals equal to or larger than 0.2 sec, one has $R(t) = Q(t)$, since the fast phase is complete after 0.2 sec and presumably $P = 1$ at that time.

One could obtain $P(t)$ during the first 200 msec of recovery if the values of $Q(t)$ were known over that interval. They can be estimated on the basis of data obtained after a conditioning pulse to potentials less negative than -120 mV, during which P is expected to remain unity. It is clear from Fig. 1 that recovery from depletion follows about the same time course for $t \geq 0.2$ sec, regardless of duration and amplitude of the conditioning pulse. It will be assumed that this is approximately the case also for $t < 0.2$ sec:

$$\frac{1 - Q(t)}{1 - Q(0.2 \text{ sec})} \doteq \frac{1 - Q'(t)}{1 - Q'(0.2 \text{ sec})}, \quad (6)$$

where the primed quantities refer to recovery from a pulse to potentials more positive than -120 mV. Isolating $P(t)$ from eqns. (5) and (6) one obtains

$$P(t) \doteq \frac{R(t)}{1 - [1 - R(0.2 \text{ sec})][1 - R'(t)]/[1 - R'(0.2 \text{ sec})]}. \quad (7)$$

The right-hand side contains only measurable quantities. With

$$\nu = \frac{1 - R'(0)}{1 - R'(0.2 \text{ sec})} \quad (8)$$

the values of $P(0)$ at the end of any conditioning pulse can be obtained by

$$P(0) \doteq \frac{R(0)}{1 - \nu[1 - R(0.2 \text{ sec})]}. \quad (9)$$

In order to determine, for instance, from curve C in Fig. 1 the extent to which permeability had declined at the end of the 80 msec pulse to -161 mV, one can proceed as follows in accordance with the above treatment. The continuous line is curve A scaled to fit the point at $t = 0.2$ sec and represents recovery from depletion. Its intercept of 0.28 suggests that at the end of the pulse, depletion alone could account for a conductance decrease to $(1 - 0.28) = 72\%$ of the resting value, or that $Q(0) = 0.72$. Immediately after the pulse, however, the conductance was diminished

to $(1 - 0.47) = 53\%$; at that time the permeability therefore was presumably $0.53/0.72 = 74\%$ of its value at rest, and $P(0) = 0.74$ by eqn. (5) or eqn. (9).

Inadequacy of an alternative model

Instead of eqn. (4), one might have considered an expression of the type

$$I = (P + Q)f(V, V_{K,S}),$$

implying that the two parameters P and Q operate on separate populations of K-permeable sites. In Fig. 1, it is seen that depletion alone could reduce the current during a test pulse by at least 50% or even 60% if one extrapolates along the continuous curve E . This gives a lower limit for the fraction of the rectifier sites which reside in the T-system. If the fast process operated only on the surface, as the above expression implies, it should reduce the permeability by less than 50%, even if it could completely abolish ion transport across the surface membrane. As shown previously (Almers, 1972, Fig. 4) the fast process can lower the membrane permeability by 60–70%, indicating that it also operates in the transverse tubules.

Effect of the membrane potential on the variable P

Fig. 3 shows results from two-pulse experiments which illustrate the time- and voltage dependence of the parameter P . The ordinate gives values of P at the ends of conditioning pulses to the potential given next to each curve and of the duration given on the abscissa. P was determined as outlined above and calculated by eqn. (9). The points are fitted well by exponentials (continuous lines); the time-dependence of permeability is therefore adequately described with a variable obeying first-order kinetics. The time constants τ_p of the exponentials, and their steady-state values P_∞ are plotted against the membrane potential in Figs. 4 and 5 (open circles) together with results from similar experiments on other fibres. The curve relating P_∞ to the membrane potential is S-shaped. The potential-sensitive fraction is half maximal near -165 mV. At very negative potentials where P_∞ is small, this fraction changes about e-fold for a 12.6 mV potential change.

There is a tendency in Fig. 3 for the points obtained with the longest conditioning pulses to fall above the continuous lines, as if during these pulses, P went through a minimum and then increased again. However, depletion at the ends of these pulses must have been severe, and small variations in the correction factor ν would seriously affect the estimates of P . What looks like an increase of P with time during long pulses could be explained if recovery from short pulses proceeded somewhat faster than after long pulses. Small variations in ν are expected, since the T-system is unlikely to behave like a single compartment (see Discussion). The extent of uncertainty is illustrated by the scatter of points over the range of -120 to -160 mV in Fig. 5, which includes results from experiments

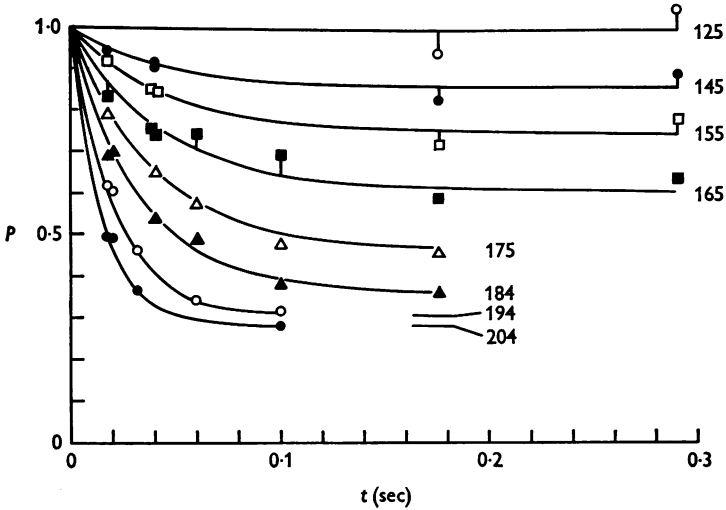


Fig. 3. Effect of membrane potential on K permeability. Ordinate, values of P at the end of conditioning pulses to potentials V and of duration t (sec). V is given in mV next to each curve. P was calculated by eqn. (9); continuous lines are exponentials fitted to the points by eye. The value of ν was the average of two determinations with 0.29 sec and 2.9 sec pulses to -95 mV. Test pulses to -145 mV. Resting and holding potential -65 mV; temperature 24°C ; $l = 0.5$ mm; fibre 84-1.2.

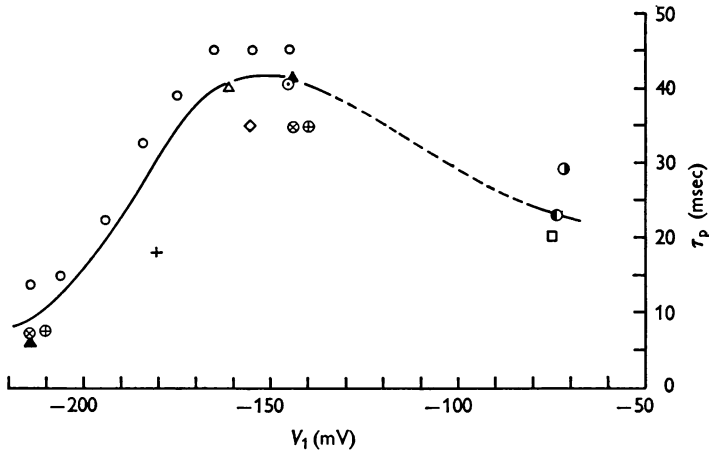


Fig. 4. Effect of membrane potential on the time constant τ_p of K-permeability change at 24°C . \bullet \square time constants of recovery from 30 msec pulses to beyond -200 mV, corrected for recovery from depletion by eqn. (7). \circ Same experiment as in Fig. 3. \triangle \diamond $+$ from other experiments of the kind illustrated in Fig. 3; details are given in entries under the same symbols in the legend of Fig. 5. \odot \otimes \oplus \blacktriangle From experiments of the kind described in Fig. 7. Experiments were carried out at temperatures ranging from 17.5 to 26°C ; the time constants at 24°C were calculated by assuming a linear relation between $\log(\tau_p)$ and $1/T$ ($^\circ\text{K}$) and a Q_{10} of 2.8.

where P_∞ was measured on the same fibre with both, long and shorter pulses. The effect of an error in ν on the estimates of P_∞ is probably less serious at more negative potentials, where the K permeability appears to shut off before substantial depletion can occur.

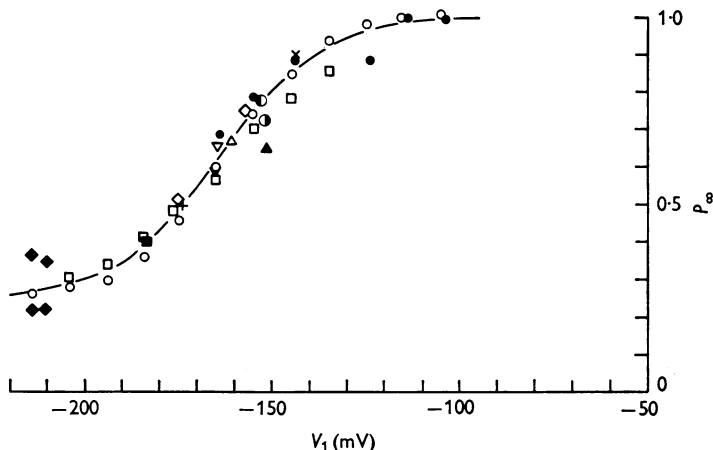


Fig. 5. Steady-state relation between the parameter $P(V)$ and membrane potential. \triangle fibre 67-4.3: ν from a 3 sec pulse to -111 mV; duration of the conditioning pulse used to measure P was $t_{CP} = 0.27$ sec. \bullet \circ \blacktriangle fibres 80-1.3, 80-1.4, 80-2.1; ν from 3 sec pulses to -124 , -122 , -122 mV, respectively; $t_{CP} = 0.475$, 0.55 , 0.2 sec, respectively. ∇ \blacktriangledown fibre 81-3.2: ν from 1.45 sec pulse to -115 mV, t_{CP} were 0.1 sec and 0.93 sec, respectively. \square \blacksquare fibre 82-1 a 1, same as in Fig. 6, where details are given. \circ fibre 84-1.2 same as in Fig. 3: ν is the average of two determinations with 0.29 and 2.9 sec pulses to -95 mV. $+$ \diamond fibres 84-2.1 and 85-1.1: ν from 1.45 sec pulses to -95 mV; points were obtained by fitting exponentials to data as in Fig. 3. \bullet \times fibre 85-3.1: ν average from determinations with 0.48 sec and 2 sec pulses to -93 mV; t_{CP} was 0.175 and 0.48 sec, respectively. \blacklozenge from four different fibres from experiments of the kind illustrated in Fig. 7. All experiments were carried out at temperatures ranging from 17 to 24° C. Holding and resting potentials ranged from -60 to -65 mV.

Errors due to non-uniformity of the membrane potential are probably small in these experiments. The extent of uniformity along the end of the fibre is determined by the electrode spacings l , which varied between 0.3 and 0.5 mm. At the end of conditioning pulses, $-(V_2 - V_1)$ was usually in the range of 4 – 7 mV, and always less than 10 mV. During a displacement in V_1 of 90 mV, $(V_2 - V_1) = 10$ mV corresponds to a length constant of $l/0.273$. By linear cable theory, the total longitudinal span of potentials over the length $2l$ was therefore less than about 13 mV in the above experiments. This is unlikely to cause serious error, if one considers that neither P_∞ nor the steady-state membrane current (and hence depletion) during hyperpolarization beyond -110 mV vary very steeply with the membrane potential. The effect of radial potential decrement in the T-system can be estimated by using the model of Adrian *et al.* (1969). With a resistivity of the tubular lumen of 300Ω cm (Schneider, 1970), the model predicts that in a fibre of 80μ diameter and tubular wall conduct-

ance $\bar{G}_w = 0.45$ mho/cm² fibre volume, a fall in \bar{G}_w by 75% would reduce the total tubular conductance by 75%. At extremely negative potentials, P_∞ might therefore fall as low as 0.22 instead of the value of about 0.25 indicated in Fig. 5. Distortion of the P_∞ curve due to radial potential decrement should also be insignificant, since the relevant measurements are made under conditions where the membrane conductance is small.

The effect of changes in P on the current-voltage relations

In the experiment of Fig. 6, instantaneous and steady-state current-voltage curves (curves *A* and *B*) and the steady-state relation between P and the membrane potential (curve *E*) were determined on the same fibre, the latter in the manner outlined above. Curves *C*, *D* and *F* were derived from these measurements. Curve *C* was obtained by multiplying curve *A* by curve *E* and gives the steady-state current-voltage relation expected if only the membrane permeability and not the tubular K concentration had changed during hyperpolarization. It shows a marked negative slope. Curve *D* results from dividing curve *B* by curve *E* and show the steady-state current-voltage curve corrected for the permeability change. In contrast to curve *B*, it shows no negative slope, indicating that the variation of membrane permeability as determined by the above method can account for this effect.

Curve *F* was obtained by dividing curve *D* by curve *A* and indicates to what fraction of its initial value the current has been reduced in the steady state due to depletion alone. It falls to a minimum of about 0.4, indicating that at least 60% of the initial K conductance must reside in the tubules. Curve *F* shows a slight tendency to increase again at very negative membrane potentials. Such an effect would be expected, since the steady-state currents diminish in that range and depletion should become less severe.

However, the rather rapid decline of inward current at these potentials makes accurate extrapolation to the beginning of pulses difficult; the negative end of curve *A* and consequently of curve *F* is therefore somewhat uncertain.

An increase in curve *F* at very negative potentials was also observed where the steady-state current did not indicate a negative slope conductance. This can be explained if ions other than K begin to carry appreciable current in that potential range. Although most fibres tolerate relatively short hyperpolarizations to beyond -200 mV, a slow and irreversible increase in membrane conductance always occurs during longer pulses at potentials more negative than -160 mV and presumably represents partial loss of the membrane's selectivity due to dielectric break-down. It is conceivable that during the long (2 sec) pulses used to determine the steady-state curve (*B*), this process in some fibres contributes to a small extent already at less negative potentials, and masks the negative slope of the potassium current-voltage relation.

Temperature and the kinetics of permeability change

Fig. 7 illustrates an experiment to study the matter. The fibre was first hyperpolarized to -140 mV for 1 sec. By this time, the membrane current had reached a steady state, and depletion should have been close to maximal. The fibre was then further hyperpolarized for varying duration to -210 mV, which produced a further decline of membrane conductance.

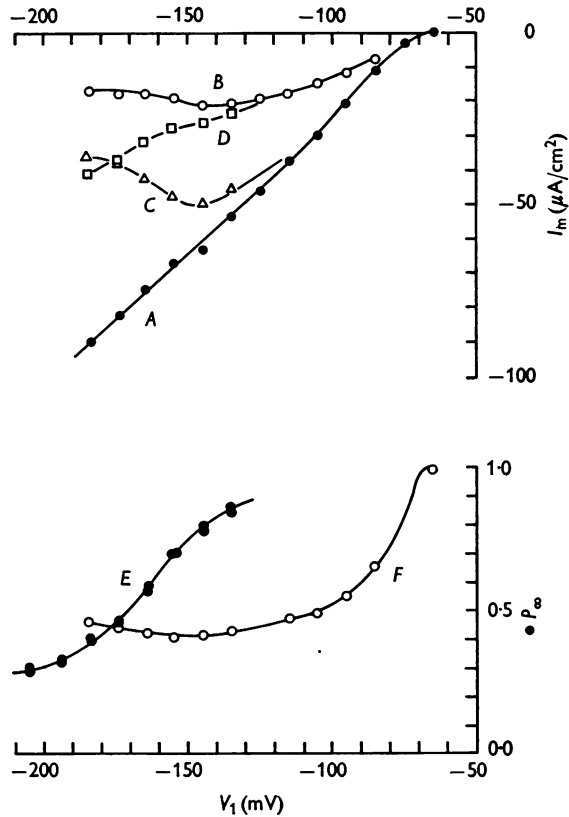


Fig. 6. Experiment illustrating the effect of K^+ permeability change on current-voltage relations. *A*, instantaneous current-voltage relation from the resting potential; values were obtained by extrapolating to the beginning of the pulse from semilogarithmic plots of the decaying parts of the currents. *B*, steady-state current-voltage curve from currents at the end of 2 sec pulses. *E*, steady-state relation of $P(V)$ versus potential in this fibre: ν from a 3 sec pulse to -115 mV; the conditioning pulses lasted 0.17 sec except for those to -194 , -204 and -214 mV which lasted 0.1 and 0.08 sec respectively. Test pulses were to -155 mV. *C* is curve *A* multiplied by curve *E*; *D* is curve *B* divided by curve *E*; *F* is curve *D* divided by curve *A*. Resting and holding potential, -65 mV; temperature 22.5°C ; $a = 0.5$ mm, $b = 0.92$ mm; fibre 82-1 a 1.

Upon return to -140 mV, this process was reversed, and inward current increased along a monotonic curve to the steady-state value which is appropriate for that potential. Since one would not expect the tubular contents to have changed significantly during the short pulse to -210 mV

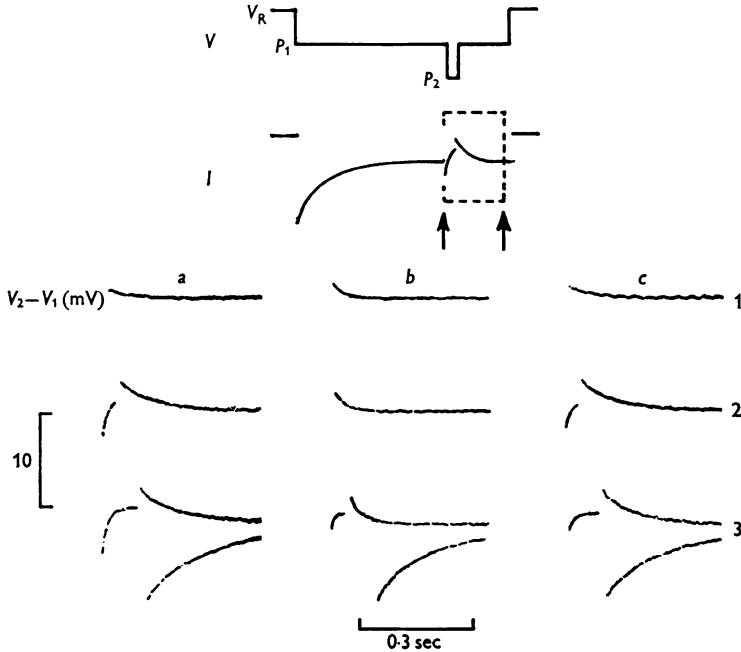


Fig. 7. Effect of temperature on the kinetics of the variable P . Top: drawing which illustrates the pulse sequence used to obtain the records at the bottom. Resting potential $V_R = -60$ mV, $P_1 = -140$ mV, $P_2 = -210$ mV. Bottom: records of $(V_2 - V_1)$ illustrating changes in membrane permeability during the time interval marked by two arrows in the drawing at the top. In row 3, the currents during the initial portions of P_1 are also shown. $(V_2 - V_1)$ is negative throughout, i.e. membrane current is inward at all times. At the 'base lines' in column a , for example, values of $(V_2 - V_1)$ were -6.4 , -6.6 and -6.5 mV in 1, 2, 3 respectively. Resting and holding potential between pulse sequences -60 mV. Temperature 16.3°C in columns a and c and 25.7°C in column b . $l = 0.5$ mm; ordinate scales for I_m are $1\text{ mV} \approx 2.8\ \mu\text{A}/\text{cm}^2$ at 16.3°C and $1\text{ mV} \approx 3.7\ \mu\text{A}/\text{cm}^2$ at 25.7°C .

and the subsequent recovery at -140 mV, the rates of decline and recovery of inward current should give an estimate of the rates of permeability change. Fig. 8 is a semi-logarithmic plot of the current 'tails' at -140 mV which followed the pulses to -210 mV. At a given temperature the experimental points are well fitted by straight lines which have about the same slope, although there was a slight indication that recovery from long pulses was a little slower. Apart from this rather small effect,

however, it appears that the changes observed are predominantly due to a single process which can be fitted by first-order kinetics.

To investigate the effect of temperature, the half-times of recovery and decline at around -140 to -145 and -210 to -215 mV were compared in the same fibre at two different temperatures. The results from four such

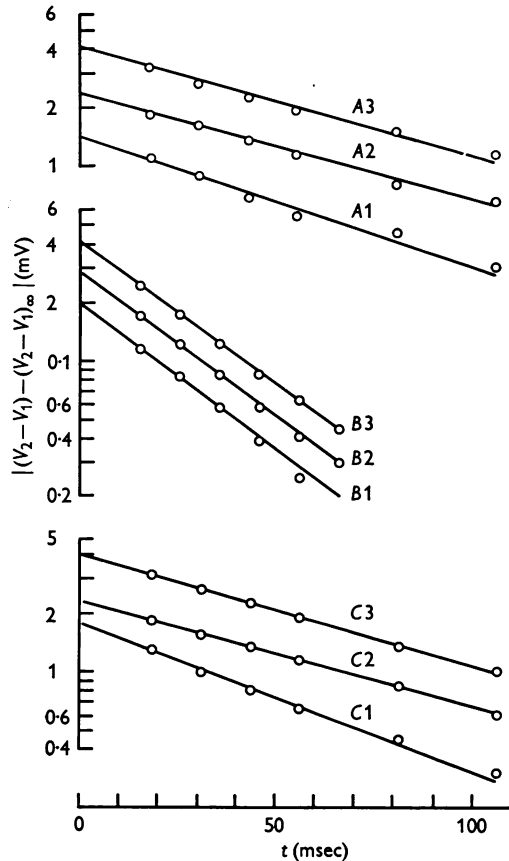


Fig. 8. Semilogarithmic plot of the current 'tails' in Fig. 7. Ordinates, displacement of $(V_2 - V_1)$ from its steady-state value $(V_2 - V_1)_\infty$ at -140 mV; abscissa, time after the end of the pulse to -210 mV. The straight lines were drawn through the points by eye. Labels refer to the corresponding traces in Fig. 7, i.e. A 1 in this Figure refers to trace α , 1 in Fig. 7.

experiments are given in Table 2. Rates of decline were obtained from currents during hyperpolarizations to -210 and -215 mV of sufficient duration to lead to a steady state; rates of recovery at each temperature are the means of at least two observations per run at each temperature with short (5–10 msec) and long (40–100 msec) hyperpolarizations. The

Q_{10} 's for decline and recovery are between 2.8 and 3.0, and similar to those found for the kinetics of permeability changes in nerve (Frankenhaeuser & Moore, 1963).

The above experiment also gives a measure of the steady-state value of P at -210 mV. From simultaneous pen recorder tracings it appears that the steady-state current at -140 mV in Fig. 7 *a3* was $(V_2 - V_1) = -6.5$ mV. By extrapolation on the semi-logarithmic plot, the current immediately after the pulse to -210 mV was found to be $(V_2 - V_1) = -2.5$ mV. Assuming again that the tubular K concentration did not change significantly, the

TABLE 2. Effect of temperature on kinetics of the variable P

Fibre no.	P_1 (mV)	P_2 (mV)	Q_{10} of recovery	Q_{10} of decline
86-2.2	-144	-214	2.69	2.33
87-1.1	-140	-210	2.89	2.76
87-1.2	-144	-214	3.18	3.26
87-1.3	-144	-194	3.03	2.84
Mean \pm s.e. of mean	—	—	2.95 ± 0.10	2.80 ± 0.19

P_1 , potential during the first step and that at which recovery is measured; P_2 , potential during the second step which produces the decline in conductance. Resting potentials were between -60 and -64 mV. Q_{10} 's were calculated between 10 and 20° C. They correspond to Arrhenius activation energies of 17.8 kcal/mole at -140 to -144 mV and 16.8 kcal/mole at -194 to -214 mV.

pulse is found to have reduced inward current at -140 mV to $2.5/6.5 = 38.4\%$; if P at -140 mV is taken as 0.86 from Fig. 4, it follows that P at the end of the pulse was 0.33. The results from four such experiments are included in Fig. 5 (filled diamonds). There appears to be good agreement with the values obtained with the earlier method.

Experiments at higher external K concentrations

It was concluded previously (Almers, 1972) and appears from Fig. 5 that at potentials less negative than -120 mV, depletion alone causes the decline of inward current. It is of interest whether the behaviour of fibres in media with other K concentrations is consistent with this view. Fig. 9*c* illustrates the decline of inward current or its absence in four different media. All experiments were carried out in the cold in order to avoid contractile repriming (Hodgkin & Horowicz, 1960*a*; Heistracher & Hunt, 1969; Caputo, 1971) during the pulses in those solutions where fibres had resting potentials less negative than -60 mV. Fig. 9*a, b* were obtained in solutions with $[K]_o = 190$ mM (solution *D*) and $[K]_o = 117$ mM (solution *C*), resting potentials were near zero. Fig. 9*c* is from an experiment with

$[K]_o = 40$ mM (solution *B*); the resting potential was -31 mV. Current traces obtained in these experiments at potentials between -86 and -91 mV (arrows) will be considered first. In Fig. 9*c*, inward current at -91 mV is reduced to about $\frac{1}{2}$ within 3 sec but changes only slightly at potentials of -86 and -90 mV in Fig. 9*a, b*. This is to be expected, since

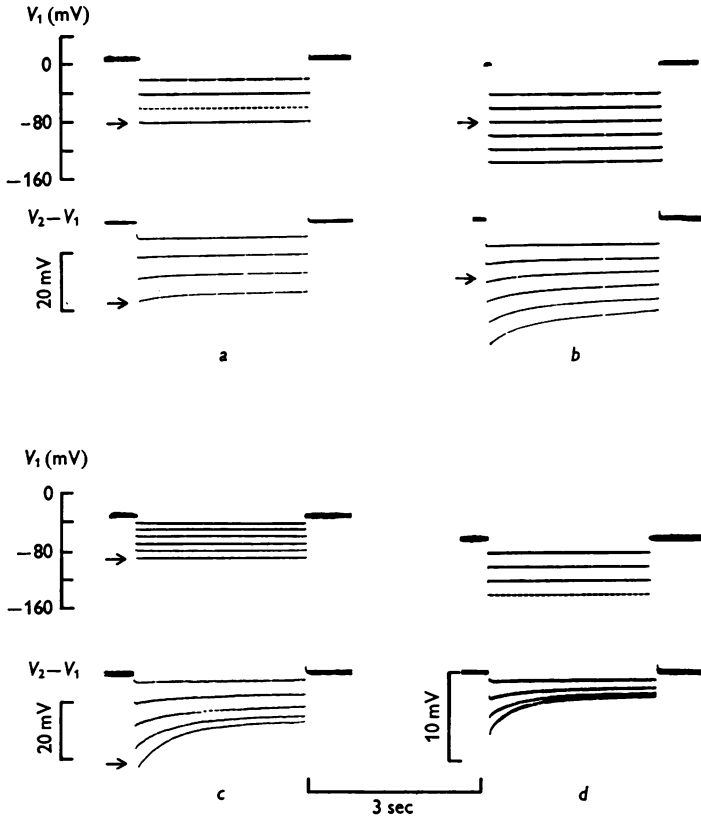


Fig. 9. Inward currents at different K-concentrations $[K]_o$. (a) $[K]_o = 190$ mM (solution *D*); resting and holding potential $+4$ mV; (b) $[K]_o = 117$ mM (solution *C*); resting and holding potential -6 mV; (c) $[K]_o = 40$ mM (solution *B*); resting and holding potential -31 mV; (d) $[K]_o = 10$ mM (solution *A*); resting and holding potential -62 mV; Temperatures between 3.7 and 4.9° C; $l = 0.5$ mm in all cases; 1 mV ≈ 1.9 μ A/cm 2 .

the parameter P should not change at these potentials, and any decline of inward current should be due to tubular depletion. Depletion can occur under the conditions of Fig. 9*c*, where potassium in the tubules is being exchanged for sodium during the passage of inward current, but only to a small extent in isotonic K-saline (Fig. 9*a, b*) or not at all, if anions are

excluded from the lumen of the T-system by, for instance, fixed negative charges.

The lowermost traces in Fig. 9*b*, *d* were obtained at membrane potentials of -146 and -142 mV, respectively. In this potential range, there is appreciable decline of inward current also in isotonic K-saline. Again, this is to be expected, since P diminishes in that range. Decline of inward current at about the same potentials is much more marked in Fig. 9*d* ($[K]_o = 10$ mM, solution A) because depletion as well as changes in P contribute.

A quantitative comparison of the data at $[K]_o = 117$ mM with Fig. 5 is complicated by the presence of a very slow component which contributes to the decline of current in K-rich solutions. Preliminary experiments (unpublished) indicate that at room temperature, the half-times of this process range from 10 to 50 sec; the effect is only partly reversible. The extreme slowness of the process alone makes it unlikely that it is in any way related to the permeability changes investigated here. It can be corrected for by drawing straight lines through the later parts of the traces in Fig. 9*b* and taking the difference between these lines and the earlier part of the traces as that part of the current which declines due to changes in the parameter P .

If one considers that in Fig. 9*b*, $(V_2 - V_1)$ was as negative as -50 mV and consequently the membrane potential very non-uniform, it appears that the decline of current at $[K]_o = 117$ mM in so far as it is attributable to changes in P , does not conflict with Fig. 5. Also, the results in Fig. 9 are consistent with the view that P depends on the membrane potential rather than the difference between membrane- and K-equilibrium potential. At $[K]_o = 117$ mM, the decline, which on the basis of its time course can be attributed to changes in P , amounts to perhaps 10% of the initial current when the fibre is hyperpolarized by -100 to -125 mV (trace below the arrow) from the resting potential of -6 mV. On the other hand at $[K]_o = 10$ mM, hyperpolarizing by -110 mV from resting potentials between -60 and -65 mV reduces P_∞ by about 50% (Fig. 5).

DISCUSSION

Besides being an 'instantaneous' or at least very rapid function of the difference between membrane- and K-equilibrium potential (Hodgkin & Horowitz, 1959; Adrian & Freygang, 1962*b*), K-inward currents show a comparatively slower decline with time which is in part due to a permeability change. The purpose of the present experiments was to characterize the voltage- and time-dependence of the latter. The conclusion is that it can be described in terms of a variable P which diminishes upon hyper-

polarization and obeys first-order kinetics. Experiments obtained at different K-concentrations gave no indication that P is determined also by the external K-concentration, as is the so-called 'instantaneous' behaviour of the rectifier. The variable is analogous to, but not identical with, a similar variable introduced by Adrian *et al.* (1970*a*) to describe the decline of K-inward currents, the difference being that these authors did not consider the effects of changes in tubular K-concentration which occur during and after hyperpolarization.

The inward rectifier in fast skeletal muscle fibres appears to be similar in many ways to that found in sheep Purkinje fibres (Noble & Tsien, 1968). As in the present system, a voltage- and time-dependent permeability variable – called s by Noble & Tsien – was found there to operate on a K-channel with inwardly rectifying current-voltage characteristic. As pointed out already by Adrian *et al.* (1970*a*), however, the effects of temperature on the parameters s and P are different. Temperature has twice as large an effect on the kinetics of s than of P , the Q_{10} 's being 6 and about 3, respectively. Also, the steady-state relation between P and the membrane potential spans a much wider range of potentials than that of Noble & Tsien's parameter s , and P affects a larger fraction of the rectifying K conductance.

The variable Q as a measure of $[K]$ in the T-system

In the analysis it was assumed that the extent of recovery from depletion during the first 0.2 sec is approximately constant. This would hold rigorously only if the T-system behaved like a single compartment. Otherwise, especially the initial time course of recovery will depend to some extent on the radial concentration profile at the end of the depleting pulse. In order to gain some idea of the magnitude of such effects, one needs a relation between membrane conductance and the tubular K-concentration.

Experiments in Cl-free media like those employed here have shown that at a sufficiently negative fixed potential of, e.g. -150 mV, initial inward currents depend approximately linearly on the external K-concentration between 2 and 10 mM (Almers, 1971). It appears from curve F in Fig. 6 and column 5 in Table 4 that depletion alone can reduce the K conductance by more than 50%, and that therefore most of the K conductance must reside in the tubules. Consequently, it is reasonable to assume that the linear current-concentration dependence also holds for the T-system. If the surface/volume ratio of the tubules is constant over the fibre cross-section, and if potential decrements between surface and T-system may be neglected, it follows that at a potential of about -150 mV, current through the T-system should depend linearly on the average K-concentration $[\hat{K}]_T$ there, whatever the concentration profile in the T-system.

This can be shown as follows. Consider a cylindrical fibre segment of unit length and radius a . Inside the segment, consider a ring of thickness dr , unit length and radius r where $0 \leq r \leq a$. If $dr \ll r$, its volume is $2\pi r dr$. If $f(r)$ is the tubular K-concentration at distance r from the centre and $h(r)$ the fraction of the fibre volume occupied by the T-system there, one has for the amount of K, dK_T , enclosed by the T-system in the cylindrical ring

$$dK_T = 2\pi r h(r) f(r) dr.$$

The average tubular K-concentration $[\hat{K}]_T$ is defined as the total amount of K contained in the T-system divided by the latter's volume. By integration of the above equation, one has

$$[\hat{K}]_T = \frac{1}{a\rho} \int_0^a r h(r) f(r) dr,$$

where ρ is the fraction of the fibre volume occupied by the T-system. Assuming that at a fixed potential, the current i_T is linearly related to the tubular K^+ -concentration $f(r)$, and also that the potential in the T-system is uniform,

$$i_T = gA f(r),$$

where A is the membrane area and g a proportionality constant. Assume that the surface/volume ratio R of the tubules is independent of r . The membrane area dA of the T-system contained in the cylindrical ring is then $dA = 2\pi r R h(r) dr$, and the current di_T across it

$$di_T = gR 2\pi r h(r) f(r) dr.$$

The tubular current referred to a unit fibre volume, j_T , can then be obtained by integration,

$$j_T = \frac{gR}{a} \int_0^a r h(r) f(r) dr$$

which is a linear function of $[\hat{K}]_T$:

$$j_T = gR\rho[\hat{K}]_T.$$

The variable $Q(t)$ was defined previously as that fraction to which the conductance is reduced on account of depletion alone. Provided it is measured at potentials near -150 mV, one has therefore

$$Q(t) \doteq (1-z) + z[\hat{K}]_{T,t}/[K]_o \tag{10}$$

where $[\hat{K}]_{T,t}$ is the average tubular K-concentration at time t , $[K]_o$ the concentration in the external medium and z the tubular fraction of K conductance when $[\hat{K}]_{T,t} = [K]_o$, i.e. at rest. Numerical calculations (unpublished) have shown that (10) is a good approximation even if one allows potential decrements of the expected magnitude to occur in the T-system, and that the two sides of eqn. (10) are unlikely to have deviated by more than 0.3 in the present experiments.

One can now discuss the validity of the important assumption contained in eqn. (6) concerning the time course or recovery from depletion during the first 0.2 sec. Calculations were performed (W. Almers & P. Barry, un-

published) using the T-system model of Adrian *et al.* (1969). Schneider's (1970) value of $300 \Omega \text{ cm}$ for the resistivity of the T-system lumen was adopted, and the diffusion constant for potassium ions was correspondingly taken to be one third of that in aqueous solution, or $5.7 \times 10^{-6} \text{ cm}^2/\text{sec}$. Assuming 75% of the initial potassium conductance to be due to the T-system and calculating from measured conductances the conductances \bar{G}_w of the tubular wall (Adrian *et al.* 1969), one obtains a fairly good reconstruction of the decline of inward current during hyperpolarization not beyond -120 mV . During subsequent recovery, there is also good agreement between the calculated time course of $[\hat{K}]_{T,t}$ and that observed experimentally for $Q(t)$. It appears from the calculations that (6) is a very good approximation under conditions where currents during depleting pulses have reached a steady state. After pulses of 2 sec duration from a resting potential of -65 mV to -110 mV and -150 mV , for example, ν as defined by eqn. (8) differed by only 1%. The model predicts that recovery should be accelerated and consequently ν increased after shorter pulses which did not lead to a steady state. The calculations showed, however, that after a 0.2 sec pulse to -150 mV , ν is still within 4% of the value found after 2 sec pulses to -110 mV . After pulses of 50 msec duration, ν exceeds the values obtained after 2 sec pulses by as much as 11%, but this is of comparatively little consequence for the estimate of $P(0)$ by eqn. (9) since depletion is only slight in that case and $(1 - Q(0.2 \text{ sec}))$ small. In an experiment of the kind shown in Fig. 3, the calculated variations of ν would cause the estimate of $P(0)$ by eqn. (9) to be too low by 0.06 after a 0.2 sec pulse to -150 mV and by 0.02 after a 50 msec pulse to that potential.

Errors of this kind will be largest if diffusion across the surface and within the T-system occur at the same rate, as expected in the purely 'distributed' T-system models of Adrian *et al.* (1969) and Schneider (1970), and would be smaller if there were a barrier at the mouth of the tubules (Peachey & Adrian, 1972) which makes ionic exchange between T-system and external medium slow relative to the rate of diffusion within the T-system. In this case, radial concentration gradients would be smaller and the behaviour of the T-system would approach that of a single compartment.

Extent of K depletion and magnitude of the depleted space

It is of interest how the conductance changes attributed to alterations of the tubular K-concentration in this and the previous paper (Almers, 1972) compare with present views about the space ρ enclosed by the T-system (Hodgkin & Horowicz, 1960*b*; Peachey, 1965) and the fraction of K conductance residing there (Eisenberg & Gage, 1969). Making a number of simplifying assumptions, one can estimate from the present data these

T-system parameters and also $[\hat{K}]_T$ during hyperpolarization, if one considers that because of eqn. (10), $Q(t)$ should directly yield the time-course of $[\hat{K}]_T$.

The analysis is based on (i) initial current $j(0)$ and (ii) steady-state current $j(\infty)$, both referred to unit fibre volume and measured during a long pulse not beyond -120 mV, and (iii) the initial rate of recovery of $Q(t)$ after that pulse. It is found experimentally, that the time course of $Q(t)$ during the first 100 msec – and consequently that of $[\hat{K}]_T$ – can be fitted rather well by an exponential function. Let its time constant be τ . Since re-entry of K-ions into the T-system proceeds initially almost exclusively by diffusion (Almers, 1972), one can write the approximate equation

$$\frac{d[\hat{K}]_T}{dt} \doteq \frac{1}{\tau} ([K]_o - [\hat{K}]_T); \quad 0 \leq t \leq 100 \text{ msec} \quad (11)$$

and, using (10)

$$\frac{d[\hat{K}]_T}{dt} \doteq \frac{[K]_o}{\tau} \frac{1-Q}{z}. \quad (12)$$

In the steady state, tubular current referred to a unit fibre volume, $j_T(\infty)$, should be approximately equal to the charge carried by the K ions which enter the T-system by diffusion, since the transport number for K ions at the mouth of the tubules is likely to be small:

$$j_T(\infty) \doteq \frac{\rho F [K]_o}{\tau} \frac{1-Q}{z}, \quad (13)$$

where ρ is the fraction of the fibre volume occupied by the T-system, F is the Faraday and Q the value of $Q(t)$ at the end of the conditioning pulse. Solving for ρ and considering that $z < 1$ and $j_T(\infty) < j(\infty)$, one obtains the inequality

$$\rho < j(\infty)\tau/F[K]_o(1-Q). \quad (14)$$

The right-hand side of (14) is an upper limit for the space enclosed by the T-system. Values obtained from eight experiments are given in Table 3, column 6. In the mean, the T-system space should be less than 0.7% of the fibre volume.

If one assumes a value for ρ , one can estimate $[\hat{K}]_{T,\infty}$, $j_T(\infty)$ and z as follows. Since the current across the surface during a low-amplitude pulse is constant and given by $(1-z)j(0)$, one has

$$j_T(\infty) = j(\infty) - (1-z)j(0). \quad (15)$$

Isolating $j_T(\infty)$ between (13) and (15) one obtains

$$j_T(\infty) \doteq -A/2 + \frac{1}{2}[A^2 + 4F\rho[K]_o(1-Q)j(0)/\tau]^{\frac{1}{2}}, \quad A = j(0) - j(\infty). \quad (16)$$

TABLE 3. T-system space and tubular fraction of K conductance. Experiments with pulses of low amplitude

Fibre no.	1 V_p (mV)	2 $j(0)$ (mA/cm ²)	3 $j(\infty)$ (mA/cm ²)	4 (1-Q)	5 τ (sec)	6 ρ_{\max} (%)	7 $j_T(\infty)$ (mA/cm ²)	8 z	9 $[\hat{K}]_{T,\infty}$ (mM)
67-4.3	-111	13.3	6.5	0.482	0.482	0.67	3.67	0.788	3.88
82-1a1	-115	18.5	8.7	0.506	0.341	0.61	5.27	0.815	3.79
89-1.3	-102	13.1	6.7	0.388	0.325	0.58	4.26	0.812	5.22
89-1.6	-116	19.2	9.4	0.439	0.423	0.94	4.15	0.725	3.94
89-1.7	-103	13.0	7.0	0.343	0.344	0.73	3.83	0.754	5.46
89-1.8	-105	23.5	13.1	0.355	0.168	0.64	7.87	0.777	5.43
89-1.9	-105	17.1	9.3	0.380	0.307	0.78	4.84	0.740	4.86
89-1.10	-103	16.0	8.4	0.371	0.270	0.64	5.06	0.786	5.28
Mean \pm s.e.	—	—	—	—	—	0.70	4.86	0.775	4.73
of mean	—	—	—	—	—	0.04	0.48	0.012	0.26

All data were obtained from two-pulse experiments of the kind illustrated in Fig. 1. V_p is the potential during the conditioning pulse, which lasted between 2 and 3.5 sec and was of sufficient duration for the current to attain a steady state. $j(0)$ is the initial current, $j(\infty)$ the final current during the pulse. Recovery was investigated in the usual manner by giving test pulses to potentials between -145 and -161 mV; the exact values for each fibre are equal to those given in Table 4, column 1. $Q = I_2(0)/I_2(10 \text{ sec})$ where $I_2(0)$ and $I_2(10 \text{ sec})$ are the initial currents during test pulses given 0 and 10 sec after the end of the conditioning pulse. τ is the time constant of the exponential function which fits $[1 - I_2(t)/I_2(10 \text{ sec})]$, *vs.* t initially and is given by $\tau = t_1/\log_e\{[I_2(10 \text{ sec}) - I_2(0)]/[I_2(10 \text{ sec}) - I_2(t_1)]\}$ where t_1 varied between 40 and 63 msec. ρ_{\max} is the right-hand side of eqn. (14) and is the upper limit of the T-system space. The remaining quantities were estimated by assuming the latter to be 0.3% of the fibre volume. $j_T(\infty)$ is the steady-state tubular current during the conditioning pulse estimated by eqn. (16), z the initial K conductance due to the T-system calculated by eqn. (15) and $[\hat{K}]_{T,\infty}$ the final average tubular K-concentration by eqn. (10). Resting and holding potentials between -61 and -67 mV; temperatures between 21 and 24°C.

Except for ρ , the right-hand side contains only measurable quantities. Knowing $j_T(\infty)$, z can be calculated from (15) and $[\hat{K}]_{T,\infty}$ from (10). Values for z and $j_T(\infty)$, $[\hat{K}]_{T,\infty}$ at the end of low-amplitude pulses of long duration are given in Table 3, columns 8, 7 and 9 respectively. Assuming $\rho = 0.3\%$ of the fibre volume (Peachey, 1965), it is calculated there that $[\hat{K}]_{T,\infty}$ fell to about 5 mM during pulses to -103 to -116 mV, and that the tubular fraction of the K conductance is about 78%.

If one assumes a linear current- $[\hat{K}]_T$ relation to hold also when $P < 1$, one can extend this analysis to pulses of larger amplitude, provided one corrects for the decline in permeability which occurs in that case. Table 4 was obtained from experiments on the same fibres as in Table 3, but with pulses to about -150 mV. The analysis was based on the following measurements: (i) the initial current $j(0)$ and (ii) the steady-state current $j(\infty)$ during a long pulse to about -150 mV, (iii) the value P_∞ of the permeability variable P at the end of that pulse, and (iv) the initial time constant τ of recovery from depletion by a low-amplitude pulse. Assuming that τ is the same after a pulse to -150 mV, one can use (13) to estimate the steady-state tubular current $j_T(\infty)$ at the end of that pulse. The value of Q at that time is given by $j(\infty)/j(0)P_\infty$, and one can evaluate the right-hand side of (14) as before. Results are given in column 6 of Table 4. In the mean, the upper limit of the T-system space is 0.74% of the fibre volume, which is in good agreement with the results from low-amplitude pulses given in Table 3, column 6.

The values of Q at the ends of the pulses are tabulated in column 5 of Table 4. They indicate to which fraction of the initial value the conductance had declined due to depletion alone, and give an upper limit for the surface contribution to K^+ -permeability. Since $z < 1$, the quantity Q also indicates an upper limit for $[\hat{K}]_{T,\infty}$ by eqn. (10). It is seen that pulses to about -150 mV reduce the average tubular K^+ -concentration by more than 50%.

Assuming a value for ρ , one can again determine $j_T(\infty)$, z and a more precise value for $[\hat{K}]_{T,\infty}$. Correcting for the decline in permeability during the pulse to -150 mV, one can modify eqn. (15):

$$j_T(\infty) = j(\infty) - (1-z)P_\infty j(0) \tag{17}$$

and obtains for $j_T(\infty)$

$$j_T(\infty) \doteq -\bar{A}/2 + \frac{1}{2}[\bar{A}^2 + 4F\rho[K]_o(1-Q)j(0)P_\infty/\tau]^{\frac{1}{2}}, \quad \bar{A} = j(0)P_\infty - j(\infty). \tag{18}$$

The value of z can be calculated by (17) and $[\hat{K}]_{T,\infty}$ by (10). Results for $j_T(\infty)$, z and $[\hat{K}]_{T,\infty}$ at the end of long pulses to -150 mV are given in columns 7, 8 and 9 of Table 4. It was assumed that $\rho = 0.3\%$ as before.

TABLE 4. T-system space and tubular fraction of K conductance. Experiments with pulses of large amplitude

Fibre no.	1 V_p (mV)	2 P_∞	3 $j(0)$ (mA/cm ²)	4 $j(\infty)$ (mA/cm ²)	5 Q	6 ρ_{\max} (%)	7 $j_T(\infty)$ (mA/cm ²)	8 z	9 $[\hat{K}]_{T,\infty}$ (mM)
67-4-3	-161	0.65	28.9	7.84	0.402	0.66	4.381	0.822	2.74
82-1 a.1	-145	0.79	31.5	10.4	0.418	0.63	5.99	0.822	2.94
89-1-3	-150	0.76	32.0	11.3	0.460	0.71	6.10	0.787	2.15
89-1-6	-150	0.77	33.4	13.1	0.510	1.17	4.91	0.681	2.82
89-1-7	-150	0.80	33.2	13.3	0.500	0.95	5.83	0.720	3.07
89-1-8	-150	0.88	51.1	16.2	0.354	0.44	12.20	0.912	2.92
89-1-9	-150	0.83	39.0	13.4	0.418	0.73	6.90	0.798	2.69
89-1-10	-150	0.74	40.6	13.0	0.430	0.63	7.47	0.817	3.02
Mean \pm s.e.	—	—	—	—	0.437	0.74	6.72	0.795	2.92
of mean	—	—	—	—	0.018	0.08	0.86	0.025	0.06

Data from the same fibres as in Table 3, and from similar experiments, except that conditioning pulses of larger amplitude were used. They lasted between 0.9 and 2 sec. V_p , potential, $j(0)$ initial current and $j(\infty)$ steady-state current during the conditioning pulse. V_p was also the potential during the test pulses used to determine Q and τ in Table 3 and P_∞ , except for fibre 82-1 a.1, where test pulses were to -155 mV. P_∞ is the value of P at the end of the pulse and was determined in the usual manner by eqn. (9). $Q = j(\infty)/j(0)P_\infty$ and is the fraction to which the conductance was reduced at the end of the pulse on account of depletion alone. It gives an upper limit for the surface fraction of the K conductance. It also gives an upper limit for the average tubular K^+ -concentration $[\hat{K}]_{T,\infty}$ at the end of the pulse as a fraction of the external K^+ -concentration. ρ_{\max} is the right-hand side of (14) as before. Assuming $\rho = 0.3\%$, $j_T(\infty)$ is given by eqn. (18), z by eqn. (17) and $[\hat{K}]_{T,\infty}$ by eqn. (10).

In the mean, $j_T(\infty) = 6.7 \text{ mA/cm}^2$ and $[\hat{K}]_{T,\infty}$ fell to about 3 mM. Both quantities would be expected to vary with fibre diameter, and some of the scatter could probably be accounted for in that way. The value of $z = 0.795$ is in good agreement with the result $z = 0.775$ obtained with low-amplitude pulses.

Unfortunately, the value of ρ is subject to some uncertainty. Peachey & Schild (1968) have reported that the estimate of 0.3% might be on the low side, the correct value being possibly 30% higher. Also, it has been reported that the volume of the T-system in isotonic sulphate solutions similar to those employed here, might be 1.7 times that in normal Ringer fluid (Freygang, Goldstein, Hellam & Peachey, 1964). If the data are re-analysed assuming $\rho = 0.6\%$, the mean conductance due to the tubules is 95% in Table 3 and 93% in Table 4. The values for $[K]_{T,\infty}$ at the ends of the pulses are then 6 mM in Table 3 and 4 mM in Table 4.

The use of eqns. (11) and (13) for estimating steady-state tubular currents introduces several further possible sources of error. Firstly, the transport number for K ions at the mouths of the tubules has been neglected. On the basis of the T-system model of Adrian *et al.* (1969) one would further expect (W. Almers & P. Barry, unpublished calculations) that the rate of recovery from depletion $1/\tau$ diminishes appreciably even during the first 50 msec, and that such a change would be difficult to detect on the basis of data such as curve *A* in Fig. 1. The initial rate of recovery could therefore be larger than was assumed in eqn. (11) on the basis of measurements made 0 and 40 to 63 msec after a depleting pulse. Thirdly, recovery after a pulse to -150 mV should in such a model proceed somewhat faster initially than after a pulse of lower amplitude, although the good agreement between Table 3, columns 6 and 8, and Table 4, columns 6 and 8, indicates that any such difference should be only slight. The latter two effects should be rather less serious if there is a diffusion barrier at the mouths of the tubules (Peachey & Adrian, 1972), as one might expect from the observation that tubules become narrow and tortuous before they reach the fibre surface and open into the extracellular space (Franzini-Armstrong, 1970) and from the apparent paucity of such openings suggested by the results of Huxley & Taylor (1958).

All three factors mentioned above would contribute to making the right-hand side of (14) larger than ρ , and would also result in the steady-state current being larger than estimated by eqn. (13). The tubular fraction of K^+ -conductance could therefore be somewhat larger than the value of about 80% given in Tables 3 and 4.

The above analysis permits the following conclusions. (i) The space enclosed by the T-system is less than 0.8% of the fibre volume. This is

clearly consistent with the lower limit of 0.2–0.5% given by Hodgkin & Horowicz (1960*b*) and the value of 0.3% obtained by Peachey (1965). (ii) The T-system contributes about 80% and possibly more to skeletal muscle's K^+ -permeability. The data are consistent with the notion that rectifier sites occur with the same density on surface and T-system membranes.

A previous estimate by Eisenberg & Gage (1969) that the tubular fraction of K^+ -conductance is 50–75%, does not seem strikingly inconsistent with the present results, if one considers the difference in methods and experimental conditions, and that the contribution of the T-system probably depends on the fibre diameter (Nakajima & Hodgkin, 1970).

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