J. Physiol. (1963), **169**, *pp.* 91–115
With 12 *text-figures*
Printed in Great Britain

DEPENDENCE OF RESTING AND ACTION POTENTIALS ON INTERNAL POTASSIUM IN PERFUSED SQUID GIANT AXONS

BY T. NARAHASHI*

From the Department of Physiology, Duke University Medical Center, Durham, North Carolina, U.S.A.

(*Received* 28 *January* 1963)

Several attempts have so far been made to change the intracellular concentrations of ions in muscle fibres and axons. This was achieved by soaking tissues in ion-free or hypotonic solutions (Tobias, 1950; Adrian, 1956; Stephenson, 1957; Koketsu & Kimura, 1960), or by injecting solutions intracellularly (Grundfest, Kao & Altamirano, 1954; Gibbs & Johnson, 1962). However, these methods did not allow one to change drastically intracellular ionic compositions at will. An attempt was also made to perfuse the frog muscle fibre internally, but the method was not satisfactory enough to examine any problems concerned (Davies, 1961).

Recently successful methods of intracellular perfusion of squid giant axons have been established by two groups (Oikawa, Spyropoulos, Tasaki & Teorell, 1961; Baker, Hodgkin & Shaw, 1961*a*, *b*). The method of the former group depends on removal of axoplasm by inserting a large capillary (300 μ in diameter), while that of the latter depends on squeezing out axoplasm.

Several recent studies by these methods have demonstrated the importance of sodium, potassium and calcium ions in membrane potential and excitation (Baker *et al.* 1961*a*, *b*; Tasaki, 1962; Baker, Hodgkin & Shaw, 1962*a*, *b*, *c*; Tasaki, Watanabe & Takenaka, 1962; Tasaki & Shimamura, 1962). For example, the resting potential was decreased by lowering the internal potassium concentration, whereas the depolarized membrane was still able to produce action potentials of large amplitude. Raising the internal sodium concentration decreased the spike height, although the spike could be restored to some extent by adjusting the external calcium and magnesium concentrations.

The purpose of the present study is dual. First of all, an attempt was made to perfuse internally medium or small-sized squid giant axons (300–500 μ in diameter) which were rather of an average size at Woods Hole, since the previous investigations by the two groups were done with

* Present address: Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Japan.

axons having a diameter of as large as 700–900 μ and there was some doubt about general applicability of the methods to smaller axons. It will be seen that the squeezing method is quite successful for such smaller axons. Secondly, attention has mainly been focused on the behaviour of membrane potentials in various potassium concentrations on both sides of the membrane, because of the previous observation that action potentials are still produced from the membrane depolarized by low internal potassium (Baker *et al.* 1962*c*; Tasaki *et al.* 1962; Tasaki & Shimamura, 1962). It was found that lowering of the internal potassium caused the conductance–potential relations of the membrane to undergo a considerable shift along the potential axis.

METHODS

Material. Experiments were performed using the giant axons of the squid, *Loligo pealii*, available at Marine Biological Laboratory, Woods Hole, Massachusetts. The diameter of the axons ranged from 300 to 600 μ; most experiments were done with the axons of about 400 μ in diameter.

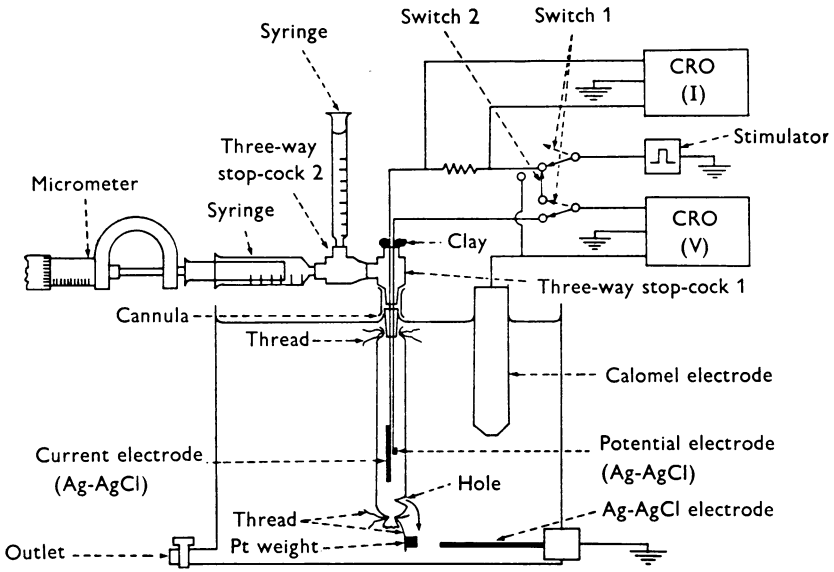

Fig. 1. Diagram of the arrangement for internal perfusion. For stimulation and recording of action potentials, switch 1 is set as shown in the lower position (solid). For measurement of resting potentials, switch 1 is set in the upper position (interrupted); the base line of the pre-amplifier may be checked by short-circuiting its input by means of switch 2.

Internal perfusion. The method of squeezing out the axoplasm was in principle the same as that developed by Baker *et al.* (1961*a*, *b*, 1962*b*). However, the method of perfusing the axon was modified in order to make the procedure simpler. The set-up of perfusion is schematically shown in Fig. 1. A giant axon of about 6 cm length was isolated, tied with a fine cotton thread at both ends, and partially cleaned of connective tissue under a

binocular microscope. A glass cannula (about 250 μ outer diameter and about 150 μ internal diameter), which had been connected with a small three-way stop-cock 1 (Fig. 1) and filled with the standard internal solution, was introduced into a cut near one end, and retied by a fine silk thread. Then the other end of the axon was also cut near the tie, and the axoplasm squeezed out on a rubber plate in three or four strokes by means of a small roller covered with rubber. The preparation was held vertically in a large chamber ($7{\cdot}5 \times 9{\cdot}0 \times 9{\cdot}0$ cm internal) which contained natural or artificial sea water with the tie on the cannula at the level of bath surface. A three-way stop-cock 2 was attached to the side arm of the stop-cock 1, and a syringe (1·5–2·0 ml. capacity) containing the standard internal solution was positioned horizontally and connected to the side arm of the stop-cock 2. The solution in this syringe was driven slowly toward the preparation by means of a micrometer. In order to move the plug of the axoplasm remaining near the cannula, it was necessary gradually to increase the internal pressure. Once the plug had been pushed out from the cut end of the axon, the internal solution could easily be moved by a very slight pressure supplied by the micrometer. Then the cut end of the axon was tied by a fine cotton thread and a small platinum weight was attached to the thread in order to hold the preparation straight. An electrode was pushed down the axis of the axon with the aid of a micromanipulator under horizontal binocular microscope observations. Excitability was maintained for 1 hr or more in the axon containing the standard solution and tied at the lower end.

In order to perfuse the axon continuously, or to change the internal solution, the following procedures were carried out: (1) An additional syringe (0·5 ml. tuberculin type) was attached vertically to the upper arm of the three-way stop-cock 2 and filled with a test solution (Fig. 1). (2) The horizontal syringe attached to the side arm of the stop-cock 2 was replaced by the one filled with the test solution. (3) The opening of the upper arm of the stop-cock 1, through which the electrode had been introduced, was covered by modelling clay in order to prevent solution from leaking out during perfusion. (4) Then the axon was cut near the lower end, (5) and the stop-cock 2 was switched so that the solution of the vertical syringe was perfused through the axon. In many cases these two procedures gave a smooth stream of internal solution through the axon. In other cases, however, some gelatin-like substance, which was probably created from the residue of the axoplasm, clogged the axon, preventing internal perfusion. In such a case the internal solution was gently pushed after rotating the stop-cock 2 so that the horizontal syringe was connected to the axon. The clogging substance was easily pushed out from the cut end of the axon by turning the micrometer. The rate of perfusion could be adjusted by the valve in stop-cock 2. In most cases the rate was of the order of 0·05–0·1 ml./min. When two or more test solutions were to be perfused, the replacement of solution was simply achieved by sucking up the old solution in the vertical syringe and pouring a new solution into it.

External solution was simply changed by draining it off through the outlet of the chamber and pouring a new solution into it. Although the amount of the internal solution coming out from the cut end of the axon was much smaller than that of the bathing fluid, the latter was usually changed after changing the internal solution in order to minimize contamination.

Measurement of membrane potential. The internal electrode consisted of two enamelled silver wires (50 μ in diameter) twisted about each other. One of them was used as a current electrode, the insulation being removed for a distance of 1·5–2·0 cm from the tip. The other was a potential electrode, the insulation being removed for about 1 mm stretch near the middle part of the current electrode. Both electrodes were carefully and daily coated electrolytically with silver chloride by the method of Moore & Cole (1963). The tips of both electrodes were waxed together. For external current electrode, an Ag-AgCl wire was dipped into the bathing fluid. The external potential electrode was a calomel electrode whose saturated KCl solution was in contact with the external medium.

In order to measure the resting membrane potential with various solutions both inside and outside the axon, the potential difference between the calomel external electrode and the internal or external solutions was assumed as zero, regardless of the kinds of the solutions, which are described below. Preliminary observations of the potential difference between Ag-AgCl electrodes and various internal solutions have shown that electrode potential was very unstable in small Ag-AgCl electrodes, whereas it was fairly stable over many hours when the surface area of electrode was large. Because the potential electrode had a small exposed area, and was not stable enough to measure the resting potential, the potential on the larger and stable current electrode was momentarily measured by the pre-amplifier to obtain the resting potential.

The procedure for measuring the resting potential was as follows: shortly before introducing the internal electrode in the axon, it was dipped into the internal solutions to be used and the potential difference between the current electrode and the reference calomel electrode was measured. These potentials ranged from 60 to 130 mV, depending on the chloride concentration. Then the electrode was inserted into the axon, and the potential differences between this electrode and the external calomel electrode were measured from time to time during the experiment. The resting potential was obtained by subtraction of the electrode potential differences. Although the electrode potentials were large in magnitude they were fairly stable, changing not more than 2–3 mV during an experiment which lasted 1–2 hr, unless strong currents (of the order of 10^{-4} A) were passed through the electrode.

Stimulation. In order to observe action potentials, a pulse of cathodal current of about 0·3 msec duration was delivered through the current electrode, while the potential change was recorded by the potential electrode. For the measurement of critical depolarization a cathodal current of 5 msec duration was applied, and the inflexion point between the rising phase of the catelectrotonic potential and the action potential was measured from the resting potential level.

Measurement of membrane resistance. Voltage–current curves were obtained by plotting the steady-state values for electrotonic potential against the intensities of applied square current. Since the applied current spread from both edges of the current electrode, membrane resistance per square centimetre could not be estimated. A 'membrane resistance' was therefore calculated from the slope of the voltage–current curve at the resting potential. However, when low-K solutions were perfused internally, a correction had to be made for *IR* drop across the internal resistance, because the conductivity of low-K internal solutions was very low. In the case where a current electrode of 50 μ diameter and 18 mm length is introduced in an axon of 400 μ diameter containing 6 mM-K solution, the total resistance of the current electrode and the internal solution is calculated as 100 Ω cm^2. The membrane resistance with 6 mM-K inside is estimated as 10 kΩ on an average, from Table 3. If it is assumed that the current does not spread beyond the edges of the current electrode, this value corresponds to 2250 Ω cm^2. It follows that the resistance of the internal solution under this condition is only about one twentieth of that of membrane resistance. In other words, the true value for the membrane resistance is 5% less than the observed value. Further, when the membrane resistance becomes smaller—for example, by delayed rectification or in the presence of high external potassium—the error caused by the internal resistance should be larger; if the membrane resistance becomes half, the error would be 10%. Other evidence for such a small amount of error due to internal resistance is that there is little, if any, rapid component at the onset of the electrotonic potential produced by a weak square current (about 10^{-5} A or less). Since the internal resistance does not contain an RC element, such a jump should have been seen if the resistance was high or if the intensity of applied current was very strong. Actually, a jump is observed with a strong current (about 10^{-4} A), as will be described below (Fig. 12). Unless otherwise stated, uncorrected values are shown.

Solutions. Principal components of the standard internal solutions were potassium sulphate, potassium chloride and buffer to hold the pH at 7·7. Some chloride ions were required in order to measure the resting potential by means of an Ag-AgCl electrode. For low-K internal solutions isotonic sucrose or isotonic glucose solution (Hodgkin & Katz, 1949) was mixed with potassium chloride solution. For K-free medium pH was adjusted by the use of tris buffer and hydrochloric acid, which also provided chloride ions to the solution. The standard internal solution referred to high-K solutions (538 or 1016 mM). The external bathing fluid was natural or artificial sea water. The compositions of solutions are given in Table 1.

Temperature. All experiments were conducted at room temperature ranging from 22 to 25° C.

TABLE 1. Compositions of solutions

Stock solutions for internal solutions (mM)

	K	Cl	SO_4	H_2PO_4	Tris	Sucrose	Glucose
Isotonic K_2SO_4	1060	—	500	30	—	—	—
Isotonic KCl	620	560	—	30	—	—	—
Isotonic sucrose	—	—	—	—	—	930	—
Isotonic glucose	—	—	—	—	—	—	980
Concentrated phosphate buffer	1200	—	—	600	—	—	—
Concentrated tris buffer	—	210	—	—	290	—	—

Proportion of mixture for internal solutions*

	Isotonic K_2SO_4	Isotonic KCl	Isotonic sucrose or glucose	Concentrated phosphate buffer	Concentrated tris buffer
1060 mM-K	100	—	—	—	—
1016 mM-K	90	10	—	—	—
538 mM-K	47·7	5·3	47	—	—
62 mM-K	—	10	90	—	—
10 mM-K	—	1·6	98·4	—	—
6 mM-K	—	0·5	99·25	0·25	—
6 mM-K tris	—	1	98	—	1
K-free	—	—	99	—	1

External solutions (mM)*

	Na	K	Choline	Ca	Cl	HCO_3
Artificial sea water	526	10	—	50	633	2·5
Choline sea water	—	10	560	50	667	2·5
K-rich sea water	—	538	—	50	635	2·5
K-free sea water	536	—	—	50	633	2·5

* Final pH: 7·7.

RESULTS

Resting and action potentials in standard internal solution

Percentage of success. The percentage of success depends largely on the axon diameter. With the axons of 500 μ or more in diameter action potentials of normal amplitude (about 100 mV) were obtained in more than about 95 % of the experiments. With the axons of around 400 μ action potentials

were obtained with about 90 % success. Even with the axons as small as 300 μ in diameter some experiments could be performed successfully.

With the standard solution internally the amplitude of the action potential decreased progressively with time, the rate of decline being largely dependent on the diameter of the axon. In the axons having a diameter of 500 μ or more a nearly constant amplitude was maintained at least for 1 hr. With the axons of 400 μ in diameter, the amplitude decreased slightly 1 hr after starting perfusion, and with the axons of 300 μ in diameter, the decline was faster. However, even after the action potential had decreased considerably in the standard internal solution, it was restored to the normal level by introducing low-K solutions internally; this is described below in detail (p. 99).

Resting and action potentials. The resting and action potentials were of the order of 60 and 100 mV, respectively, as shown in Table 2. An example of records of the action potential is illustrated in Fig. 2*A*. These values are similar to those found in intact squid axons, and agree with those found in perfused axons (Baker *et al.* 1961*a*, 1962*b*; Tasaki & Shimamura, 1962). The only marked difference between 1016 and 538 mM-K internal solutions was that the undershoot following the spike was 6 mV larger (mean) for 1016 mM-K than for 538 mM-K. This is to be expected, because the potassium equilibrium potential with 1016 mM-K inside is some 17 mV more negative than with 538 mM-K.

Some preparations which were perfused with the standard internal solution fired spontaneously. Spontaneous discharges (100–150/sec) usually lasted for 10 min or more, after which the spike height became smaller and finally the discharge stopped. Action potentials of smaller amplitude could be produced by stimulation after a prolonged spontaneous discharge.

Changes in resting and action potentials in low-K solutions

Low-K internal solutions. The resting potential decreased on replacing the standard internal solution with a low-K internal solution, the magnitude being dependent on potassium concentration. The decrease occurred fairly rapidly as soon as the dead space in the perfusion system had been cleared. A series of records of the action potential is illustrated in Fig. 2, in which it is seen that the resting potential is lowered from -58 mV with 1016 mM-K to -40 mV with 62 mM-K, and further to -29 mV with 6 mM-K. Despite these changes in resting potential, action potentials of normal amplitude were still produced by stimulation without anodal polarization. Therefore, the overshoot of the action potential with low-K solutions inside reached as high as 70–80 mV, as against the normal value of about 40 mV with the standard solution inside. The undershoot disap-

TABLE 2. Resting potential, action potential and critical depolarization

$[K]_o$	Sea water	10	10	10	Sea water	10*	10†	10	0	0	538
$[K]_i$	1016	1016	538	62	6	6	6	0	0	6	6
Resting potential											
No. of axons	6	11	28	3	5	25	22	2	5	3	15
No. of measurements	6	11	28	4	8	25	22	2	8	3	15
Mean	−60·5	−62·8	−64·3	−40·7	−24·8	−33·2	−16·5	5·5	11·1	−17·3	42·2
S.E.	±1·7	±1·7	±1·6	±2·6	±5·7	±2·2	±2·6	±12·5	±6·3	±3·0	±5·6
Action potential											
No. of axons	6	10	28	3	6	24	21	2	5	3	15
No. of measurements	6	10	28	4	9	24	21	2	8	3	15
Mean	95·8	100·8	97·1	112·2	96·8	101·0	93·6	72	94·3	133·0	0
S.E.	±3·6	±4·2	±2·2	±6·4	±4·7	±2·6	±3·8	±14	±5·9	±5·5	—
Maximum rate of rise of action potential											
No. of axons	—	—	27	—	—	22	15	1	3	3	—
No. of measurements	—	—	27	—	—	22	15	1	4	3	—
Mean	—	—	790	—	—	468	314	230	155	505	—
S.E.	—	—	±56	—	—	±42	±45	—	±47	±88	—
Maximum rate of fall of action potential											
No. of axons	—	—	27	—	—	22	15	1	—	3	—
No. of measurements	—	—	27	—	—	22	15	1	—	3	—
Mean	—	—	271	—	—	70	44	45	—	28	—
S.E.	—	—	±13	—	—	±9	±8	—	—	±8	—
After-potential											
No. of axons	6	10	28	3	6	19	13	1	—	—	—
No. of measurements	6	10	28	4	8	19	13	1	—	—	—
Mean	−22·1	−21·5	−15·6	3	10·6	7·4	8·2	5	—	—	—
S.E.	±1·0	±0·8	±1·2	±3·4	±2·2	±1·5	±1·9	—	—	—	—
Critical depolarization											
No. of axons	5	7	14	3	4	4	2	—	—	—	—
No. of measurements	5	7	14	4	4	4	2	—	—	—	—
Mean	23·2	24·5	19·5	17·5	21·0	17·5	25·5	—	—	—	—
S.E.	±1·2	±1·4	±1·0	±1·3	±0·5	±1·2	±2·5	—	—	—	—

* Initial stage of 6 mM-K internal perfusion; within 5 min after introducing 6 mM-K. † Terminal stage of 6 mM-K internal perfusion. The values were obtained later than 10 min after the start of perfusion when the axons showed the lowest resting potential and were still excitable.

peared in 62 mM-K internal solution, and converted into a slowed recovery phase in 6 mM-K.

The rising and falling phases of the action potential were slowed down by lowering internal potassium concentration (Table 2). The shape of the action potential was not kept constant throughout a prolonged period of low-K internal perfusion, being further slowed in its time course, especially in its falling phase. The resting potential was also decreased further during this period. These changes are clearly seen in Fig. 3, in which the maximum rates of rise and fall of the action potential are displayed as differential curves.

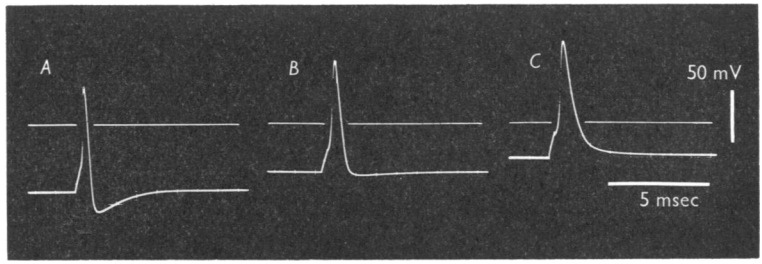

Fig. 2. Records of the action potential from an axon perfused internally with various potassium solutions. *A*, 1016 mM-K (standard solution), 5 min after the start of perfusion; *B*, 62 mM-K, 5 min; *C*, 6 mM-K, 5 min. Artificial sea water is used for the bathing fluid. Zero potential level is shown by the horizontal line.

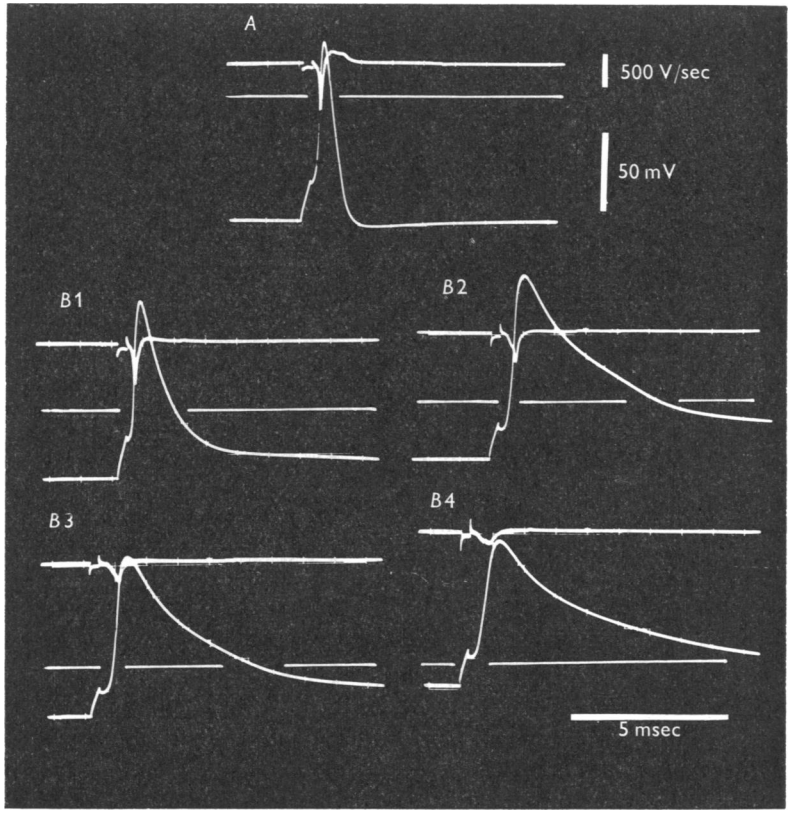

Fig. 3. Records of the action potential and its time derivative at various stages of internal perfusion with a low-K solution. *A*, 538 mM-K (standard solution), 7 min after the start of perfusion; Series *B*, 6 mM-K; *B*1, 19 min after the start of perfusion; *B*2, 43 min; *B*3, 47 min; *B*4, 53 min. Natural sea water is used for the bathing fluid. Zero potential level is shown by the horizontal line.

The mean values for the resting and action potentials are given in Table 2, in which experiments with 6 mM-K inside and 10 mM-K (artificial sea water) outside are divided into an initial and a terminal period. The former group includes measurements done within 5 min after introducing 6 mM-K internal solution, while the latter covers the lowest values obtained from the axons still excitable later than 10 min after the start of perfusion. Therefore the values of the initial period are indicative of the initial effect of low internal potassium concentration, while those of the terminal period represent the axons whose excitability is still maintained despite the minimum values of the resting potential.

It was found that the magnitude of the resting potential depends partly on the rate of perfusion when the latter is very slow. Thus, when a continuous internal perfusion with low-K solution was greatly reduced or suspended, the resting potential began to increase slowly, but when substantial rate of perfusion was resumed, the resting potential decline returned. This may imply that potassium ions continue to enter the axon under these experimental conditions.

As in the standard internal solution, spontaneous discharges were often encountered in low-K internal perfusion. However, the pattern of spontaneous discharge was different in that there were pauses between bursts of discharges. A burst of discharges lasting for several seconds occurred intermittently with a silent period which varied very much in its duration. Some preparations, which did not produce spontaneous discharge during internal perfusion with low-K solution, responded repetitively to a single stimulus. The after-discharge might last for as long as 2–3 sec and ended abruptly. Both spontaneous discharge and after-discharge occurred during the initial period of low-K perfusion, and always disappeared when the perfusion was continued.

There was a tendency for excitability to be somewhat improved by lowering the internal potassium concentration. Thus, even after the action potential had become small after a prolonged period with the standard solution inside, an internal flow of low-K solution was in most cases able to restore the action potential to nearly normal amplitude.

Low-K internal and K-free external solutions. When potassium ions were removed from the external medium while perfusing with a low-K solution internally, the falling phase of the action potential, which had already been prolonged, was greatly slowed down, forming a plateau. A series of records of the action potential is illustrated in Fig. 4. This axon was firing spontaneously with the standard solution inside (record A), but stopped firing when 6 mM-K solution was introduced and the resting potential decreased to -9 mV (record B). Upon replacing artificial sea water with K-free sea water the resting potential was increased to -25 mV, and the

falling phase of the action potential was markedly slowed (record $C1$). There appeared an oscillation on the plateau, which increased in magnitude and finally brought the membrane potential down to the resting potential level (record $C2$). In some preparations such an oscillation was lacking, but a long-lasting plateau was always observed. In record $C2$ of Fig. 4 a small undershoot followed the plateau, but this was not always observed. The oscillation became smaller with the advance of time (Fig. 4, $C4$), the transition from the plateau to the quick repolarization being smooth, resembling the cardiac action potential. The mean values for the resting and action potentials are given in Table 2; the spike amplitude is very large, the overshoot being over 100 mV, more than twice the normal value.

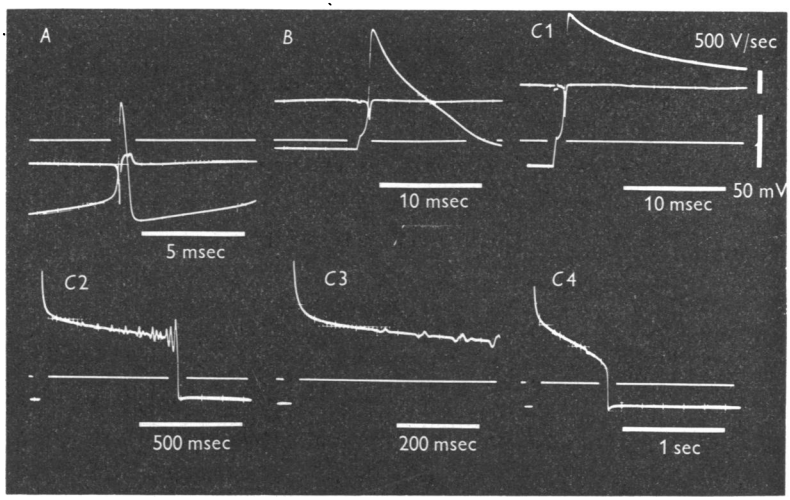

Fig. 4. Records of the action potential from an axon perfused with various potassium solutions. *A*, 538 mM-K (standard solution) inside and 10 mM-K (artificial sea water) outside, spontaneous discharge, 5 min after the start of perfusion; *B*, 6 mM-K inside and 10 mM-K outside, 11 min; Series *C*, 6 mM-K inside and K-free sea water outside; $C1$, 5 min; $C2$ and $C3$, 8 min; $C4$, 15 min. Zero potential level is shown by the horizontal line.

K-free internal solution. Records *A* to *C* of Fig. 5 show changes in action potential when the internal concentration of potassium is reduced from 538 to 6 mM and then to zero, keeping the external potassium concentration constant. The resting interior potential became more positive at the lower internal potassium concentrations, but the duration of the action potential was lengthened in 6 mM-K internal solution and shortened again to some extent in K-free internal solution. It was often observed that the resting interior potential became 10–20 mV positive when there was no potassium in the internal solution. In this case there was a slight depres-

sion of the spike height, because the overshoot of the action potential did not change (from 6 to 0 mM-K).

K-free internal and K-free external solutions. The lower half of Fig. 5 (records *D* 1–4) shows the action potentials when potassium ions are eliminated both from inside and outside the nerve membrane. When records *D* 1 and *D* 2 were compared with record *C*, which was taken with K-free solution inside and 10 mM-K sea water outside, the resting potential was slightly increased from -7 to -12 mV, and the action potential was

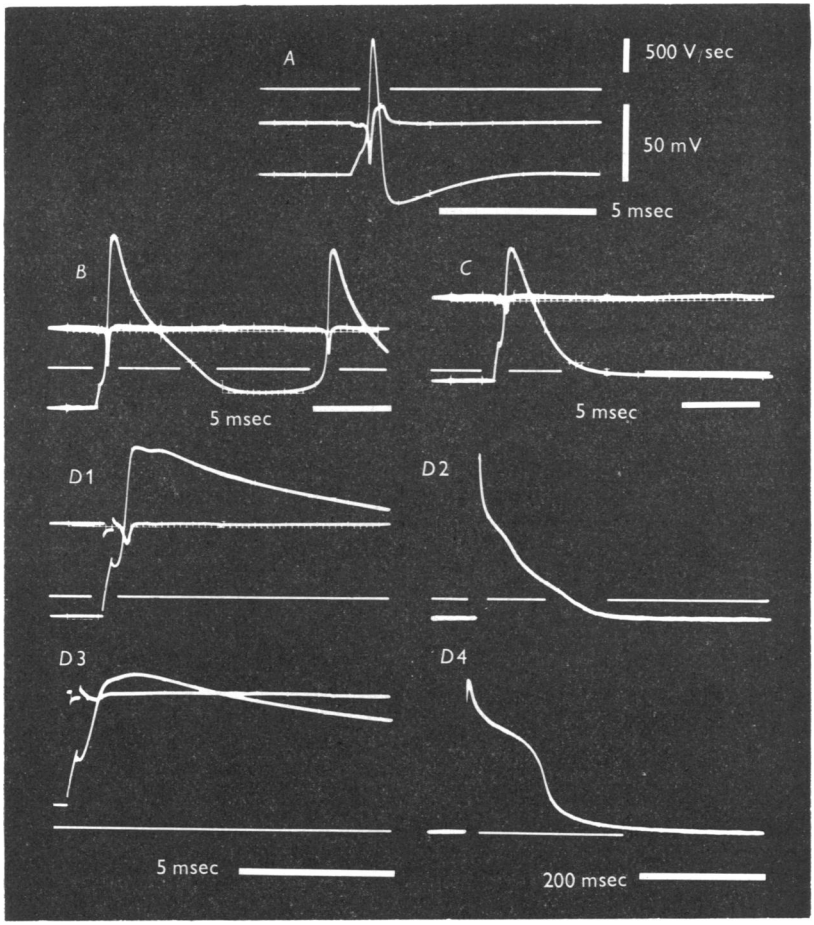

Fig. 5. Records of the action potential from an axon perfused with various potassium solutions. *A*, 538 mM-K (standard solution) inside and 10 mM-K (artificial sea water) outside, 5 min after the start of perfusion; *B*, 6 mM-K inside and 10 mM-K outside, 10 min; *C*, K-free inside and 10 mM-K outside, 5 min; Series *D*, K-free both inside and outside; *D* 1 and *D* 2, 5 min; *D* 3, 10 min; *D* 4, 14 min. Zero potential level is shown by the horizontal line.

increased in height from 88 to 114 mV and prolonged in its falling phase. The resting potential gradually declined with time from − 12 mV to + 15 mV, but action potentials were still elicited by stimulation (record *D* 3). In general, under K-free conditions on both sides of the membrane the shape of the action potential resembled that of cardiac action potential (record *D* 4).

It should be noted that the resting potential under this experimental condition varied considerably from preparation to preparation. It ranged from 12 mV, inside negative with respect to outside, to as much as 36 mV inside positive, yet action potentials of normal amplitude were produced. Therefore the overshoot of the action potential ranged from 80 to 131 mV, which was two to three times as large as the control value obtained with the standard solution inside and sea water outside. The mean values for the resting and action potentials are given in Table 2.

Thus the relation between the potassium concentrations on both sides of the membrane and the shape of the action potential can be summarized as follows: When the internal potassium concentration is low (0–6 mM), a reduction in the external potassium concentration lengthens the duration of the action potential, especially that of the falling phase.

Na-free external solution. Replacement of external sodium by choline caused inexcitability regardless of the internal concentration of potassium. It therefore seems that action potentials under varying internal potassium concentrations are carried by inward sodium current, as in intact axons.

Critical depolarization

The fact that the membrane with low potassium inside is able to produce full-sized action potentials in spite of small resting potentials leads us to the supposition that threshold for excitation may be markedly different from the normal value. However, this was not true as summarized in Table 2, in which it is seen that the critical depolarization, i.e. the amount of depolarization needed for firing, is of the same order of magnitude with low-K inside as with the standard high-K inside.

Relation between membrane potential and action potential

There is general agreement for intact axons that excitability, the ability of the membrane to undergo a sodium conductance rise upon depolarization, depends largely on the value of the preceding steady membrane potential. By the voltage-clamp technique Hodgkin & Huxley (1952*b*) showed that in squid axons a steady depolarization of 30 mV almost completely knocked out the ability of the axon to increase its sodium conductance in response to a standard test depolarizing pulse. The curve relating sodium current to membrane potential (inactivation curve) has been found to shift along the voltage axis in the squid axons exposed to

low-Ca solutions (Frankenhaeuser & Hodgkin, 1957) and in the Purkinje fibres treated with cocaine (Weidmann, 1955).

The effect of a 45 msec displacement of the membrane potential on the action potential is illustrated in Fig. 6, which shows the overshoot and the maximum rate of rise of the action potential as a function of the membrane potential. With the standard solution inside the axon, both these

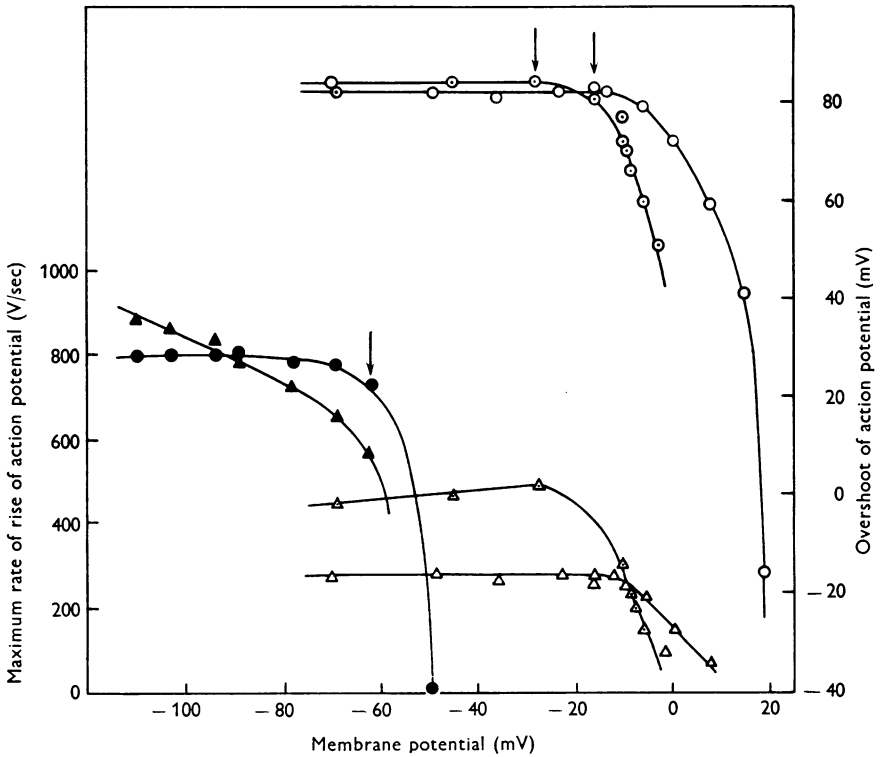

Fig. 6. The effects of the membrane potential displacement from its resting level on the magnitude (circles) and the maximum rate of rise (triangles) of the action potential. Solid symbols, 538 mM-K (standard solution) inside, 7 min after the start of perfusion; open symbols with a dot, 6 mM-K inside, 9 min; open symbols, 6 mM-K inside, 21 min. Artificial sea water is used for the bathing fluid. The resting potentials are shown by the arrows.

values were somewhat increased when the membrane potential was raised, but were decreased remarkably, resulting in complete block, when the membrane was depolarized. Similar curves were observed in low-K internal solution, but greatly shifted along the voltage axis. The magnitude of the overshoot was of course increased. The depolarization needed for the spike height to be reduced to half value was estimated as 24·2 mV with 6 mM-K inside (7–43 min after the start of perfusion, mean of fourteen

measurements) as against 12·8 mV with the standard high-K inside (mean of six measurements).

The effect of polarization on the action potential having a plateau on its falling phase was examined for only two axons. A series of records is shown in Fig. 7, in which the action potential is greatly prolonged, forming a plateau with K-free media both inside and outside the membrane (record *B*). This was the terminal period of K-free perfusion, so that the spike height was already smaller than normal and the rising phase was slow. Upon hyperpolarization the duration of the action potential was effectively shortened (record *C*). Depolarization caused a slight further slowing of the falling phase of the action potential (record *A*). The amplitude of the action potential was kept nearly constant by polarization within the limits of experiment. The effect of depolarization was examined only up to 16 mV displacement, because firing occurred beyond this level.

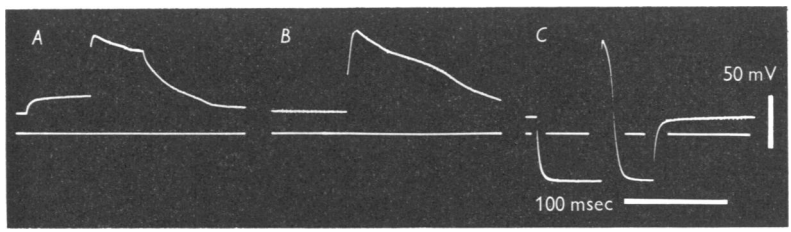

Fig. 7. Records of the action potential as affected by the displacement of membrane potential. K-free both inside and outside; 21 min after the start of perfusion. *A*, the depolarized membrane; *B*, no polarization; *C*, the hyperpolarized membrane. Zero potential level is shown by the horizontal line.

Membrane resistance

Voltage–current relations in various potassium concentrations. There is general agreement for intact axons that steady depolarization decreases membrane resistance (e.g. Hodgkin & Huxley, 1952*a*). An example of voltage–current relations is shown in Fig. 8. In this case sodium chloride in external medium was replaced by choline chloride to avoid the discharge of impulses upon depolarization. With weak intensities of polarizing current the slope resistance increased with hyperpolarization and decreased with depolarization when the standard solution was present inside the axon, whereas a nearly straight voltage–current relation, which was less steep than the above control near the resting potential level, was obtained when the internal potassium concentration was lowered to 6 mM. Delayed rectification was observed with a much greater depolarization with low-K inside than with the standard solution inside. The slope resistances in a large hyperpolarization range were almost the same between these two internal solutions. Thus, lowering internal potassium

concentration had at least two effects on membrane resistance, i.e. an increase in resting membrane resistance and a depression of delayed rectification. Numerical data on the resting membrane resistance together with its corrected values are given in Table 3. Only a few experiments were made on the effect of K-free internal solution on the membrane resistance, showing that the resistance was increased by reducing the internal potassium from 6 mM to zero.

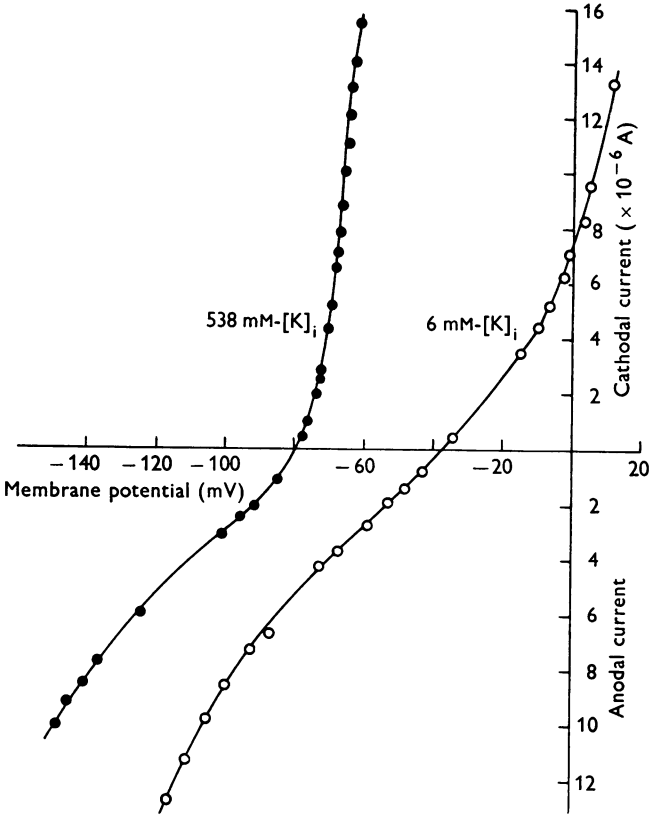

Fig. 8. Voltage–current relation in an axon perfused internally with 538 mM-K (standard solution, 11 min after the start of perfusion) and with 6 mM-K (9 min) in Na-free choline sea water.

The results raise a question whether concentrations both inside and outside the membrane determine the membrane resistance regardless of the resting potential, and whether the inside and outside of the membrane are symmetrical with respect to the effect of potassium on the resistance and to the delayed rectification. Voltage–current relations in various

TABLE 3. Membrane resistance (kΩ) in the axons perfused internally with various potassium solutions in artificial sea water

	High-K (mM) Observed		Low-K (mM) Observed			
Axon	1016	538	10	6	Corrected*	Ratio†
803A	2·25	—	—	16·0	15·2	6·8
804A	1·9	—	—	16·0	15·2	8·0
808A	—	5·9	10·0	—	9·5	1·6
808B	—	5·8	—	8·9	8·5	1·5
808C	—	9·8	—	13·2	12·5	1·3
808D	—	5·2	—	6·8	6·5	1·3
809B	—	4·2	—	16·6	15·8	3·8
821B	—	1·8‡	—	2·4‡	2·3	1·3
821C	—	4·2‡	—	7·1‡	6·7	1·6
825C	—	7·6	—	4·0	3·8	0·5
828A	—	5·8	—	8·0	7·6	1·3
831A	—	2·4	—	6·7	6·4	2·7
901A	—	12·5	—	10·0	9·5	0·8
901B	—	7·4	—	14·5	13·8	1·9
Mean	2·1	6·1	10·0	10·0	9·5	2·5

* Values corrected for internal resistance; observed values × 0·95.
† Low-K corrected value/high-K observed value.
‡ Na-free choline sea water outside.

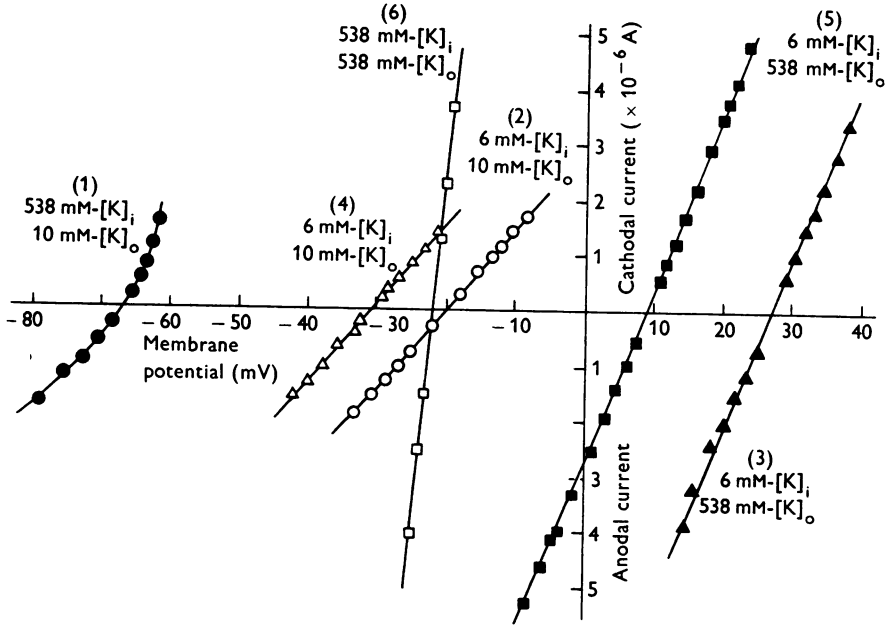

Fig. 9. Voltage–current relation in an axon perfused with various potassium solutions. Measurements were made in the order numbered, and 8 min (1), 16 min (2), and 6 min (3)–(6) after the start of new perfusion conditions.

internal and external potassium concentrations are illustrated in Fig. 9. It will be seen that the membrane resistance at the resting potential was highest when both internal and external potassium concentrations were low (6–10 mM, curves 2 and 4), and lowest when both concentrations were high (538 mM, curve 6). With 538 mM-K on one side and 6–10 mM-K on the other (curves 1, 3 and 5), the membrane resistance was of medium value. However, marked delayed rectification was observed only when high-K was present inside and low-K outside.

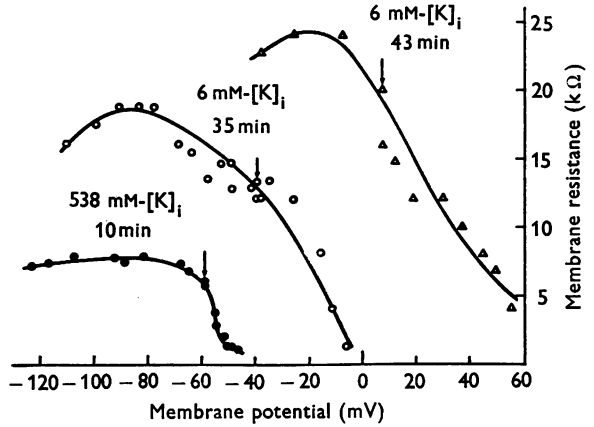

Fig. 10. The effect of displacement of the membrane potential by polarizing current on the membrane resistance. The resting potentials are shown by the arrows. The membrane resistances were estimated from the steady-state magnitudes of the electrotonic potential produced by applying small square currents on polarized membrane. Artificial sea water was used for the bathing fluid.

Effect of displacement of membrane potential on membrane resistance. Changes in the low-frequency component of the membrane resistance by polarization can be estimated from voltage–current relations, such as Fig. 8, but in order to know changes in the high-frequency membrane resistance it is necessary to apply small square pulses to the polarized membrane. A weak anodal current was applied during a prolonged polarization (40–50 msec), and the change in membrane resistance by polarization was estimated by measuring the amplitude of the anelectrotonic potential. An example of the results of such experiments is illustrated in Fig. 10, in which the membrane resistance is plotted against the membrane potential. With the standard solution inside the axon, the membrane resistance slightly increased with hyperpolarization and attained a maximum value, and effectively decreased with depolarization, attaining finally a very small value. The shape of the curve relating the membrane resistance to membrane potential was similar for low-K internal solution.

However, the curve was greatly shifted toward the higher membrane resistance as well as toward the lower membrane potential. In other words, apart from these shifts, the membrane resistance changes upon polarization in a similar way in low-K internal solution as it does in high-K internal solution. Another aspect worthy of note is that with low-K inside a greater amount of depolarization is needed for the membrane resistance to fall to a given value. This is consistent with a smaller degree of rectification mentioned above.

Hyperpolarizing response with low-K inside and high-K outside

It is well known that certain kinds of excitable tissues produce hyperpolarizing responses when anodal polarization exceeds a certain threshold, especially when the external potassium concentration is raised (e.g. Segal, 1958; Stämpfli, 1959; Tasaki, 1959; Chang & Schmidt, 1960; Reuben, Werman & Grundfest, 1961). As in the intact squid giant axons bathed in K-rich media, the axons having low-K internally and high-K externally were also found to produce hyperpolarizing responses (Fig. 12*A*). There was a certain critical point of hyperpolarization beyond which the response occurred. Beyond this the response appeared earlier with increasing hyperpolarization. Although no quantitative measurement was made, the spike phase of the hyperpolarizing response disappeared when the frequency of stimulation was increased beyond a certain rate.

However, it should be borne in mind that the error introduced by the high resistance of the internal medium is not small, because the intensity of applied anodal current was high. As described earlier, it is reasonable to assume that the initial rapid phase of the electrotonic potential in Fig. 12*A* is an indication of the *IR* drop across the internal resistance. Therefore the true magnitude of the electrotonic potential was obtained by subtracting the magnitude of the initial rapid phase from the apparent magnitude.

Some of the preparations which were internally perfused with low-K solution and bathed in high-K sea water produced an oscillatory response superimposed on an anelectrotonic potential (Fig. 11). Increasing hyperpolarization depressed the height and shortened the period of each transient depolarization (records *C–E*), resulting finally in a skip of most of the depolarization phase (record *F*). In these cases also a correction for the internal resistance has to be made in order to estimate the true value for the hyperpolarization, as has been done in the preceding section. A similar oscillation superimposed on a prolonged hyperpolarization has been observed in nodes of Ranvier bathed in K-rich media (Wright & Ooyama, 1962) and in lobster muscle fibres (Reuben *et al.* 1961). Skeletal

muscle fibres have been shown to produce a slow oscillation spontaneously in K-rich Cl-free media (Adrian, 1960).

Response to cathodal stimulus with low-K inside and high-K outside

There is evidence that the excitable membrane produces a depolarizing response in K-rich Na-free medium when a cathodal pulse is applied during a prolonged hyperpolarization (Moore, 1959; Ooyama & Wright, 1962). Moore (1959) interpreted this response in terms of the N-shaped current curve obtained when the membrane was voltage clamped.

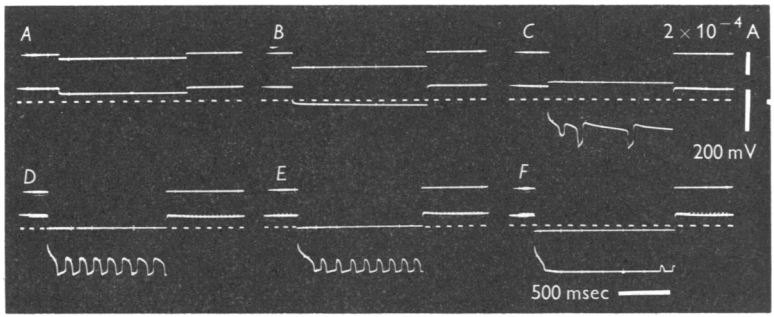

Fig. 11. Records of the oscillatory hyperpolarizing response (lower tracing) and the applied current (upper tracing) in an axon with 6 mM-K inside and 538 mM-K outside; 22 min after the start of perfusion, and 11 min after applying the high-K externally. The intensity of anodal current is increased in records *A*–*F*. Zero potential level is shown by the broken line.

It was found that similar responses can be produced in the axons internally perfused with a low-K solution in 538 mM of external potassium. A series of records is illustrated in Fig. 12. No depolarizing response was elicited when a weak cathodal pulse was applied during a prolonged hyperpolarization (records *B*1 and *B*2). With a slight increase in cathodal pulse intensity there occurred a depolarizing response of rather short duration (records *C*1 and *C*2). The depolarizing response became larger in amplitude and longer in duration with increasing stimulus intensity (series *D* and *E*).

In series *D* and *E* of Fig. 12 it was not possible to trace the terminal phase of the depolarizing response. However, it became evident that the depolarizing response elicited by a strong stimulus terminated in a sudden repolarization after forming a plateau which lasted for as long as several seconds. It was generally observed that the depolarizing response was produced regardless of the presence or absence of hyperpolarizing response.

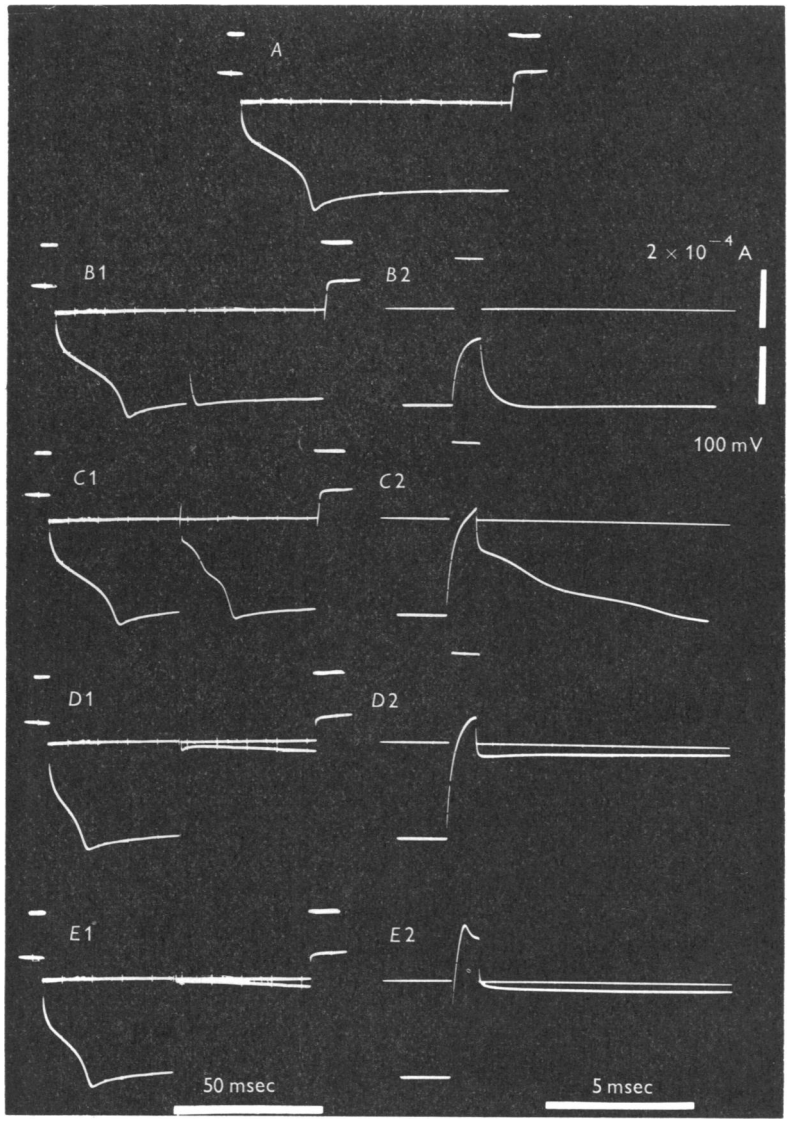

Fig. 12. Records of the depolarizing response produced by a brief cathodal pulse in the hyperpolarized membrane with 6 mM-K inside and 538 mM-K outside. The upper and lower tracings show the applied current and potential change respectively, 28 min after the start of perfusion, and 23 min after applying the high-K externally. *A*, without cathodal stimulus, showing a hyperpolarizing response; Series *B–E*, a cathodal stimulus, whose intensity is raised from *B* to *E*, is applied during the course of hyperpolarizing response. The right-hand column shows the records near the point of cathodal stimulus, which were taken with a faster sweep.

DISCUSSION

The experiments described establish that it is now possible, with a high probability of success, to perfuse internally squid giant axons with diameters of 400 μ. Axons of this size are readily available at the Marine Biological Laboratory, Woods Hole. This size of the axon is considerably smaller than the axons used successfully by Tasaki *et al.* (1962) and Baker *et al.* (1961*a*, *b*, 1962*b*, *c*), which were 500–900 μ in diameter.

Resting potential

The resting potentials with low-K inside are of the same order of magnitude as those obtained by Tasaki *et al.* (1962) and somewhat higher (more negative) than those of Baker *et al.* ((1962*c*) in experiments in which KCl was replaced by NaCl. For example, the resting potential with 62 mM-K inside is -40 mV, which is comparable to $-(35–45)$ mV with 50 mM-K inside in the experiments of Tasaki *et al.* (1962), but is higher than about -25 mV with 50 mM-K inside in the experiments (Fig. 2) of Baker *et al.* (1962*c*). With 6 mM-K inside the average resting potential is -33 mV at the initial stage and -16 mV at the terminal stage, both of which are considerably higher than about 0 mV obtained by interpolation in Fig. 2 of Baker *et al.* (1962*c*). Since the contribution of chloride does not seem to be large enough to account for the difference, in view of the fact that the resting potential falls only by about 5 mV when internal potassium sulphate is replaced by potassium chloride (Baker *et al.* 1962*c*), sodium ions in the internal medium might be responsible for the smaller resting potentials in the experiments of Baker *et al.*

Since the membrane resistance is increased rather than decreased by lowering the internal potassium concentration, and no data are available for potassium permeability under such a condition, it cannot be determined by calculations as has been done for Fig. 8 of Baker *et al.* (1962*c*) whether or not potassium is a major factor contributing to the resting potential under the present experimental conditions. It should, however, be added that simple calculations by equation (1) below, with an assumption that potassium permeability is kept constant ($b = 0{\cdot}08$) by lowering internal potassium, give a value of about $+30$ mV as against the observed resting potential of -33 mV with 6 mM-K inside. If the increase in membrane resistance by lowering internal potassium is assumed as indicating a decrease in potassium permeability, the deviation of the values would become more significant.

Excitability

The increase in the overshoot of the action potential by lowering internal potassium concentration is at least in part explained by the following equation, as suggested by Baker *et al.* (1962*c*):

$$V = \frac{RT}{F} \ln \frac{[K]_o + b[Na]_o}{[K]_i + b[Na]_i}, \quad (1)$$

where $[\ ]_o$ and $[\ ]_i$ are activities of potassium and sodium in outer and inner solutions, b is the permeability ratio, P_{Na}/P_K, and R, T and F have their usual meanings. Under the condition of $[Na]_i = 0$ and $[Na]_o \gg [K]_o$, the terms $[K]_o$ and $b[Na]_i$ become negligible and zero respectively, so that lowering $[K]_i$ causes the overshoot to increase.

One of the most surprising findings in the present experiments is that the axon can produce action potentials from its greatly depolarized membrane when the internal potassium concentration is significantly reduced. This confirms and extends an earlier observation by Tasaki *et al.* (1962) in which action potentials were obtained from the subnormal resting potential (35–45 mV inside negative) when the internal potassium was reduced to 50 mM, and that by Baker *et al.* (1962*c*) in which a large action potential could be elicited from the membrane depolarized to −33 mV by 87 mM-K. With low-K inside, action potential and membrane resistance are decreased by cathodal depolarization as in the intact axons or in the axons with the standard solution inside. Furthermore, critical depolarization for firing remains constant despite depolarization by low internal potassium. It follows that the relations between membrane conductance and membrane potential, including probably those for both sodium and potassium conductances, shift along the voltage axis by the amount of change in resting potential. A corollary of this result is that excitability is determined both by membrane potential and by internal potassium concentration. An assumption is, however, made that changes in internal sulphate concentration and ionic strength have no effect on excitability.

The finding that action potentials can be produced even when no potassium is present inside the axon throws some doubt on the possible role of internal potassium in triggering the mechanism of excitation. There has been a suggestion that, on passing cathodal current, calcium which occupies the negative sites of the membrane is replaced by univalent positive ions which are carried by the current from the interior of the axon, thereby raising the membrane conductance and producing an action potential (e.g. Tasaki & Shimamura, 1962; Tobias, 1958). According to this hypothesis potassium ions have been naturally expected as a carrier of the current.

Prolonged action potential and hyperpolarizing response

Some of the responses obtained with low-K inside have been shown to be reproduced by computations based on the Hodgkin-Huxley equations (FitzHugh, 1960; George & Johnson, 1961; Moore, unpublished). For example, an action potential having a prolonged plateau on its falling phase is obtained by computation when the rate of potassium conductance rise is greatly reduced, as in the actual nerve with low-K or K-free medium on both sides of the membrane. Hyperpolarizing responses, oscillatory responses superimposed on a prolonged hyperpolarization, and shortening of the plateau of the action potential by anodal current can also be reproduced by computation. This agreement supports the view that action potentials in the perfused axons with low-K inside or with K-free both inside and outside the membrane are described in terms of sodium and potassium conductances, as in intact axons (Hodgkin & Huxley, 1952*c*), though the role of potassium may be negligible or smaller than in intact axons.

The prolonged action potentials, which can be produced by applying a cathodal shock to the anodally hyperpolarized membrane with low-K inside and Na-free high-K outside, appear to be interpreted as being due to potassium conductance rise, as in intact axons (Moore, 1959; Ooyama & Wright, 1962). It then follows that the mechanism by which the potassium conductance rises upon depolarization is still operative under this low-K perfused condition.

SUMMARY

1. Intracellular perfusion of medium-sized squid giant axons (about 400 μ in diameter) has been successful.

2. With 538 mM or 1016 mM-K standard solution inside the axon, the magnitudes of the resting and action potentials were of the order of 60 and 100 mV respectively.

3. With 6 mM-K inside, the resting potential was reduced to as small as 16–33 mV inside negative but the height of the action potential remained constant though its falling phase was prolonged. Further depolarization by application of current decreased the spike height in the same manner as in the axons with 538 mM-K inside.

4. When potassium was eliminated from the external medium with 6 mM-K inside, an action potential having a prolonged plateau on its falling phase was produced from low resting potential levels. With K-free media on both sides of the nerve membrane, the resting potential was low and might reverse its sign, but prolonged action potentials were still produced.

5. Critical depolarization for firing was little changed by lowering internal potassium concentration.

6. When the internal potassium was lowered to 6 mM, the curve relating membrane resistance to membrane potential was shifted toward lower membrane potentials but the membrane resistance increased.

7. When the concentration of potassium was increased from 6–10 mM on either side of the axon membrane to 538 mM, the membrane resistance was reduced.

8. Delayed rectification was very marked with 538 mM-K inside and 10 mM-K outside, but much less marked or absent with 6 mM-K inside and 10 mM or 538 mM-K outside, or with 538 mM-K inside and outside.

9. Hyperpolarizing thresholds were observed with 6 mM-K inside and 538 mM-K outside upon anodal polarization. An oscillation was superimposed on the hyperpolarizing response on some occasions.

10. With 6 mM-K inside and 538 mM-K outside, a prolonged depolarizing response was produced when a cathodal shock was applied during steady anodal hyperpolarization.

11. It is concluded that excitability is determined both by membrane potential and by internal potassium concentration.

The author wishes to express his gratitude to Dr J. W. Moore for his valuable comments and help in the present study. Thanks are also due to Professor A. L. Hodgkin for his advice concerning the method of internal perfusion while he was staying at Woods Hole and to Dr W. Ulbricht for helpful discussion. This study was performed at the Marine Biological Laboratory, Woods Hole, Massachusetts, and was supported by grants B 3437 from the Public Health Service, the National Institutes of Health, and by B-11554 from the National Science Foundation to Dr J. W. Moore.

REFERENCES

ADRIAN, R. H. (1956). The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol.* **133**, 631–658.

ADRIAN, R. H. (1960). Potassium chloride movement and the membrane potential of frog muscle. *J. Physiol.* **151**, 154–185.

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1961*a*). Replacement of the protoplasm of a giant nerve fibre with artificial solutions. *Nature, Lond.*, **190**, 885–887.

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1961*b*). Perfusion of the giant nerve fibres of *Loligo*. *J. Physiol.* **157**, 25*P*.

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1962*a*). Some experiments with perfused nerve fibres from *Loligo forbesi*. *Proc. int. Union physiol. Sci.* **1**, 559–560.

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1962*b*). Replacement of the axoplasm of giant nerve fibres with artificial solutions. *J. Physiol.* **164**, 330–354.

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1962*c*). The effects of changes in internal ionic concentrations on the electrical properties of perfused giant axons. *J. Physiol.* **164**, 355–374.

CHANG, J. J. & SCHMIDT, R. F. (1960). Prolonged action potentials and regenerative hyperpolarizing responses in Purkinje fibers of mammalian heart. *Pflüg. Arch. ges. Physiol.* **272**, 127–141.

DAVIES, P. W. (1961). A method for measuring membrane potential of intracellularly perfused single skeletal muscle fibers. *Fed. Proc.* **20**, 142.

FITZHUGH, R. (1960). Thresholds and plateaus in the Hodgkin-Huxley nerve equations. *J. gen. Physiol.* **43**, 867–896.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.* **137**, 218–244.

GEORGE, E. P. & JOHNSON, E. A. (1961). Solutions of the Hodgkin-Huxley equations for squid axon treated with tetraethylammonium and in potassium-rich media. *Aust. J. exp. biol. Sci.* **39**, 275–294.

GIBBS, C. L. & JOHNSON, E. A. (1962). Intracellular ionic injection in rabbit ventricular fibres. *Aust. J. exp. biol. Sci.* **40**, 85–92.

GRUNDFEST, H., KAO, C. Y. & ALTAMIRANO, M. (1954). Bioelectric effects of ions micro-injected into the giant axon of *Loligo*. *J. gen. Physiol.* **38**, 245–282.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). The components of membrane conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 473–496.

HODGKIN, A. L. & HUXLEY, A. F. (1952*b*). The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 497–506.

HODGKIN, A. L. & HUXLEY, A. F. (1952*c*). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**, 37–77.

KOKETSU, K. & KIMURA, Y. (1960). The resting potential and intracellular potassium of skeletal muscle in frogs. *J. cell. comp. Physiol.* **55**, 239–244.

MOORE, J. W. (1959). Excitation of the squid axon membrane in isosmotic potassium chloride. *Nature, Lond.*, **183**, 265–266.

MOORE, J. W. & COLE, K. S. (1963). Voltage clamp techniques. In *Physical Techniques in Biological Research*, vol. **6**, ed. NASTUK, W. L. New York: Academic Press.

OIKAWA, T., SPYROPOULOS, C. S., TASAKI, I. & TEORELL, T. (1961). Methods for perfusing the giant axon of *Loligo pealii*. *Acta physiol. scand.* **52**, 195–196.

OOYAMA, H. & WRIGHT, E. B. (1962). Activity of potassium mechanism in single Ranvier node during excitation. *J. Neurophysiol.* **25**, 67–93.

REUBEN, J. P., WERMAN, R. & GRUNDFEST, H. (1961). The ionic mechanisms of hyperpolarizing responses in lobster muscle fibers. *J. gen. Physiol.* **45**, 243–265.

SEGAL, J. R. (1958). An anodal threshold phenomenon in the squid giant axon. *Nature, Lond.*, **182**, 1370.

STÄMPFLI, R. (1959). Is the resting potential of Ranvier nodes a potassium potential? *Ann. N.Y. Acad. Sci.* **81**, 265–284.

STEPHENSON, W. K. (1957). Membrane potential changes and ion movements in the frog sartorius muscle. *J. cell. comp. Physiol.* **50**, 105–128.

TASAKI, I. (1959). Demonstration of two stable states of the nerve membrane in potassium-rich media. *J. Physiol.* **148**, 306–331.

TASAKI, I. (1962). Movement of radio-isotopes across squid axon membrane at rest and during activity. *Proc. int. Union physiol. Sci.* **1**, 588–589.

TASAKI, I. & SHIMAMURA, M. (1962). Further observations on resting and action potential of intracellularly perfused squid axon. *Proc. nat. Acad. Sci., Wash.*, **48**, 1571–1577.

TASAKI, I., WATANABE, A. & TAKENAKA, T. (1962). Resting and action potential of intracellularly perfused squid giant axon. *Proc. nat. Acad. Sci., Wash.*, **48**, 1177–1184.

TOBIAS, J. M. (1950). Injury and membrane potentials in frog muscle after depleting potassium and producing other changes by soaking in potassium free salt solution or distilled water. *J. cell. comp. Physiol.* **36**, 1–13.

TOBIAS, J. M. (1958). Experimentally altered structure related to function in the lobster axon with an extrapolation to molecular mechanisms in excitation. *J. cell. comp. Physiol.* **52**, 89–125.

WEIDMANN, S. (1955). Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. *J. Physiol.* **129**, 568–582.

WRIGHT, E. B. & OOYAMA, H. (1962). Role of cations, potassium, calcium, and sodium during excitation of frog single nerve fiber. *J. Neurophysiol.* **25**, 94–109.