

THE AFTER-EFFECTS OF IMPULSES IN THE GIANT NERVE FIBRES OF *LOLIGO*

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The giant nerve fibres of *Loligo* and *Sepia* differ from most excitable tissues in that the spike of an isolated axon is followed by a brief period of hyperpolarization, which is often called the positive phase (Curtis & Cole, 1942; Hodgkin & Huxley, 1939; Weidmann, 1951). There is evidence that the positive phase occurs because the permeability of the membrane to potassium increases during the second half of the spike, and does not at once return to its resting value (Hodgkin & Huxley, 1952*d*). Since the resting potential of an isolated axon may be 15-30 mV less than the equilibrium potential for potassium ions, the persistence of the state of increased potassium permeability during the refractory period raises the membrane potential above its resting value and generates a positive phase. This suggestion is supported by the observation that the membrane potential during the positive phase is markedly affected by small changes in the concentration of potassium outside the fibre (Hodgkin & Katz, 1949*a*, Hodgkin & Keynes, 1955). Thus, increasing the external potassium concentration from 0 to 20 mM, which decreases the resting potential by only about 10 mV, reduces the membrane potential during the positive phase by 30 mV.

The sensitivity of the positive phase to changes in potassium concentration forms the basis for interpreting the observations described in this paper; the starting-point was provided by an experiment in which the effects of calcium-deficient solutions were being examined. Like many previous observers we found that reducing the concentrations of calcium and magnesium made the giant axon of *Loligo* spontaneously active. When fibres were near the point at which they fired continuously they often gave long trains of impulses in response to a single shock. In these trains we noticed that the positive phases of the spikes were not of equal size, but declined during the initial stages of the

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discharge. In order to find out whether this effect was caused by a lack of calcium, we examined the effect of repetitive stimulation in fibres surrounded by a normal ionic medium. Somewhat to our surprise we found that the positive phases at the beginning of a train of impulses were still not constant, but declined exponentially to a new level with a time constant of 30–100 msec (Fig. 1). This effect developed at the same rate as the slow depolarization resulting from the addition of successive negative after-potentials (cf. Shanes, 1949*a*, 1952 and Shanes, Grundfest & Freygang, 1953). Since both the positive phase and the resting potential are reduced by an increase in potassium concentration we compared the effect of potassium with that of repetitive stimulation. These measurements showed that both the change in positive phase and the slow depolarization were matched by an increase in potassium concentration. This is regarded as evidence that the after-effects were caused by a rise in potassium concentration in the immediate vicinity of the membrane. The apparent rise produced by a single impulse at 18° C was about 1.6 mM, but much larger concentrations could be built up by stimulating the nerve at high frequencies. After activity the excess potassium disappeared along an exponential curve with a time constant of 30–100 msec.

In order to explain these results, it may be supposed that the potassium ions which leak out of the axon during activity do not diffuse freely from the fibre, but are restrained by an unselective barrier between the excitable membrane and the external solution. One form of this hypothesis is to suppose that the external barrier or membrane is not in direct contact with the excitable membrane, and that potassium ions accumulate in the aqueous space between the two membranes. Since the total quantity of potassium leaving 1 cm² of membrane in one impulse is known to be about 4×10^{-12} mole at 18° C (Shanes, 1954; Keynes & Lewis, 1951), it is possible to estimate the permeability of the outer layer and the distance between the outer layer and the excitable membrane. This calculation (see p. 350) indicates that the space between the two membranes is of the order of 300 Å, and that the outer layer should have an electrical resistance of about 5 Ω.cm². These results are mentioned now because it is helpful to keep two points in mind when considering the experiments. The first is that the quantity of potassium leaving the fibre in one impulse would only cause an appreciable rise in concentration if these ions were confined in an exceedingly small space (cf. Shanes *et al.* 1953). The second is that an unselective layer with a resistance of only 1/200 of that of the resting membrane would be sufficient to cause an appreciable rise in potassium concentration during a train of impulses. In this connexion it should be said that there is independent evidence for the existence of a layer with a resistance of the order of 5 Ω.cm² between the excitable membrane and the external solution (Hodgkin, Huxley & Katz, 1952; and below, p. 365).

METHOD

In all essential respects the apparatus and method were similar to those described by Hodgkin & Katz (1949*a*). Giant axons, 500–670 μ in diameter, were isolated from *L. forbesi* and cleaned by the usual methods. All small nerve fibres were removed and branches were left as long as possible. No attempt was made to remove the layer of connective tissue (about 20 μ in thickness) which clings tightly to the axon.

A long microelectrode of the type shown in fig. 2*c* of Hodgkin & Katz (1949*a*) was introduced through a cannula and thrust down the axis of the fibre for 20–30 mm. The electrode was about 100 μ in diameter, and was filled with 0.57 M-KCl.

The axon was surrounded by about 100 ml. of fluid, and solutions were changed by draining the cell completely, washing once and then filling with a new solution. With cleaned fibres the changes in potential produced by different concentrations of potassium were complete within less than the time required to change the solutions (about 30 sec).

Natural sea water was used in some of the experiments; in others we employed an artificial chloride sea water of the composition shown in Table 1. The potassium concentration was changed at the expense of sodium, the sum of $[Na]_0$ and $[K]_0$ being kept at 470 mM.

The voltage clamp records discussed on p. 361 were taken in 1949 during the course of the work described by Hodgkin *et al.* (1952).

TABLE 1. Composition of chloride sea water

	mg-ions/l.
Na	460
K	10.4
Ca	11.4
Mg	55
Cl	603
HCO ₃	0.6

RESULTS

The usual method of measuring the change in positive phase was to apply shocks at 50/sec, and to record the beginning of a train of impulses with a gain at which the positive phase occupied several centimetres on the oscillograph screen. A typical record is shown in Fig. 1 A. At the gain and time base speed employed, the spike was too large and rapid to register on the film, and the positive phases, which are shown as upward deflexions, appear as vertical lines with well-defined peaks. With this method of recording it is evident that the membrane potential reached in successive positive phases was not constant, but declined from an initial value of 65.5 mV to a steady level of 60 mV with a time constant of about 100 msec. Each impulse was followed by a negative after potential which built up to a steady level and declined exponentially when the stimulus was switched off (Fig. 1 B). Fig. 2 was obtained on another fibre in which the time constant of the effect was about 35 msec instead of 100 msec as in Fig. 1. Comparison of A and B in Fig. 2 suggests that the positive phase and the base-line between impulses decreased slowly after the initial exponential decline was complete; this is discussed further on p. 355. A variant of the method was to use a short train of impulses as in Fig. 4; this had the advantage that the beginning and end of the train could be seen on

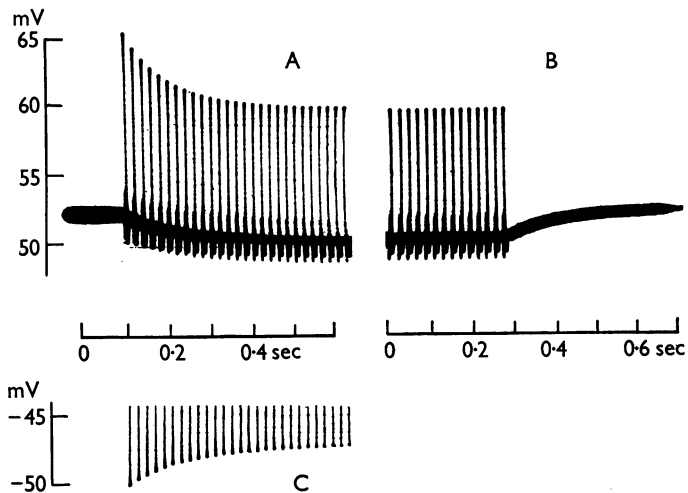


Fig. 1. Records showing positive phases and negative after-potentials at the beginning (A) and end (B) of a train of impulses of frequency 50/sec and duration about 1 sec. Record C shows the tips of the spikes at the same gain but with the input displaced by 100 mV. (The spike is too large and rapid to register on the film in records A and B.) The vertical scale gives the potential difference between the external solution and the internal electrode. The time base is linear in this and all subsequent records. Temperature 19.8°C , axon 6. Natural sea water, $[\text{K}]=10\text{ mm}$. Note that in this paper depolarizations are shown as downward deflexions and hyperpolarizations as upward deflexions. Some vertical lines have been retouched in this and other figures.

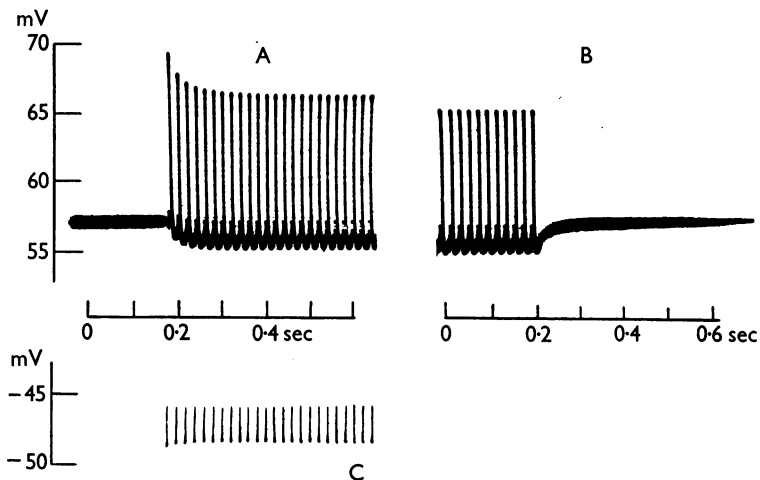


Fig. 2. Details as in Fig. 1 except that the axon had a shorter time constant. Temperature 18.2° , axon 8, artificial sea water, 10.4 mm-K .

the same record and that there was no danger of subjecting the nerve to excessive stimulation.

In addition to the change in positive phase and in the level of the membrane potential between impulses there was an alteration in the peak potential reached during the spike. This is illustrated by Fig. 1 C, which shows the tips of the spikes recorded on the same gain as in Fig. 1 A but with the membrane potential offset by 100 mV. The record shows that the peak potential at the crest of the spike changed exponentially with about the same time constant as the positive phase. The magnitude of this effect varied greatly between different fibres and was smallest in axon 8 (Fig. 2 C) which was an unusually stable preparation.

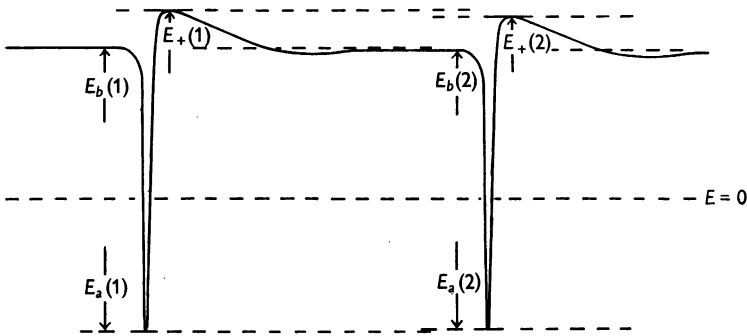


Fig. 3. Diagram showing quantities measured in first two spikes of a train of impulses at a frequency of 50/sec. The drawing is approximately to scale but the difference between the two impulses has been slightly exaggerated.

Nomenclature

Fig. 3 shows some of the quantities which can be measured in a record of a train of impulses:

$E_+(1)$ is the potential difference between the external solution and the axoplasm at the crest of the first positive phase.

$E_b(1)$ is the potential difference across the membrane immediately before the first impulse.

$E_a(1)$ is the p.d. across the active membrane at the crest of the first spike.

$E_+(n)$, $E_b(n)$ and $E_a(n)$ are the same p.d.'s measured for the n th impulse of the train. Another quantity which was often used was the amplitude of the positive phase relative to the base-line immediately before the impulse. This is defined by

$$V_+(n) = E_+(n) - E_b(n).$$

The nomenclature for changes in potential during the train of impulses is

$$\Delta_1(E) = E(2) - E(1),$$

$$\Delta_n(E) = E(n+1) - E(1).$$

The effect of changing the external concentration of potassium

The effects seen in Figs. 1 and 2 are similar to those produced by a rise in potassium concentration from the initial value of 10 mm to a steady level of 13–15 mm (cf. Hodgkin & Katz, 1949*a*, table 7). In order to examine this point further, trains of impulses were recorded at external potassium concentrations of 0, 5.2, 10.4, 15.6 and 20.8 mm. These records are illustrated by Fig. 4.

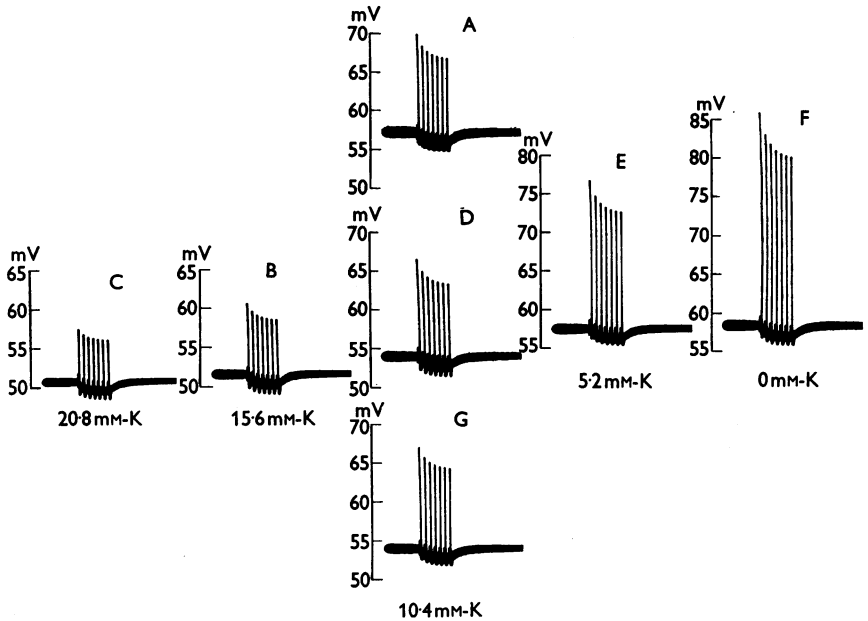


Fig. 4. Effect of external potassium concentration on the positive phase during short trains of impulses; frequency 50/sec. The sequence in which the records were taken was A (10.4 mM-K), B (15.6 mM), C (20.8 mM), D (10.4 mM), E (5.2 mM), F (K-free), G (10.4 mM). Axon.8*b*, temperature 17–18° C. Since the amplitude in K-free was just too large for the film, the record in F was made by joining two photographs at the crest of the third positive phase; other records taken at lower amplification showed that no error has been introduced by this procedure.

Qualitatively, it is plain that the amplitude of the positive phase decreased with increasing potassium and that the change produced by the passage of impulses or by a rise in external potassium concentration was greatest with the low potassium solutions. The time constant of the effect was the same in all five solutions.

In order to work out this experiment, a suitable way of measuring the positive phase must first be chosen. The variable which gave the most consistent results when the external potassium concentration was altered was the amplitude (V_+) of the positive phase relative to the base-line immediately

before the spike. This quantity could be measured more accurately over a period of time than the absolute membrane potential during the positive phase (E_+) since it was not subject to drifts in electrode potential.

Table 2 gives the results of measuring the series of records in the film from which Fig. 4 was made. It shows the amplitude of the first positive phase, $V_+(1)$, the difference between the first and second positive phase, $\Delta_1 V_+$, and the difference between the first and seventh positive phase, $\Delta_6 V_+$.

TABLE 2. Effect of potassium concentration on amplitude of positive phases at the beginning of a train of impulses

Series	[K] ₀ (mM)	$V_+(1)$ (mV)	$\Delta_1 V_+$ (mV)	$\Delta_6 V_+$ (mV)	Δ_1 [K] (mM)	Δ_6 [K] (mM)	Temperature (° C)
A	10.4	12.6	-0.98	-2.04	1.1	2.5	17.5
B	15.6	9.1	-0.50	-1.10	0.9	2.1	17.5
C	20.8	6.7	-0.25	-0.47	0.8	1.9	18.0
D	10.4	12.6	-1.08	-2.21	1.3	2.8	17.0
E	5.2	19.1	-1.52	-3.07	1.2	2.4	17.8
F	0	27.4	-2.21	-4.46	1.2	2.6	17.5
G	10.4	13.1	-0.89	-1.76	1.0	2.2	17.8

The results in this table were obtained from the experiment illustrated in Fig. 4.

[K]₀ is the potassium concentration in the external fluid.

$V_+(1)$ is the amplitude of the first positive phase.

$\Delta_1 V_+$ is the difference between the first and second positive phases.

$\Delta_6 V_+$ is the difference between the first and seventh positive phases.

Δ_1 [K] is the local rise in potassium concentration produced by 1 impulse. These figures were obtained from $\Delta_1 V_+$ and the curve in Fig. 5; they have not been corrected for loss of potassium during the 20 msec interval between the first and second impulses.

Δ_6 [K] is the corresponding rise produced by 6 impulses.

The frequency of the train of impulses was 50/sec.

The relation between the external potassium concentration and the amplitude of the first positive phase is shown in Fig. 5. This curve can be used to calculate the rise in potassium concentration near the membrane during a train of impulses. For example, in the top row of Table 2 the difference between the first and second positive phase is 0.98 mV; from Fig. 5 this corresponds to a rise of 1.1 mM in the potassium concentration near the membrane. Values obtained by this method are given under Δ_1 [K] in Table 2. They appear to be fairly consistent, but suggest that the outflow of potassium during electrical activity is reduced by a rise in the external concentration of potassium. This is reasonable since potassium-rich solutions decrease the spike and should therefore reduce the quantity of potassium lost in each impulse.

The values for the rise in potassium concentration produced by 6 impulses are given under Δ_6 [K] in Table 2. The time constant for the build-up of the potassium concentration was calculated from the ratio Δ_6 [K]/ Δ_1 [K] by equation (7) (p. 367), and was found to be within the range 31–39 msec throughout the experiment. The time constants for the build-up and decay of the negative after-potential were 33 and 36 msec respectively in this experiment.

The other quantities available for analysis in the experiment illustrated by Fig. 4 were the change in base-line, ΔE_b , and the change in spike potential ΔE_a . These results are given in Table 3. For completeness, the change in membrane potential during the positive phase, ΔE_+ , has been included, but these columns do not contain any new information since E_+ is the sum of E_b and V_+ .

TABLE 3. Effect of potassium concentration on potentials during trains of impulses

Series	$[K]_0$ (mM)	$E_b(1)$ (mV)	$\Delta_1 E_b$ (mV)	$\Delta_6 E_b$ (mV)	$E_a(1)$ (mV)	$\Delta_1 E_a$ (mV)	$\Delta_6 E_a$ (mV)	$E_+(1)$ (mV)	$\Delta_1 E_+$ (mV)	$\Delta_6 E_+$ (mV)
A	10.4	+57.2	-0.56	-1.12	-45.2	+0.19	+0.38	+69.8	-1.54	-3.16
B	15.6	+51.7	-0.54	-1.06	-46.8	+0.28	+0.47	+60.8	-1.04	-2.16
C	20.8	+50.8	-0.42	-0.94	-42.6	+0.38	+0.66	+57.5	-0.67	-1.42
D	10.4	+54.0	-0.45	-0.95	-47.6	+0.19	+0.41	+66.6	-1.53	-3.16
E	5.2	+57.5	-0.47	-1.02	-45.7	+0.10	+0.26	+76.6	-1.99	-4.09
F	0	+58.4	-0.57	-1.23	-46.1	+0.12	+0.22	+85.8	-2.78	-5.69
G	10.4	+54.1	-0.43	-0.96	-44.4	+0.19	+0.40	+67.2	-1.32	-2.72

The results in this table were obtained from the experiment illustrated in Fig. 4.

$[K]_0$ is the potassium concentration in the external fluid.

$E_b(1)$ is the base line before the first impulse, i.e. the resting potential.

$E_a(1)$ is the membrane potential at the crest of the first spike.

$E_+(1)$ is the membrane potential at the crest of the first positive phase.

Δ_1 and Δ_6 are the changes produced by 1 and 6 impulses respectively.

In order to see whether the results in Table 3 were consistent with a rise in potassium concentration, the three variables E_b , E_+ and E_a were first plotted against potassium concentration as in Fig. 6. The points for the resting potential were reasonably well fitted by a straight line of slope 0.425 mV/mm and this relation has been used to calculate $\Delta[K]$ from the negative after-potential. These values are shown in Table 4 under the columns headed 'From E_b ', and are plainly in good agreement with those obtained from E_+ . This agreement is evidence that both the negative after-potential and the change in positive phase were produced by a rise in the potassium concentration immediately outside the membrane; 20 msec after one impulse the potassium concentration appeared to be about 1 mM greater than in the resting condition.

Owing to irregular drifts in electrode potential, the curve relating spike potential to potassium concentration was not sufficiently accurate to allow $\Delta[K]$ to be calculated from ΔE_a by the methods described in the previous paragraphs. The approximate values in Table 4 were obtained in the following way. Over the range from zero potassium concentration to 20.8 mM, E_a varied between -46.1 and -42.6 mV. At 10.4 mM-K, $dE_a/d[K]$ should therefore be about 3.5 mV/20.8 mM = 0.17 mV/mm; since $\Delta_1 E_a$ is 0.19 mV, $\Delta_1[K]$ is 0.19/0.17 = 1.1 mM. The close agreement with the value calculated from E_b and E_+ may be fortuitous, but it is evident that the change in spike potential was about equal to that produced by a solution containing 1 mM more potassium and 1 mM less sodium. The change in E_a is discussed further on p. 352.

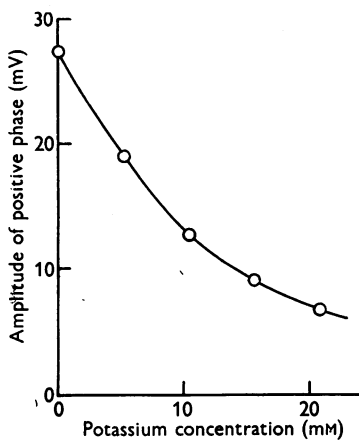


Fig. 5.

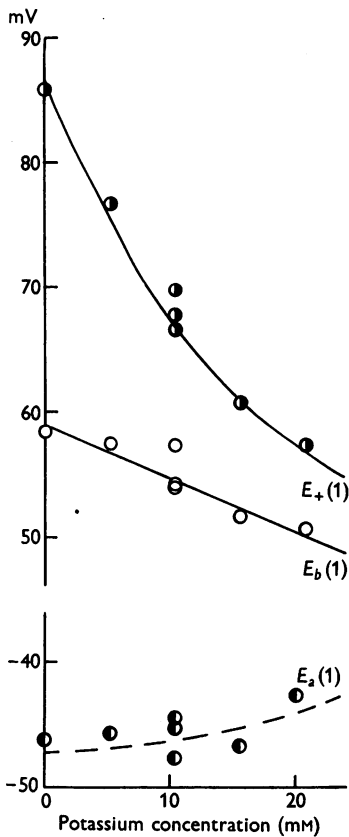


Fig. 6.

Fig. 5. Relation between potassium concentration in the external fluid and the amplitude of the positive phase, $V_+(1)$, plotted from Table 2. The point at 10.4 mM-K is the mean of the values obtained in series A, D, and G.

Fig. 6. Relation between potassium concentration and membrane potential: (1) at maximum of first positive phase, $E_+(1)$; (2) in resting state, $E_b(1)$; (3) at crest of first spike, $E_a(1)$; plotted from Table 3. The shape of the dotted curve for $E_a(1)$ is based on other experiments.

TABLE 4. Rise in potassium concentration during a train of impulses, calculated from Table 3 and Fig. 6

Series	[K] ₀ (mM)	Rise in potassium concentration (mM)					
		After 1 impulse			After 6 impulses		
		From E_b	From E_+	From E_a	From E_b	From E_+	From E_a
A	10.4	1.3	1.2	1.1	2.6	2.6	2.3
B	15.6	1.2	1.1	—	2.5	2.3	—
C	20.8	1.0	0.9	—	2.2	2.0	—
D	10.4	1.1	1.2	1.1	2.2	2.6	2.4
E	5.2	1.1	1.2	—	2.4	2.4	—
F	0	1.3	1.2	—	2.9	2.6	—
G	10.4	1.0	1.0	1.1	2.3	2.2	2.4

*The permeability of the outer layer and the apparent thickness of the space
outside the membrane*

The results described in the preceding section indicate that the potassium concentration outside the membrane in axon 8*b* was 1.2 mM greater 20 msec after a single spike than it was before. Later in this paper evidence will be described which shows that the excess potassium disappeared in an exponential manner, and that different methods of measuring the time constant were in reasonable agreement. In axon 8*b* the time constant was 34 msec, so that the excess potassium concentration immediately after the spike would have been $1.2 \text{ mM} \times \exp(20/34) = 2.16 \text{ mM}$. From the data of Shanes (1954) and Keynes & Lewis (1951) the potassium leakage per impulse may be taken as about $4 \times 10^{-12} \text{ mole/cm}^2$ at 18° C. In order to produce a rise of 2.16 mM these potassium ions must be confined within a space of thickness θ , given by the equation

$$\begin{aligned}\theta &= \frac{4 \times 10^{-12} \text{ mole/cm}^2}{2.16 \times 10^{-6} \text{ mole/cm}^3} \\ &= 1.9 \times 10^{-6} \text{ cm} \equiv 190 \text{ \AA} \equiv 19 \mu\mu.\end{aligned}$$

Concentration differences should be equalized by diffusion within a few microseconds in a watery space of this thickness so that it is fair to suppose that the potassium concentration in the space is uniform, as has been tacitly assumed in the calculation. For reasons given on p. 370, it is best to regard θ as an empirical quantity which need not correspond exactly to an anatomical space.

On p. 367 the time constant τ for the disappearance of excess potassium ions is shown to be related to the thickness of the space (θ) and the permeability constant of the outer layer (P) by the equation

$$\tau = \theta/P.$$

In axon 8*b* $\tau = 34$ msec, so that $P = 5.6 \times 10^{-5} \text{ cm/sec}$.

A different method of working out the permeability of the outer layer is to calculate the mean rise in potassium concentration during steady stimulation. In axon 8*b* the excess concentration of potassium 20 msec after the 6th impulse was estimated as 2.7 mM. At this time the nerve was very nearly in a steady state so that the mean potassium concentration may be taken as

$$\frac{2.7}{20} \int_0^{20} \exp\left(\frac{t}{34}\right) dt = 3.7 \text{ mM}.$$

With a stimulation frequency of 50/sec the mean outflow is $2 \times 10^{-10} \text{ mole/cm}^2 \cdot \text{sec}$ so that the permeability should be

$$P = \frac{2 \times 10^{-10}}{3.7 \times 10^{-6}} = 5.4 \times 10^{-5} \text{ cm/sec}.$$

The two methods necessarily agree to within the limits introduced by rounding errors because the mean concentration during a steady stimulation was derived from one of the quantities (2.7 mM) used in calculating the time constant.

Collected results

Table 5 summarizes the results of all the experiments carried out at room temperature with a normal ionic medium. The quantities $\Delta_1[K]$, θ and P were calculated in the manner described in the previous section. Calibrations with different potassium concentrations were made only in axons 3 and 8. In the remaining experiments we used the curve in Fig. 6 to work out the results. The outflow of potassium per impulse was taken as 4.0×10^{-12} mole/cm² in all fibres. An alternative method of measuring θ , which does not depend on this assumption, is described on p. 362.

TABLE 5. Collected results

Axon no.	Diameter (μ)	Temperature ($^{\circ}$ C)	$V_+(1)$ (mV)	$\Delta_1 E_+$ (mV)	$\Delta_1 E_b$ (mV)	τ (msec)	$\Delta_1[K]$ (mM)	θ (10^{-6} cm)	P (10^{-5} cm/sec)
1	—	20	12.1	-1.36	-0.46	57	1.2	3.3	5.8
2	500	19	14.5	-1.15	-0.30	55	1.3	3.1	5.7
3	620	19	11.4	-1.36	-0.49	120	1.3	3.1	2.6
4	—	18	14.9	-2.40	-0.63	60	2.7	1.5	2.5
5	—	17	15.6	-1.26	-(0.4)	(32)	(1.8)	(2.3)	(7.2)
6	570	20	13.3	-1.06	-0.36	101	1.0	3.8	3.8
7	590	18	16.6	-0.62	-(0.2)	(22)	(1.2)	(3.4)	(15.5)
8a	670	18	12.3	-1.40	-0.62	38	1.9	2.1	5.5
8b	670	17	12.8	-1.54	-0.51	34	2.2	1.9	5.6
Mean	600	18	13.7	-1.35	-0.44	58	1.6	2.7	6.0
S.E. of mean	—	—	—	—	—	—	—	0.3	1.3

$V_+(1)$ is the amplitude of the first positive phase in a train of impulses.

$\Delta_1 E_+$ is the difference between the membrane potentials at the peaks of the first and second positive phase; the interval between stimuli was 20 msec, except in axon 1 where it was 8 msec.

$\Delta_1 E_b$ is the negative after-potential measured (or calculated) at the same time as $\Delta_1 E_+$.

$\Delta_1[K]$ is the rise in potassium concentration immediately after the spike calculated from $\Delta_1 E_+$ and τ .

θ is the apparent width of the space outside the excitable membrane.

P is the apparent permeability of the layer outside this space.

A potassium outflow of 4×10^{-12} mole/cm² per impulse has been assumed throughout.

The values opposite axons 8a and 8b were obtained at different times on the same fibre.

Values enclosed in parentheses were difficult to measure because of the small size of the negative after-potential.

The method of measuring the rise in potassium concentration produced by a single impulse may introduce a small error if there is an appreciable leakage of potassium in the resting state. In applying the curve in Fig. 6 to calculate $\Delta[K]$ it is assumed that a rise in potassium concentration in the external fluid produces the same effect on E_+ as a rise in potassium concentration in the space between the excitable membrane and the external layer. This will not be true if the resting potassium leakage varies with the external potassium concentration. In order to assess the error we shall assume, somewhat arbitrarily, that there is no resting leakage into the 20 mM-K solution and that the leakage into a K-free solution is 100 pmole/cm². sec (this is about three times the corresponding rate in *Sepia* axons—Hodgkin & Keynes, 1954). The concentration difference required to give a

flow of 100 pmole/cm².sec through a layer of permeability 5×10^{-5} cm/sec is 2 mm. This means that the potassium concentration inside the external layer will be 2 mM in a K-free solution and 20 mM in a 20 mM-K solution. The upshot is that the relation between E_+ and the potassium concentration near the membrane would be about 10% steeper than the relation between E_+ and the potassium concentration in the bulk of the external fluid. Since the latter relation was used in the calculations $\Delta[K]$ and P would be 10% too high and θ 10% too low. Another possible error mentioned on p. 368 would not affect $\Delta[K]$ or P , but would again make θ about 10% too low.

In calculating $\Delta[K]$ from the measured change in membrane potential (ΔE_+ or ΔE_0) it has been tacitly assumed that the local rise in potassium concentration did not produce a potential difference across the external layer. This is reasonable if the outer layer is unselective. The junction potential between 500 mM-KCl and 500 mM-NaCl is about 5 mV so that a 1 mm change in potassium concentration in an unselective layer would give only 0.01 mV.

The change in spike potential

It has been shown that the potential reached by successive spikes in a train of impulses alters with about the same time constant as the positive phase and the displacement of the membrane potential between impulses. The change in spike potential (E_a) was not measured in many experiments, but appeared to be highly variable. In axon 6 (Fig. 1) $\Delta_1 E_a$ was 0.5 mV, whereas axon 8, which was an exceptionally stable preparation, gave a value of 0.19 mV with 10.4 mM-K and 0.12 mV with a K-free solution. The change in E_a may be partly explained by the decrease in external sodium concentration resulting from the entry of sodium into the axon from a confined space. If the sodium entry were equal to the potassium exit the change in sodium concentration would alter the spike potential by about 0.06 mV. Another factor which could give larger changes in E_a is the inactivation of the sodium-carrying system resulting from the depolarizations produced by increasing concentration of potassium (Hodgkin & Huxley, 1952c; Weidmann, 1955). This effect might be variable because (1) the peak potential reached during the spike does not depend on the sodium permeability if this is high compared with the permeability to other ions, (2) the relation between inactivation and membrane potential is S-shaped so that changes in potential have little effect if the resting potential is high. The combined effect of these two factors is that $\Delta_1 E_a$ should increase as the external potassium concentration rises or the resting potential falls. The effect of an increase in potassium concentration in raising ΔE_a is plainly seen in Table 3.

The relative constancy of the spike potential, and the fact that the changes which did occur were explained by alterations in the external potassium and sodium concentrations, mean that there is no need to postulate any appreciable change in the sodium concentration inside the membrane. This is interesting because it indicates that sodium ions must be able to diffuse freely from the inside of the membrane, and that there is no inner layer comparable to the outer layer which holds up potassium.

The time course of the after-effects

Shanes (1949*a*) found that the negative after-potential of the squid giant fibre has an exponential time course. Our results confirm this, as may be seen from Fig. 7 in which the negative after-potentials produced by 1, 2 and 7 impulses, spaced 20 msec apart, are plotted on a semi-logarithmic scale. The exponential nature of the after-potentials is shown by the fact that the observations are fitted by straight lines of about the same slope. The time constant found by this method was 36–37 msec.

Records like those in Fig. 1 indicate that the after-potentials build up exponentially during a train of impulses with about the same time constant as they decline after activity. This conclusion is borne out by the observations in Fig. 7. Thus the intercept at $t=0$ is 0.68 mV for 1 impulse and 1.57 mV for 7 impulses. From equation (7) (p. 367) the time constant for the build-up should be given by the value of τ which satisfies the equation

$$\frac{1 - \exp(-140/\tau)}{1 - \exp(-20/\tau)} = \frac{1.57}{0.68}.$$

This gave $\tau = 36$ msec as against the value of 36–37 msec for the decline.

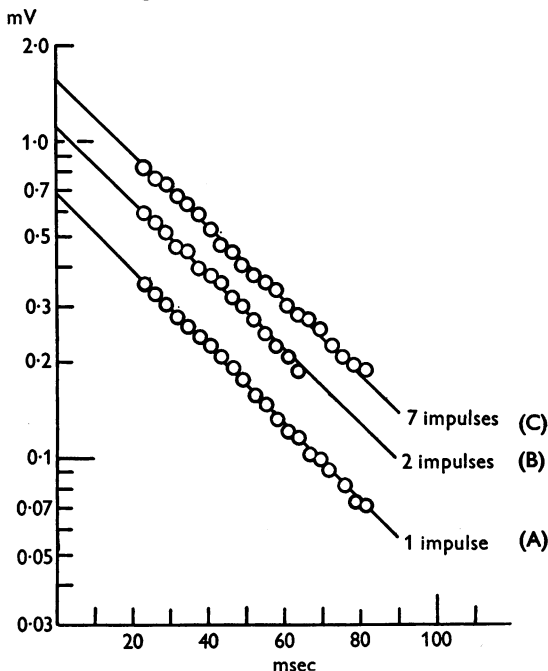


Fig. 7. Time course of negative after-potentials. Axon 8, temperature 18° C; artificial sea water, 10.4 mm-K. The ordinate is the potential in mV and the abscissa is the time in msec after the last spike in the series. The line for 1 impulse corresponds to a time constant of 36 msec; those for 2 and 7 impulses to one of 37 msec (the difference is almost certainly not significant). The line for 1 impulse is based on records taken at higher gain than those for 2 or 7 impulses. The straight lines were drawn through the points by eye.

A corollary to these results is that the negative after-potentials should add in a linear manner. The gain at which the records in Fig. 7 were taken was not sufficient to allow this point to be tested accurately, but the prediction is approximately fulfilled. Thus the intercept of lines A, B and C are 0.68, 1.10 and 1.57 mV respectively; 20 msec later the values are 0.39, 0.55 and 0.91 mV respectively. If B (2 impulses) is obtained by superposition the calculated value is $0.68 + 0.39 = 1.07$ mV as against the observed value of 1.10 mV. Since a steady state has been achieved by 7 impulses (line C) it follows that the amount by which C declines in 20 msec must be equal to the contribution of a single impulse. This is also fulfilled since $1.57 - 0.91 = 0.66$ as against the observed value of 0.68 mV.

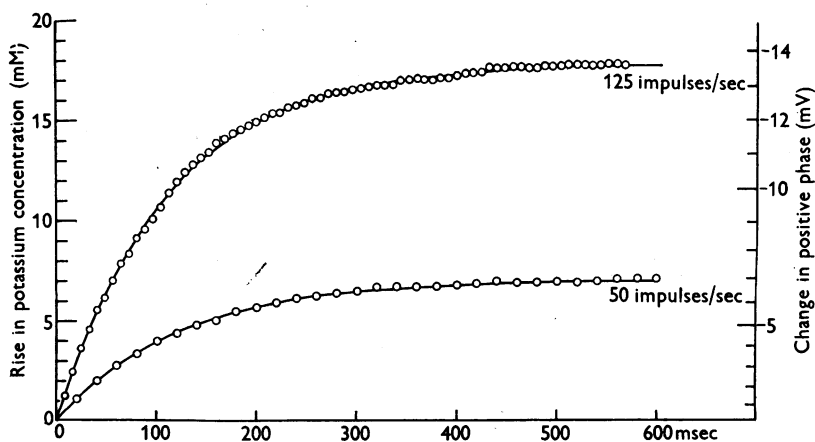


Fig. 8. Change in positive phase and potassium concentration during trains of impulses of frequency 125/sec and 50 sec. The abscissa is the time in msec from the first impulse. The right-hand ordinate scale gives ΔE_+ , i.e. the difference between the peak membrane potential during the positive phase of any impulse and that during the first impulse. The left-hand scale gives the rise in potassium concentration calculated from ΔE_+ by a calibration curve similar to that in Fig. 6. The smooth curves were drawn from the equation $y = A[1 - \exp(-t/\tau)]$ with $A = 17.8$ mM (upper curve) or 7.0 mM (lower curve) and $\tau = 110$ msec (upper curve) or 120 msec (lower curve). The amplitude of the first positive phase was 11.1 mV (upper curve) or 11.6 mV (lower curve). Axon 3; temperature 19° C; artificial sea water, 10.4 mM-K.

Since the relation between potassium concentration and resting potential is linear when the former is small (Fig. 6 and Shanes, 1949*b*) the exponential nature of the after-potentials suggests that the potassium concentration must rise and fall in an exponential manner. In order to test this point further, the variation of the positive phase during a train of impulses was examined by the method illustrated in Fig. 8.

Stimulation frequencies of 125/sec or 50/sec were employed and the potential

during the positive phase (E_+) was measured in the usual way. When the right-hand scale in Fig. 8 is employed the points give the difference between the positive phase of any impulse and the positive phase of the first impulse. These observations were converted into changes in potassium concentration by a calibration curve similar to that in Fig. 6. The linear scale on the left-hand side of Fig. 8 gives these values. The smooth curves, which are clearly a good fit to the observations, were drawn from the equation

$$y = A[1 - \exp(-t/\tau)],$$

where τ had a value of 110 msec in the upper curve and 120 msec in the lower curve. The difference between the two time constants is not regarded as important since it could have arisen from a small error in the shape of the calibration curve. The steady concentration built up after a large number of impulses is determined by the value of A ; this was 17.8 mM for stimulation at 125/sec and 7 mM for stimulation at 50/sec—a ratio of 2.56. If the time constant τ were very long compared to the interval between spikes, the ratio should be 125/50 = 2.5. A more exact calculation based on equation (7) (p. 367) gave a theoretical ratio of 2.64 when τ was taken as 115 msec for both curves, and one of 2.40 when the observed values of 110 and 120 msec were employed.

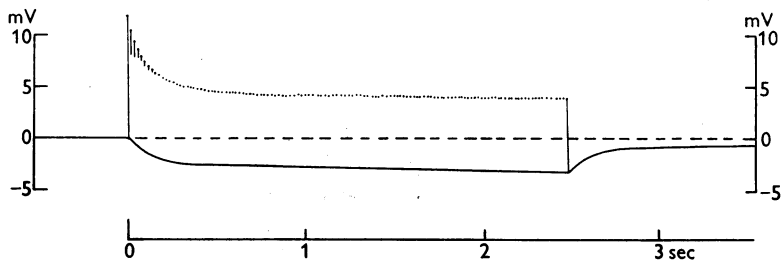


Fig. 9. Tracing showing slow depolarization and variation of the positive phase during a train of impulses at 50/sec lasting 2.5 sec. Axon 3, temperature 19° C; artificial sea water, 10.4 mM-K. A continuous line has been drawn through the base-line between impulses; the tips of the positive phases are shown by dots.

When trains of impulses lasting several seconds were used there was evidence of a gradual increase in potassium concentration after the initial rise was complete. This is illustrated by Fig. 9, which shows that there was a progressive shift in base-line and in the positive phase during the period of 2.5 sec in which the stimulus was applied. An effect of this kind is to be expected because potassium ions have to diffuse through about 20μ of connective tissue before reaching the external fluid, and this will cause a slow rise in potassium concentration. Considerable variation is likely because the thickness and possibly the consistency of the connective tissue changes from one fibre to the next.

The records described so far show the decline of the positive phase during a train of impulses, but they do not give any information about the way in which the underlying process recovers after activity. According to the present hypothesis this should have the same time constant as the negative after-potential. Two methods of testing this conclusion were used. In one case a gap was left in a train of impulses as in Fig. 10. This showed that the potential at the peak of the positive phase increased as the gap was widened. On working out the experiment the negative after-potential was found to have a time constant of 112 msec, while the positive phase recovered with one of 113 msec. The close agreement is probably a coincidence but there would seem to be little doubt about the correlation between the negative after-potential and the amplitude of the positive phase.

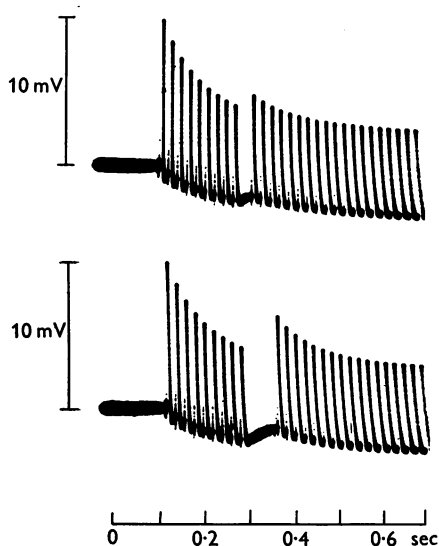


Fig. 10. Effect of leaving a gap in a train of impulses. Axon 6; temperature 13.0° C; natural sea water, 10 mM-K. Frequency of stimulation, 50/sec.

The other way of examining the recovery of the positive phase was to use two shocks and vary the interval between them. This procedure, which was used on only one occasion, gave the results shown in Fig. 11. In this experiment the negative after-potential had an amplitude of 0.63 mV 20 msec after a single impulse, and an exponential time constant of 60 msec. The observations of the recovery of the positive phase of the second impulse were also fitted by an exponential curve with the same time constant.

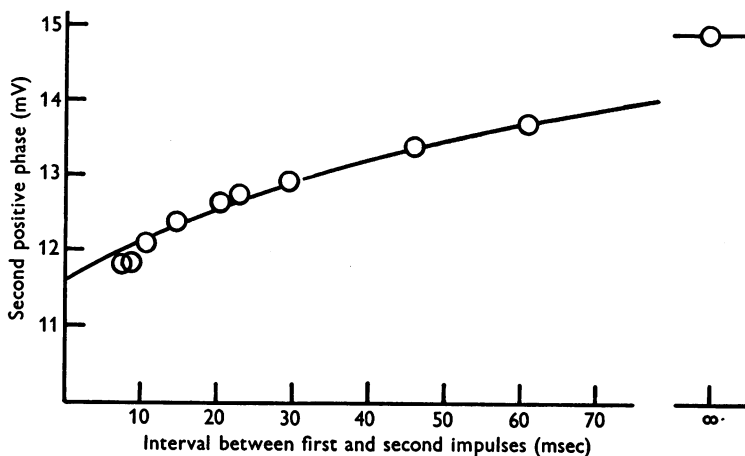


Fig. 11. Effect of varying interval between 2 impulses on the positive phase of the second impulse. Abscissa, interval between spikes; ordinate, membrane potential at crest of second positive phase minus resting potential. The smooth curve is drawn according to the equation $y = 14.9 - 3.3 \exp(-t/60 \text{ msec})$. Axon 4, temperature 18°C , natural sea water, 10 mm-K.

TABLE 6. Time constants of after-effects in msec

Axon no.	Temperature ($^\circ \text{C}$)	τ_1	τ_2	τ_3	τ_4	$\bar{\tau}$
1	20	57	—	—	—	57
2	19	55	59	61	—	58
3	18.8	120	118	123	—	120
4	18	—	—	60	60	60
5	16.8	32	—	—	—	32
6	27.3	80	75	76	—	77
	19.5	101	101	96	—	99
	13.0	115	101	112	113	110
	10.2	148	140	141	—	143
7	17.9	22	(25)	(27)	—	22
8a	26.0	31	25	29	—	28
	18.1	38	31	32	—	34
	13.3	42	43	37	—	41
	8	47	—	43	—	45
8b	17	34	33	36	—	34

τ_1 is the time constant obtained from the decline in positive phase (ΔE_+) during a train of impulses. τ_2 and τ_3 were obtained from the build-up and decline of the negative after-potential (ΔE_b); τ_4 from the recovery of the positive phase after one or many impulses. $\bar{\tau}$ is the mean time constant. Owing to the small size of the negative after-potentials in axons 5 and 7 no figures for τ_1 or τ_3 could be obtained in the former case and only doubtful ones in the latter.

Comparison of time constants obtained by different methods

The rise and fall in the apparent potassium concentration near the membrane was normally obtained in three different ways, namely:

- (1) The decline of successive positive phases during a train of impulses,
- (2) The shift of base-line during the train,
- (3) The negative after-potential at the end of the train.

The results obtained by these methods are given in Table 6. This shows that

the time constants vary considerably between fibres, but that the different methods gave approximately the same answer in one fibre. The fact that all three effects vary in the same way from one fibre to the next is strong evidence that they depend on a common process such as the rise and fall of potassium concentration near the membrane. There are small discrepancies in individual experiments, but there is no evidence of a systematic difference. The discrepancies are probably explained by: (1) the negative after-potential was small and its early time course was obscured by the damped oscillations which follow the spike; and (2) the existence of a slow 'creep' (p. 355) makes any exact measurement of an exponential time constant slightly arbitrary.

The influence of temperature

If the after-effects of impulses are caused by a local rise in the concentration of potassium they should decrease when the temperature is raised since this reduces the outflow of potassium per impulse (Shanes, 1954). On the other hand, the time scale of the after-effects should change relatively little with temperature, since the diffusion of potassium through an unselective layer of fairly high permeability is unlikely to have a high temperature coefficient. Both predictions are easily tested over the range 15–27° C; below 10° C the duration of the positive phase and refractory period increase to such an extent that the after-effects are difficult to interpret. The records in Figs. 12 and 13 illustrate the influence of temperature on the after-effects in a fibre with a fairly long time constant.

Temperature and time scale. The two experiments summarized in Table 7 suggest that the time constant of the after-effects was increased about 1.3-fold when the temperature was lowered by 10° C; this is in contrast to the spike and positive phase whose durations are increased about 3-fold by cooling 10° C (Hodgkin & Katz, 1949*b*).

It is interesting to compare the temperature coefficient of the rate at which potassium appears to diffuse away from the membrane with the temperature coefficient of diffusion in an aqueous solution. According to the Nernst formula the diffusion coefficient D should be equal to RTU/F , where U is the mobility. From conductivity tables $U_{20^\circ\text{C}}/U_{10^\circ\text{C}}$ is 1.23 for 0.5M-KCl, so that $D_{20^\circ\text{C}}/D_{10^\circ\text{C}} = 1.27$. This is not significantly different ($P = 0.13$) from the results in Table 7, which gave a ratio of 1.34 (S.E. of mean, 0.04) for a change of 10° C.

Temperature and the positive phase. The results under $\Delta_1 E_+$ in Table 7, and the records in Fig. 13, suggest that cooling increases the difference between the first and second positive phases in a train of impulses. This is consistent with an increased outflow of potassium per impulse at low temperatures, but the results could not be worked out quantitatively because cooling reduces the amplitude of the first positive phase, and is therefore bound to alter the relation between the positive phase and the external potassium concentration. This

means that curves such as those in Fig. 6, which were determined only at room temperature, could not be used for calculating the rise in potassium concentration at other temperatures.

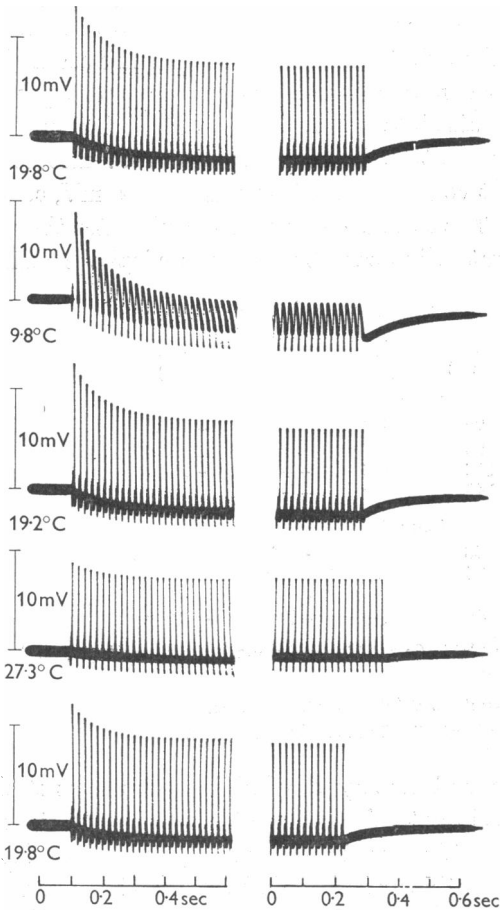


Fig. 12.

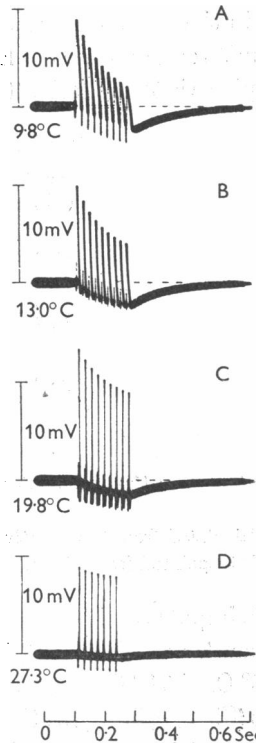


Fig. 13.

Fig. 12. Effect of temperature on after-effects. The records were taken in the order shown, starting at the top. Frequency of stimulation 50/sec; axon 6; natural sea water, 10 mM-K.

Fig. 13. Effect of temperature on after-effects. The records were taken in the order B, C, A, D. Frequency of stimulation 50/sec; axon 6; natural sea water, 10 mM-K.

The variation of the positive phase of a single impulse seen in Table 7 agrees with previous observations, which showed that the positive phase had a maximum at about 25° C, and was reduced to about half by warming to 35° C or cooling to 5° C (Hodgkin & Katz, 1949*b*). The decline on warming, which was seen in one of the axons in Table 7, is probably a consequence of the marked

decrease in spike amplitude which occurs near 30° C. The decline between 20 and 0° C is more interesting since the spike increases slightly as the temperature is reduced over this range. Cooling is now thought to decrease the positive phase in two different ways. In the first place Hodgkin & Katz (1949*b*) found that the resting potential remained practically constant or even increased slightly as the temperature fell from 20 to 0° C. The equilibrium potential for potassium, which determines the membrane potential during the positive phase, should be proportional to absolute temperature and therefore ought to decrease by 2 mV when the temperature falls from 20 to 10° C. The reduction in the amplitude of the positive phase over this range is 4–5 mV, so that 2–3 mV remain to be explained. The second factor which could alter the positive phase is that a fall of temperature increases the potassium leakage during a

TABLE 7. Influence of temperature on after-effects

Temperature (° C)	$\bar{\tau}$ (msec)	$V_+(1)$ (mV)	$\Delta_1 E_+$ (mV)	$\Delta_1 E_b$ (mV)
Axon 6				
27.3	77	8.9	-0.34	-0.12
19.5	99	13.3	-1.06	-0.36
13.0	110	9.8	-1.52	-0.54
10.2	143	9.2	-1.54	-0.42*
Axon 8				
26.0	28	15	-0.76	-0.50
18.1	34	12.3	-1.40	-0.62
13.3	41	8.8	-1.76	-0.62
8	45	7.3	-1.71	-0.40†

$\bar{\tau}$ is the mean time constant given in Table 6: see Table 5 for definition of $V_+(1)$, $\Delta_1 E_+$ and $\Delta_1 E_b$.

* Calculated from the negative after-potential following 7 impulses.

† Extrapolated from observations made at 40–80 msec after stimulus.

single impulse and prevents the membrane hyperpolarizing as much as at high temperatures. In axon 8 the rise in potassium concentration was about 2 mM at 18° C. At 8° C the potassium outflow per impulse should be about twice as great (Shanes, 1954), so that the positive phase should be reduced by an amount equivalent to an excess of 2 mM in the potassium concentration; according to Fig. 6, which was also obtained on axon 8, this would be 2.4 mV.

Temperature and the negative after-potential. The negative after-potential was increased by cooling from 27 to 12–15° C (Fig. 13), but at lower temperatures it probably decreased (Table 7). The increase is consistent with a greater potassium outflow at 12–15° C than at 27° C; the decrease below 12° C may occur because the positive phase lasts so long that it obscures the negative after-potential. Another possibility is that the depolarizing effect of a small rise in potassium concentration may be less at low temperatures.

The polarization effect in voltage clamp experiments

Hodgkin *et al.* (1952) found that the potassium current which flowed outwards under a cathode was not maintained but declined as a result of a polarization effect. The records in Fig. 14 (which were taken in collaboration with Mr A. F. Huxley in 1949) illustrate the effect. The decline in potassium current

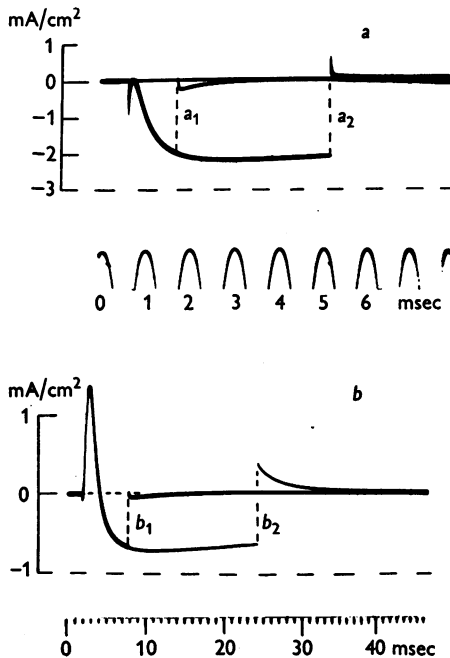


Fig. 14. Records of membrane current densities during a voltage clamp lasting for two different durations. Inward current is shown as an upward deflexion. The external fluid was natural sea water (10 mM-K). Record *a*: axon (ii) Table 8—temperature 20° C, depolarization of 71 mV. This axon had been used for some time and was in poor condition. Record *b*: axon (iii) Table 8—temperature 8° C, depolarization of 36 mV.

is perceptible in *a*₂ and is more marked with the longer pulse used in *b*₂. The effect is correlated with a change in current at the end of the pulse. After a relatively brief depolarization as in *a*₁ the tail of current at the end of the pulse was outward; after a longer pulse it was inward, as in *b*₂; with a pulse in which the total quantity of potassium was intermediate as in *a*₂ there might be practically no current at the end of the pulse.

The polarization effects illustrated in Fig. 14 are explained by assuming that there is an unselective barrier to diffusion between the excitable membrane and the external solution (cf. Hodgkin & Huxley, 1952*b*, pp. 494–5). When internal potassium ions are carried through the excitable membrane they raise

the concentration in the space between the two membranes. This reduces the force driving potassium ions outwards and causes the current to fall. The rise in potassium concentration also accounts for the changes in current at the end of the period of depolarization. After a brief period of depolarization as in a_1 the current is outward because the resting potential (E_r) is less than the equilibrium potential for potassium ions (E_K). After a long pulse (for example b_2) the current is inward because E_K is less than E_r ; this current is carried inward by potassium ions which left the fibre and accumulated in the space during the period of depolarization.

Records like those in Fig. 14 can be used to calculate the apparent thickness of the space in which potassium ions accumulate. The potassium conductance of the membrane is defined by the equation

$$g_K = \frac{I_K}{E - E_K},$$

where g_K is the potassium conductance per unit area;

I_K is the current density (inward current positive);

E is the potential difference across the membrane (external minus internal potential);

E_K is the equilibrium potential for potassium ions.

Using the observation that g_K is a continuous function of time (Hodgkin & Huxley, 1952*b*) it follows that the potassium currents immediately before and immediately after the end of the pulse are related by

$$\frac{I'_K}{I''_K} = \frac{E' - E_K}{E'' - E_K},$$

where E' or I'_K and E'' or I''_K are the potentials or currents immediately before and immediately after the end of the pulse. Since $E'' = E_r$ and $E' = E_r + V$, where V is the applied potential, this relation can be written

$$\frac{I'_K}{I''_K} = \frac{-V + (E_K - E_r)}{(E_K - E_r)}.$$

In record a_1 of Fig. 14, I'_K is 2.00 mA/cm² and I''_K is found by extrapolating the exponential tail of potassium current to be 0.33 mA/cm². With an applied potential of -71 mV this gives a value of +14 mV for $E_K - E_r$. In the case of record a_2 the same procedure gave a value of -1.8 mV for $(E_K - E_r)$ at the end of the pulse. In order to estimate the rise in potassium concentration from these figures we used the observation that in *Sepia* axons the apparent equilibrium potential for potassium decreases by 9 mV when the potassium concentration is raised from 10 to 20 mM, and obeys the equation for a potassium electrode at higher concentrations (Table 9 of Hodgkin & Keynes, 1955). Values obtained by this method are given under $\Delta[K]$ in Table 8. The quantity

of potassium ions (ΔQ_K) which caused the rise in potassium concentration was obtained from the difference between the time integrals of the potassium currents during the two pulses. The apparent thickness of the space in which potassium ions accumulated was then calculated from the relation $\theta = \Delta Q_K / \Delta [K]$. (No allowance need be made for loss of potassium through the outer layer since the pulses considered were short compared with the time constant.) The mean value of θ obtained by this method, 290 Å, was not significantly different from that of 270 Å found previously. The agreement is satisfactory because the sources of error in the two methods are likely to be different. The voltage clamp method was probably less accurate but it had the advantage that it did not require any assumption about the quantity of potassium ions leaving the fibre during an impulse.

The decline in outward current during a steady depolarization cannot easily be treated quantitatively since there is no information about the relation between potassium concentration and conductance at a fixed membrane potential. A tentative approach is to suppose that the potassium current is determined by the equation

$$I_K = P_K \{ [K]_o - [K]_i \exp(-EF/RT) \},$$

where P_K is a function of E but is independent of the external and internal concentrations of potassium ions ($[K]_o$ and $[K]_i$). This equation should apply to any system in which the ions move independently (Hodgkin & Huxley, 1952*a*) and to certain systems in which there is interaction—for example the theoretical model considered by Hodgkin & Keynes (1955). Using the equation as a basis for calculation it follows that a change of potassium potential from E_K to E_K^* will alter the potassium current from I_K to I_K^* where

$$\frac{I_K}{I_K^*} = \frac{1 - \exp[(E_K - E) F/RT]}{1 - \exp[(E_K^* - E) F/RT]}.$$

In record *b* of Fig. 14, in which the applied potential was -36 mV, $(E_K - E)$ changes from 39 to 24 mV during the interval of 16.2 msec between the end of the short pulse and the end of the long pulse. Substituting these values in the preceding equation it is found that the potassium current should decrease by 21% in 16 msec. The decline seen in the second half of record *b* 2 is equivalent to about 15% in 16 msec, but this figure may be too small because the decline due to 'polarization' will not have its full effect until the potassium permeability has reached its maximum value. The agreement in order of magnitude is regarded as evidence that the polarization effect arises from an increase of potassium concentration, but further experiments are needed in order to place the effect on a fully quantitative basis.

One limitation of the theory put forward by Hodgkin & Huxley (1952*d*) on the basis of voltage clamp records is that the equations do not predict a

TABLE 8. Changes in potassium equilibrium potential in voltage clamp experiments

Axon	Diameter (μ)	Temperature ($^{\circ}$ C)	Depolarization (mV)		Duration (msec)		Mean outward potassium current (mA/cm 2)		Q_K (pmole/cm 2)		ΔQ_K (pmole/cm 2)	$(E_K - E_r)$ after pulse (mV)		ΔE_K (mV)	$\Delta[K]$ (mM)	θ (10^{-6} cm)
			A	B	A	B	A	B	A	B						
(i)	480	23	13	54	3.9	3.9	0.056	1.00	2.2	40.6	38.4	18.3	5.5	-12.8	14	2.7
	480	23	13	71	3.9	3.9	0.056	1.52	2.2	61.4	59.2	18.3	-1.2	-19.5	21	2.8
(ii)	485	20	71	71	1.13	4.60	1.30	1.96	15.2	93.5	78.3	14.2	-1.8	-16.0	17	4.6
(iii)	520	8	7	36	22.4	5.76	0.0082	0.36	1.9	21.4	19.5	12.0	3.0	-9.0	10	2.0
	520	8	7	43	22.4	5.76	0.0082	0.47	1.9	28.0	26.1	12.0	0.7	-11.3	12	2.1
(iv)	605	22	113	113	0.50	0.95	2.00	3.16	10.4	32.0	21.6	12.5	6.3	-6.2	7	3.1
	605	22	113	113	0.50	1.52	2.00	3.72	10.4	58.6	48.2	12.5	-1.5	-14.0	15	3.2
															Mean	2.9
															S.E.	0.3

This table gives the potassium potential measured after (A) a short or a small pulse and (B) a longer or stronger pulse. $E_K - E_r$ is the difference between the apparent equilibrium potential for potassium ions and the resting potential. $\Delta[K]$ is the rise in potassium concentration calculated from ΔE_K by the method given in the text. Q_K is the total quantity of potassium flowing outwards during the pulse calculated as $\frac{1}{F} \int_0^{t'} -I_K dt$, where F is the Faraday, $-I_K$ is the outward potassium current and t' is the duration of the pulse. ΔQ_K is the difference between the quantities of potassium moving outwards in pulse B and pulse A. $\theta = \Delta Q_K / \Delta[K]$ and is the apparent thickness of the space in which potassium accumulates. θ was not worked out in one case because the duration of pulse B (22.0 msec) was too long for the method to be applicable.

negative after-potential. It is now evident that this limitation arose because the analysis took no account of the polarization effect observed with large outward currents. If this had been allowed for correctly, it seems reasonably certain that the calculated action potentials would have been followed by a negative after-potential and other after-effects of the kind described in the present paper.

Comparison of the permeability and electrical resistance of the outer layer

The experiments in Table 5 give an average value of 6.0×10^{-5} cm/sec for the potassium permeability of the outer layer, the standard error of the mean being 1.3×10^{-5} in nine experiments.

The permeability (P) is related to the diffusion coefficient in free solution (D) by $P = DA/L$, where L is the thickness of the layer and A is a factor representing the amount by which diffusion is restricted. In a 0.5 M solution of KCl, D is about 1.5×10^{-5} cm²/sec at 18° C (Landolt & Börnstein, 1931) so that $L/A = 0.25$ cm. If the layer is assumed to be unselective, A must be the same for all ions. Hence the electrical resistance of the outer layer should be $\rho L/A$, where ρ is the specific resistance of sea water. At 20° C, ρ is about 22 Ω . cm so that the electrical resistance of the outer layer should be 5.5 Ω . cm² (s.e. 1.2). This estimate may now be compared with the figure given by Hodgkin *et al.* (1952) for the electrical resistance of the layer outside the excitable membrane. In Table 1 of that paper the average value of the quantity r_s is given as 0.68 times the standard fluid resistance r_{cd} which had a value of 74 Ω when filled with sea water at 20° C. The mean fibre diameter in these experiments was 557 μ and the length exposed to current was 0.70 cm. Part of the resistance r_s arose in the external fluid and axoplasm. At the time, these components were calculated as 30% and < 25%. Since the method of calculation only gave an upper limit for the latter quantity we made further measurements with the apparatus used by Hodgkin *et al.* to determine both components more directly. The measurements did not require an axon, and consisted in determining r_s/r_{cd} with the cell filled with artificial sea water. Tests with a probe were also made in order to find out what fraction of the potential differences occurred within 300 μ of the spiral electrode. Using these values and taking the specific resistance of axoplasm as 1.4 times sea water (Cole & Hodgkin, 1939) we obtained revised figures of 30% for the external component of r_s and 16% for the internal component. The remaining 54% is taken as the electrical resistance of the layer outside the excitable membrane. On this basis the electrical resistance of the outer layer is found to be

$$74 \Omega \times 0.68 \times 0.54 \times \pi \times 0.0557 \text{ cm} \times 0.7 \text{ cm} = 3.3 \Omega \cdot \text{cm}^2.$$

The standard error of the mean of this value is 0.3 Ω . cm². The mean value is somewhat lower than that calculated from the potassium permeability, but

the difference is not statistically significant. In view of the variability between fibres, the difficulty of the measurements and the great difference in the methods, it is thought that the agreement in order of magnitude is satisfactory.

The existence of a layer with a resistance of 3–5 $\Omega \cdot \text{cm}^2$ outside the excitable membrane may help to explain a discrepancy between the values of axoplasm resistivity obtained at high frequencies with transverse electrodes (Curtis & Cole, 1938) and those obtained with direct or alternating current flowing parallel to the nerve fibre (Cole & Hodgkin, 1939). In the former paper the axoplasm resistivity is given as 1.5–6.9 times that of sea water with a mean of 4.2; in the latter it is stated to about 29 $\Omega \cdot \text{cm}$ or 1.4 times sea water. Curtis & Cole's high-frequency resistance was obtained by extrapolating along a circle which fitted the points up to about 200 kc/s. If a layer outside the excitable membrane had an impedance of only 1 $\Omega \cdot \text{cm}^2$ at 100–200 kc/s it would raise the apparent specific resistance of the axoplasm by 40 $\Omega \cdot \text{cm}$ in a 500 μ axon (see equation (2) of Curtis & Cole, 1938). It therefore seems possible that the relatively high values of axoplasm resistivity found by Curtis & Cole, and perhaps the bump shown at the high-frequency end of the circle diagram (their fig. 2) may be caused by a layer outside the excitable membrane.

THEORY

Hypothesis 1: finite space and very thin barrier to diffusion

The simplest way of examining the results is to suppose that the diffusion of potassium ions away from the fibre is restricted by a very thin outer layer which is separated from the excitable membrane by an aqueous space about 300 Å thick. On this basis the concentration of potassium ions in the aqueous space will be governed by

$$\theta \frac{dy}{dt} = M - Py, \quad (1)$$

where y is the excess concentration of potassium in mole/cm³, θ is the thickness of the space in cm, t is time in seconds, P is the permeability of the outer layer to potassium ions in cm/sec, M is the outflow of potassium ions through the excitable membrane in mole $\cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$. In writing this equation it has been assumed that the potassium concentration at any given moment is constant throughout the space; this is reasonable because differences of concentration would be eliminated by diffusion within a few microseconds in an aqueous space of thickness 300 Å.

During the falling phase of the spike the outward flow of potassium through the excitable membrane should be large compared with the movement through the outer layer, that is $M \gg Py$. The excess concentration of potassium immediately after the spike will therefore be given by

$$y_0 = Q/\theta, \quad (2)$$

where Q is the integral of M over the spike and is the total outward movement of potassium in one impulse. To obtain the subsequent time course of y we shall assume as a first approximation that the inward transfer through the excitable

membrane is small compared with the transfer through the outer layer, that is $Py \gg M$. On this basis

$$\theta \frac{dy}{dt} = -Py, \quad (3)$$

and for a single impulse

$$y = y_0 \exp \frac{-t}{\tau}, \quad (4)$$

where

$$\tau = \theta/P. \quad (5)$$

The time course of the potassium concentration during a train of impulses can be obtained by superposition. At a fixed time interval t_2 after the n th impulse of a regular train spaced at intervals of t_1 , the potassium concentration will be

$$y = \frac{Q}{\theta} \left(\exp \frac{-t_2}{\tau} \right) \sum_{r=1}^{r=n} \exp - \frac{(r-1)t_1}{\tau}, \quad (6)$$

or

$$y = \frac{Q}{\theta} \left(\exp \frac{-t_2}{\tau} \right) \left\{ \frac{1 - \exp(-nt_1/\tau)}{1 - \exp(-t_1/\tau)} \right\}. \quad (7)$$

In order to deal with the situation where the permeability of the resting membrane is comparable with that of the outer layer we take the inward transfer through the excitable membrane as P' , where P' is the effective permeability coefficient for inward movement of potassium ions through the excitable membrane. P' may depend in a complicated way on the external and internal potassium concentrations and the potential difference across the membrane. However, for sufficiently small changes of external potassium concentration it becomes a constant so that equations (4), (6) and (7) apply if the time constant is taken as $\theta/(P + P')$ instead of θ/P .

An estimate of P' can be obtained from the resting potassium conductance which will be taken as 0.23 m-mho/cm² for a nerve surrounded by a solution containing 10 mM-K (see Hodgkin & Huxley, 1952*b*, table 5). If the external potassium concentration is increased by 1 mM the equilibrium potential for potassium decreases by 2.5 mV and the resting potential decreases by 0.4 mV (Fig. 6). The inward flux of potassium produced by this change will be

$$2.1 \times 10^{-3} \times \frac{0.23 \times 10^{-3}}{96500} = 5.0 \times 10^{-12} \text{ mole.cm}^{-2}.\text{sec}^{-1}.$$

On dividing this quantity by 1 mM ($\equiv 10^{-6}$ mole.cm⁻³) a value of $P' = 5 \times 10^{-6}$ cm/sec is obtained. This is less than one-tenth of the average permeability of the outer layer so it is not unreasonable to neglect the inward transfer through the excitable membrane.

At first it might be thought that the values given in Table 5 for the permeability (P) of the outer layer would be too high because they had been calculated by equation (5), which assumes no inward flow through the excitable membrane. On reflexion it is clear that the error will be in θ , not P . If P'

were 10% of P , 10/11 of the potassium ions which leak out through the excitable membrane during an impulse would appear in the external solution and the remaining 1/11 would return to the fibre. In estimating θ , Q should therefore be taken as 10% higher than the value obtained by Shanes (1954). This would give a new value, θ^* , which would be 10% greater than the values in Table 5. Recalculation of P from the revised equation, $(P + P') = \theta^*/T$ then gives exactly the same value as that obtained before.

The argument is easiest to see in the example of steady stimulation given on p. 350. In this case Shanes's measurements indicate a mean outflow of 2×10^{-10} mole.cm⁻².sec⁻¹; our experiments give a mean rise in potassium concentration of 3.7×10^{-6} mole.cm⁻³. Assuming that these figures are correct it follows that the permeability of the outer layer must be 5.4×10^{-5} cm/sec, whatever the permeability of the excitable membrane.

Hypothesis 2: finite diffusion barrier and no space

The experiments described in this paper could not easily be explained without postulating a barrier to diffusion between the excitable membrane and the external solution. The evidence for the existence of an aqueous space is much less direct. So far all that has been shown is that the experimental results can be fitted by a theory in which a space is assumed to exist. The purpose of this section is to consider whether the results might not also be explained by a system in which the outside of the excitable membrane is assumed to be in direct contact with a uniform layer of thickness l .

The concentration of potassium ions in this layer would obey the equation

$$\frac{\partial y}{\partial t} = D \frac{\partial^2 y}{\partial x^2}, \quad (8)$$

where y is the excess concentration of potassium at a distance x from the inner edge of the layer, and D is the diffusion coefficient.

A quantity per unit area (Q) of potassium ions is assumed to be liberated instantaneously at $t=0$ and $x=0$; thereafter the boundary conditions are taken as

$$\frac{\partial y}{\partial x} = 0 \quad \text{at } x=0,$$

$$y = 0 \quad \text{at } x=l.$$

At $x=0$ the solution $y_{x,t}$ which fits these boundary conditions becomes

$$y_{0,t} = \frac{Q}{\sqrt{(\pi Dt)}} \{1 - 2e^{-t^2/Dt} + 2e^{-4t^2/Dt} - 2e^{-9t^2/Dt} + \dots\}, \quad (9a)$$

or

$$y_{0,t} = \frac{2Q}{l} \{e^{-\pi^2 Dt/4l^2} + e^{-9\pi^2 Dt/4l^2} + e^{-25\pi^2 Dt/4l^2} + \dots\}. \quad (9b)$$

The two series have the same sum but $9a$ converges rapidly when t is small, whereas $9b$ converges rapidly when t is large. For t small, equation $9a$ becomes

$$y_{0,t} = \frac{Q}{\sqrt{(\pi Dt)}} \tag{10a}$$

For t large, equation $9b$ becomes

$$y_{0,t} = \frac{2Q}{l} e^{-D\pi^2 t/4l^2} \tag{10b}$$

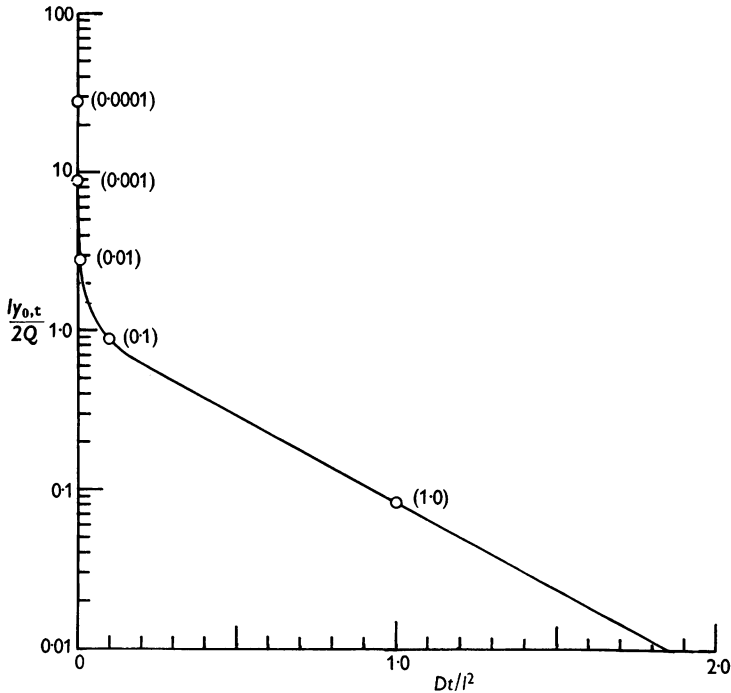


Fig. 15. Plot of equation (9). The numbers against the circles give the value of the abscissa.

The expression on the right-hand side of equation (9), which is plotted on a semilogarithmic scale in Fig. 15, becomes reasonably exponential for $t > l^2/5D$, but deviates markedly at short times. No evidence of this kind of deviation was found in the present experiments, but in many cases the measurements were made too long after the spike for any deviation to be expected. Thus the experiments in Fig. 7 are consistent with equation (9) since the observations do not begin until two-thirds of a time constant after the spike.

In the experiment of Fig. 8, in which the time constant was about 115 msec, it was possible to obtain a critical comparison between the two hypotheses. Here the excess potassium concentration 8 msec after a single impulse was 1.25 mM, whereas it was 1.10 mM 20 msec after a single impulse. The ratio of

these two values, 1.14, agrees reasonably with that of 1.10 predicted by equation (4), but is definitely less than the ratio of 1.58 predicted by equation (9).

The strongest evidence against the second hypothesis is that it is not consistent with the behaviour of the nerve at short times. If the quantities l and D are calculated from the data in Table 5 by means of equation 10*b*, average values of $l = 5.4 \times 10^{-6}$ cm and $D = 2 \times 10^{-10}$ cm²/sec are obtained. This value of D implies that a rectangular pulse of potassium current of amplitude 1 mA/cm² and duration 0.4 msec would raise the potassium concentration by 17 mM; 0.1 msec after the pulse the excess concentration would be 11 mM; 1 msec later the concentration would be 4 mM. These values (which were obtained from the integral of equation (10*a*)) are not consistent with the results obtained by the voltage clamp procedure. Thus in the experiment of fig. 11 of Hodgkin & Huxley (1952*b*), the apparent equilibrium potential for potassium remains constant to within about 1 mV for 1 msec after a brief pulse in which the total outflow of potassium was about the same as that in the theoretical example just considered.

Another reason for rejecting the second hypothesis is that it predicts improbable concentrations of potassium at the end of the spike. Taking the outflow of potassium during the spike to be the function of time calculated by Hodgkin & Huxley (1952*d*, fig. 18), and using the superposition method to calculate the potassium concentration from equation (10*a*), it is found that the concentration would reach a peak during the latter part of the falling phase and would decrease as t^{-3} during the positive phase. If D were 2×10^{-10} cm²/sec, the peak rise in potassium concentration would be about 12 mM; at the beginning of the positive phase the excess concentration would be 9 mM and 0.5 msec later it would be 6 mM. This seems an improbable result because the high potassium concentration would obliterate the positive phase and would make the end of the spike depend on the rate at which potassium diffused away from the membrane. Since the diffusion process has a low temperature coefficient, the rate of change of membrane potential should not have the high temperature coefficient observed by Hodgkin & Katz (1949*b*).

Although the second hypothesis cannot be applied in its more extreme form there is evidently need for caution in accepting the value of θ obtained by the first hypothesis. All the evidence could probably be explained satisfactorily by combining the first and second hypothesis and assuming a space of 50–100 Å. For these reasons the value of θ given in Table 5 cannot be expected to correspond exactly to an anatomical space.

As a by-product the theoretical methods used in rejecting the second hypothesis were also used to calculate the rise in potassium concentration expected if these ions could diffuse freely from the surface membrane. If there were no external barrier, and if the diffusion coefficient near the excitable membrane were 1.5×10^{-5} cm²/sec, as in an aqueous solution, the maximum

rise in potassium concentration would be only 0.05 mM; 10 msec after the spike the value would be 0.006 mM. These values are very small compared with those observed in the present experiments.

DISCUSSION

The selectivity of the excitable membrane for potassium

In cephalopod axons, the membrane potential during the positive phase and the apparent equilibrium potential for potassium ions do not obey the Nernst equation if the concentration of potassium in the external solution is less than about 20 mM (Hodgkin & Keynes, 1955; Hodgkin & Huxley, 1952*b*). One explanation of the deviations is that the potassium-carrying system in the membrane is not perfectly selective and that other ions such as sodium begin to compete effectively when the potassium concentration is less than one-twentieth of the sodium concentration. The experiments described here indicate that an alternative explanation of the deviations is to be found in the fact that the potassium concentration immediately outside the excitable membrane is not necessarily the same as that in the external solution. For example, if a fibre in which the potassium permeability of the outer layer is 5×10^{-5} cm/sec, were losing potassium at 100 pmole/cm² per sec, the potassium concentration near the membrane would be 2 mM higher than that in the external solution. Since a single impulse raises the potassium concentration by a further 1–2 mM, it is unreasonable to expect the membrane potential during the positive phase to obey the Nernst equation at concentrations below about 20 mM. Further work is needed to clear up this problem, but it seems likely that the selectivity of the potassium-carrying system in the membrane will prove to be greater than has previously been supposed.

The negative after-potential

The explanation of the negative after-potential discussed in this paper is similar to that proposed by Shanes (1951). More recently, Shanes *et al.* (1953) argued that the negative after-potential could not arise solely from an increase in potassium concentration because the potassium ions liberated in one impulse would not have much effect if they were distributed uniformly throughout the extra-cellular space. The objection disappears if one modifies the original theory by postulating a layer just outside the excitable membrane which prevents potassium ions diffusing freely from the fibre. This hypothesis works well for squid fibres in a normal medium, but there are difficulties in supposing that the negative after-potential always arises in this manner.

As in other tissues (Gasser, 1937; Graham & Gasser, 1931) the negative after-potential of squid nerve is greatly increased by veratrine (Shanes, 1952). Although veratrine is known to increase the leakage of potassium per impulse (Shanes, 1951) this is probably not the sole cause of the increased negative after-

potential. In the first place veratrine may increase the negative after-potential of the squid axon five to tenfold (Shanes, 1952), whereas the potassium leakage of crab nerve is not more than doubled (Shanes, 1951). In the second place Shanes (1952) showed that two constituents of 'veratrine', veratridine and cevadine, have different effects. Veratridine gives a negative after-potential with a time constant of several hundred milliseconds; cevadine one which is shorter than that produced by veratridine but somewhat longer than that of the unpoisoned fibre. These observations are hard to reconcile with the present hypothesis, and it seems more likely that the enhanced negative after-potential in veratrine arises in a different manner from that in the unpoisoned fibre. The question could probably be settled by repeating the present experiments on squid axons which had been poisoned with veratridine or cevadine; until this has been done it seems unwise to speculate further.

Although the negative after-potentials of vertebrate A fibres are not totally unlike those in the squid axon, there are several differences which make it difficult to apply the present hypothesis to myelinated fibres. In the first place the after-potentials of mammalian A fibres do not seem to add in a linear manner when the nerve is stimulated repetitively (e.g. Gasser, 1937, fig. 79). Whether the non-linearity is to be attributed to the process underlying the negative after-potential or to the development of the positive after-potential is not clear, but the situation is plainly more difficult to analyse than in squid fibres. Another line of evidence comes from studies with metabolic inhibitors. Gasser (1937) and Lorente de Nó (1947) have provided convincing evidence that the negative after-potential is closely linked with oxidative metabolism and that anoxia or carbon monoxide (Schmitt & Gasser, 1933) depress the after-potential more than the spike. If the nerve is allowed to recover after a period of anoxia, the after-potential far over-shoots its original size (Gasser, 1937; Lorente de Nó, 1947). These facts are not easily reconciled with the present hypothesis, but do not rule it out completely since anoxia might reduce the leakage of potassium per impulse or the sensitivity of the membrane to a small rise in potassium concentration. Another somewhat remote possibility is that agents which change the negative after-potential might do so by altering the properties of the Schwann cell or of some other layer outside the excitable membrane.

Comparison with electron microscopy

In a recent article Geren & Schmitt (1954) have published excellent electron-micrographs of the surface of the giant axon of *Loligo*. Fig. 16 is an attempt to represent some of their results in a line drawing. Owing to the complexity and variability of the Schwann cell layer this figure should not be regarded as typical and is reproduced here only in order to label some of the structures seen in Geren & Schmitt's photographs.

At the surface of the axoplasm there is a sharply defined layer or membrane (*a*) which stains with osmic acid and appears to be about 50 Å thick. Outside this, and spaced at a distance of about 100 Å, there is a similar membrane (*b*₁) at the inner border of the Schwann cell. The protoplasm of the Schwann cell layer appears to be interrupted by cracks; these are about 100 Å wide and are lined on both sides with an osmiophilic layer, *b*₂, which is continuous with the layer at the inner border of the Schwann cell. At the outer surface of the Schwann cell there is a single membrane, *b*₃, which is continuous with *b*₂ and *b*₁. The appearance of the double membranes *ab*₁ or *b*₂*b*₂ is similar to that of the double membranes at the surface of mitochondria (e.g. fig. 6 of Geren & Schmitt, 1954). The double membranes *b*₂*b*₂ often run parallel to the axon

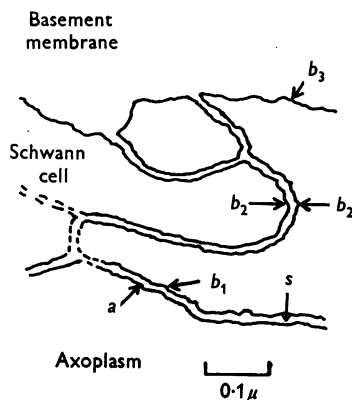


Fig. 16. Line drawing of part of Schwann cell and surface of squid axon, based on electron-micrograph of Geren & Schmitt (1954), fig. 3. The dotted lines represent structures not clearly seen in Geren & Schmitt's fig. 3, but shown in their fig. 2. *a*, *b*₁, *b*₂ and *b*₃ are dense osmiophilic layers. Much of the Schwann cell layer is thicker and more complicated than in this figure.

surface for considerable distances, and should perhaps be regarded as intracytoplasmic layers rather than as cracks between overlapping processes of the Schwann cell.

A tentative basis for our results is to suppose that layer *a* in Fig. 16 is the excitable membrane and that the region in which potassium ions accumulate is the space (*S*) between the layers *a* and *b*₁. In the photographs published by Geren & Schmitt the thickness of *S* is about 100 Å, which is less than the value of 270 Å obtained in the present work. This is not a serious objection because the argument on p. 370 indicates that 270 Å may be too large, and no allowance has been made for possible shrinkage in Geren & Schmitt's sections. However, the suggestion must be regarded as highly speculative, because there is no evidence that the space labelled *S* is an aqueous phase.

Our experiments suggest that potassium ions escape from the space outside

the excitable membrane through an outer layer which is unselective and which has an electrical resistance of about $4 \Omega \cdot \text{cm}^2$. This may be explained by supposing either that the Schwann cell membrane (*b*) has a relatively high permeability or that ions escape through cracks in the Schwann cell layer. In lobster fibres (Geren & Schmitt, 1954) there is little evidence of intracytoplasmic layers or cracks, and here it would seem either that ions must pass through the Schwann cell membranes or that there are channels which have not been observed. The same may be true of squid fibres, but the possibility that ions pass through the spaces between the osmiophilic layers cannot at present be excluded on dimensional grounds. The cracks are sometimes longer and more tortuous than is shown in Fig. 16, but even if the total length were as much as 3μ and the aqueous space in the crack as little as 50 \AA there would only need to be one crack every 3μ in order to give a resistance of $3\text{--}4 \Omega \cdot \text{cm}^2$.

SUMMARY

1. Previous work has shown that the spikes of isolated squid axons are followed by a positive phase, during which the membrane potential approaches the equilibrium potential for potassium ions and is markedly affected by small changes in the external potassium concentration.

2. At the beginning of a train of impulses the positive phases were not constant but declined exponentially to a steady level with a time constant of 30–100 msec.

3. The decline in the positive phase was correlated with the slow depolarization resulting from the addition of successive negative after-potentials.

4. Although there was considerable variation between fibres the following effects had about the same time constant in any one fibre:

(*a*) The decline of successive positive phases at the beginning of a train of impulses.

(*b*) The exponential build-up of the slow depolarization at the beginning of a train of impulses.

(*c*) The recovery of the positive phase when a gap was made in a train of impulses.

(*d*) The disappearance of the residual depolarization after one or many impulses.

5. Both the change in positive phase and the residual depolarization were satisfactorily matched by an increase in potassium concentration.

6. The same increase in potassium concentration also accounted for the small change in spike potentials seen during a train of impulses.

7. The match between the effects of potassium and the cumulative effects of a train of impulses held over a range of potassium concentrations which had different effects on positive phase, resting potential and spike.

8. The apparent rise in potassium concentration after one impulse at 18° C was about 1.6 mM; the excess potassium then disappeared exponentially with a time constant of 30–100 msec.

9. Warming decreased the after-effects and moderate cooling increased them. This is consistent with an increase in potassium leakage at low temperatures. The time constant with which the after-effects disappeared was not greatly affected by temperature, the Q_{10} being about 1.3.

10. In order to explain the results it is necessary to suppose that potassium ions cannot diffuse freely from the excitable membrane but are restrained by an external barrier to diffusion.

11. The apparent thickness of the space between the excitable membrane and the external barrier was calculated as about 300 Å. The permeability of the external barrier appeared to be about 6×10^{-5} cm/sec.

12. The polarization effect in voltage clamp experiments probably arises from an increase in the potassium concentration immediately outside the membrane; analysis of the effect also gave a value of about 300 Å for the space in which potassium ions accumulate.

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