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AFTER-POTENTIALS IN MAMMALIAN NON-MYELINATED NERVE FIBRES

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Grundfest & Gasser (1938) described a negative and a positive after-potential following the spike in mammalian sympathetic C fibres. We have studied these after-potentials in an attempt to interpret them in terms of ionic mechanisms.

Frankenhaeuser & Hodgkin (1956) found in giant fibres that the *negative* after-potential is caused by an increase in the potassium concentration around the excitable membrane following activity. The increased potassium concentration occurs because the potassium ions which leak out during activity cannot diffuse freely away from the membrane but are restrained by an external barrier to diffusion. Frankenhaeuser & Hodgkin suggested that this barrier is formed by the Schwann cell layer surrounding the axon. In mammalian non-myelinated fibres a burst of activity also leads to an increased potassium concentration near the excitable membrane (Ritchie & Straub, 1957), suggesting that, as in giant axons, the negative after-potential following a single impulse might be caused by local accumulation of potassium. The vertebrate non-myelinated fibres are contained in invaginations of the Schwann cells (Nageotte, 1922; Gasser, 1955; Hess, 1956; Robertson, 1957*a*). However, calculation shows that the Schwann cell sheath would provide less than 1/200 of the barrier to diffusion required to account for the rate of decline of the negative after-potential, if the gap in the Schwann cell, seen on electron micrographs, were an aqueous phase. This discrepancy may be interpreted as indicating that there is another diffusion barrier in addition to the Schwann cell. This barrier may lie in the space around the axon and the mesaxon, which have been found to contain a highly hydrated gel (Robertson, 1957*b*); moreover, diffusion may be restricted by the amorphous dense material, described as basement membrane (Gasser, 1955; Robertson, 1957*a*), around the Schwann cell.

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Following the negative after-potential there is a *positive* after-potential. The positive after-potential is similar to the larger hyperpolarization which is observed after a burst of repetitive activity. Ritchie & Straub (1957) suggested that the post-tetanic hyperpolarization is caused by an increased active potassium uptake. It will be shown that the positive after-potential after a single impulse can be explained in the same way.

Apart from the negative and the positive after-potentials described by Grundfest & Gasser (1938) we have observed, on preparations of sympathetic C fibres bathed in potassium-free solution and kept at low temperature, a brief period of positivity preceding the negative after-potential. Similar, but longer lasting periods of positivity immediately following the spike have been observed on other types of mammalian non-myelinated fibres and described by Bishop (1934) as the 'positive after-effect associated with the first phase' in vagus nerve fibres, and by Gasser (1950, 1955) as the 'post-spike positivity' in dorsal root C fibres. These periods of positivity observed in various types of C fibres suggest a period of hyperpolarization in the individual fibres identical with the 'positive phase' observed in isolated giant axons (Hodgkin & Huxley, 1939; Curtis & Cole, 1942; Weidmann, 1951). This phase has been explained by Hodgkin & Huxley (1952) on the basis of the persistence of a state of increased potassium permeability after the spike.

METHODS

The technique used was similar to that described by Ritchie & Straub (1956*a*, 1957). Adult lop-eared rabbits were anaesthetized with urethane given as a 25% (w/v) solution into the marginal ear vein (1·6 g/kg). The cervical sympathetic trunk was removed and cleaned under a dissecting microscope. One of the subdivisions of the trunk was isolated and its epineurium removed over a length of about 1 cm, the other subdivisions being cut away. The preparation was mounted in the apparatus described by Stämpfli (1954) and modified by Ritchie & Straub (1956*a*) for recording resting and action potentials from C fibres. The preparation lay in a horizontal insulating capillary 7 mm long, containing sucrose solution, while each end of the nerve was suspended in a vertical tube, through which Locke's solution was flowing. The effects of solutions with different ionic composition (potassium, lithium) were tested by substituting them for the Locke's solution at one side of the sucrose insulator. A pair of Ag–AgCl electrodes, connected over a KCl–agar bridge to the Locke's solution on either side of the insulating horizontal tube, recorded the potential difference between one Locke's–sucrose interphase and the other. The apparatus with the nerve in position is illustrated elsewhere (Straub, 1956). With this technique a high outside resistance is produced between the recording electrodes so that a large fraction of the absolute values of the changes in resting potential and of the slow components of the action potential are recorded. The electrodes were connected to a pair of low grid-current cathode followers and a differential DC amplifier.

The nerve was stimulated through circular electrodes forming part of the inside wall of one of the vertical tubes. The stimulator had an RF output unit (Schmitt, 1948) to isolate the stimulus from earth and to minimize stimulus artifact. The distance between stimulating and recording electrodes was approximately 6 mm.

Monophasic action potentials were recorded from the C fibres after the sucrose solution in the insulating tube had been flowing for about 5 min. The monophasic records were obtained from a defined spot because the conduction of impulses was stopped at the interphase between the

Locke's solution and the sucrose solution, in which lack of sodium ions prevented impulse conduction. This interphase was well defined because small desheathed bundles were used. With the usual method of killing one end of an undesheathed nerve by crushing, heating or treating it with KCl or cocaine it has been found difficult to record monophasically from C fibres (Bishop, 1934; Gasser, Richards & Grundfest, 1938; Gasser, 1950).

The temperature was measured with a thermocouple bathed in the Locke's solution a few millimetres from the Locke's–sucrose interphase on the stimulated side.

The composition of the Locke's solution was: (mM) NaCl, 154; KCl, 5·6; $CaCl_2$, 2·2; $NaHCO_3$, 1·9; dextrose, 5. It was equilibrated with 2% CO_2 and 98% O_2 and had at 30° C a pH of 6·6. The potassium concentration was changed by replacing Na by K; in the Li-Locke's solution LiCl was substituted for the NaCl of the Locke's solution. The sucrose solution contained sucrose (A.R.) 10% (w/v) in distilled water which had been passed over an ion-exchange resin. The sucrose solution had a specific resistance of more than 10^5 Ω cm.

RESULTS

The three types of after-potentials following a single impulse in sympathetic C fibres are seen in Figs. 1–3. Fig. 1 illustrates the negative after-potential which follows the spike in C fibres, when bathed in Locke's solution. On records taken with a slower time base and a higher amplification than that used in the record of Fig. 1, the positive after-potential becomes evident, as is seen in Fig. 2. The brief period of positivity preceding the negative after-potential in preparations bathed in potassium-free solution is illustrated in Fig. 3.

The negative after-potential

Time course. The negative after-potential from C fibres declined approximately exponentially. This is shown in Fig. 4 where the height of a negative after-potential is plotted on a logarithmic scale against time. From about 20 msec up to 150 msec after the spike the points on the graph can be fitted by a straight line; later the slope becomes steeper, presumably owing to the relatively increased contribution of the factors giving rise to the positive after-potential. From ten different nerve bundles thirty-five after-potentials were plotted in this way; they gave results similar to that seen in Fig. 4. The exponential decline of the negative after-potential has also been observed on squid giant fibres by Shanes (1949*a*) and Frankenhaeuser & Hodgkin (1956).

Results of measurements of the time constant of the decline of the negative after-potential in C fibres from the ten nerve bundles are summarized in Table 1. For a given temperature the time constant varied considerably among the ten preparations. The reason may lie in differences in the fibre population amongst the C fibres in the bundle and in the presence of a small proportion of B fibres. These also had a negative after-potential but with time constants shorter than those recorded for C fibres. Since in the conditions of our experiments these B fibres must have been stimulated together with the C fibres, the time constant for C fibres alone would have been somewhat longer than the mean value of 150 msec given in Table 1.

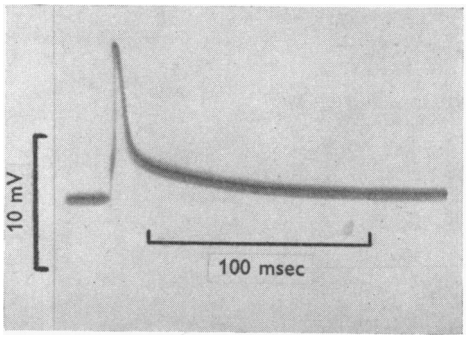

Fig. 1. Monophasic record of the compound action potential set up in a bundle of nerve fibres from the rabbit's cervical sympathetic trunk. The main component of the action potential is a C potential derived from non-myelinated fibres; the other component, the small B potential, is just visible in the rising phase of the spike. The falling phase of the spike is followed by the negative after-potential. Temperature 24° C.

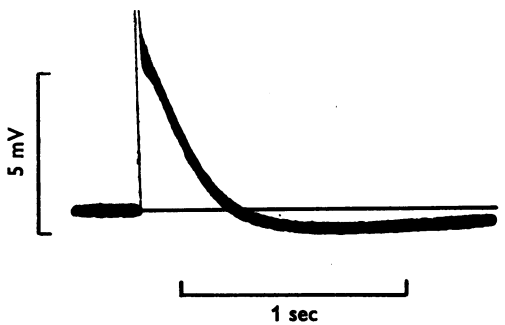

Fig. 2. The negative and the positive after-potentials recorded from a bundle of C fibres from the rabbit's cervical sympathetic trunk. The record was taken with a high amplification so that the spike was too large to be completely registered. The inserted base line shows the position of the resting potential. Temperature 21·7° C.

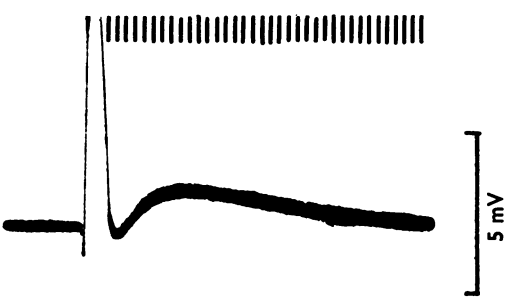

Fig. 3. The 'period of positivity' preceding the negative after-potential, recorded from a bundle of C fibres of the rabbit's cervical sympathetic trunk bathed in a potassium-free solution. Temperature 10·5° C; time 20 msec.

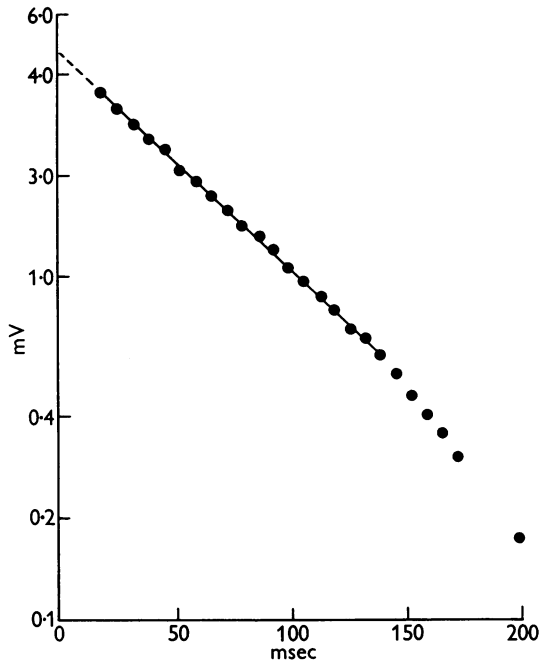

Fig. 4. The time course of the negative after-potential recorded from a bundle of C fibres from the rabbit's cervical sympathetic trunk. The ordinate represents the potential in mV on a logarithmic scale, the abscissa is the time in msec after the peak of the spike. The straight line corresponds to a time constant of 69 msec; temperature 36·5° C.

TABLE 1. Time constant (τ) of the negative after-potential

Preparation no.	τ (msec)	Temp. (° C)	τ (msec)	Temp. (° C)
1	216	20·0	58	36·8
2	74	21·0	—	—
3	244	20·0	83	37·0
4	138	20·3	50	37·2
5	115	20·8	55	37·0
6	205	21·0	—	—
7	159	21·0	—	—
8	135	21·0	—	—
9	64	20·0	—	—
10	150	20·0	44	37·0
Mean	150	20·5	58	37·0

Effect of potassium ions. When the Locke's solution at one side of the sucrose insulator was changed to one in which the sodium was replaced by various amounts of potassium, the resting potential changed rapidly and reached a new steady level in about 3 min. The nerve was then stimulated and the after-potential recorded; such experiments were performed on five bundles. In Fig. 5 the after-potentials from one nerve bundle bathed in solutions of different potassium concentrations are plotted as in Fig. 4. Changing the

potassium concentration had no appreciable effect on the time course of the negative after-potential. The same result was obtained in all five experiments. In each of them the change in resting potential was measured and plotted against potassium concentration. The relation between the potassium concentration and the resting potential was linear from 0 to 12·5 mM potassium. Results similar to these were obtained by Frankenhaeuser & Hodgkin (1956) on giant fibres, suggesting that in both giant fibres and mammalian non-myelinated fibres the same mechanism is responsible for the negative after-potential, i.e. accumulation near the excitable membrane of potassium ions released during the action potential.

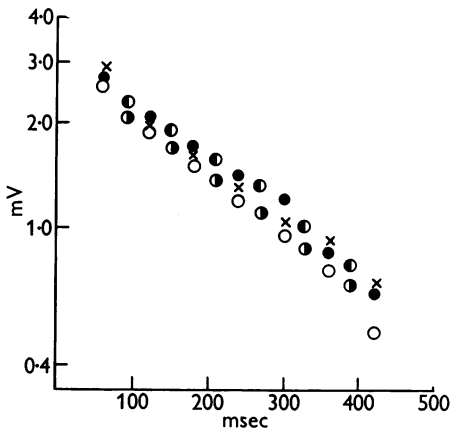

Fig. 5. The effect of potassium ions on the time course of the negative after-potential. The ordinate represents the potential in mV on a logarithmic scale, the abscissa is the time in msec after the peak of the spike. The points represent the height of the negative after-potential recorded when the bundle of C fibres was bathed in solutions with various potassium concentrations (mM): ○, 0·0; ●, 5·6; ◐, 7·5; ◑, 10·0; ×, 12·5. Temperature 25° C.

The increase in potassium concentration near the membrane immediately after the spike can be estimated from the height of the after-potential in Locke's solution, extrapolated to the end of the spike, as shown in Fig. 4, and the curve relating potassium concentration to membrane potential. On this curve a potassium concentration can be found which produces a depolarization equal in magnitude to the height of the negative after-potential at the end of the spike; this concentration gives the potassium concentration at the membrane. By subtracting the initial potassium concentration from this value the increase in potassium concentration is obtained. The results for four nerve bundles are given in Table 2. The mean value for the increase in potassium concentration was 3·35 mM.

Estimation of the net potassium efflux per impulse. The increase in potassium concentration after the spike is likely to be limited primarily to the space

between nerve fibre and Schwann cell, which on electron micrographs is about 145 Å thick (Robertson, 1957*a*). Assuming that the potassium ions liberated during the impulse are confined to this space, the net potassium efflux can be estimated by multiplying the increase in potassium concentration (3·35 mM) by the thickness of the space. The potassium efflux estimated by this method would be $4{\cdot}9 \times 10^{-12}$ mole/cm^2 per impulse at 24° C. This value of 4–5 pmole/cm^2 per impulse for C fibres agrees reasonably well with the potassium efflux measured at approximately the same temperature with other methods on

TABLE 2. Calculated rise in potassium concentration (ΔK) near the membrane at the end of the spike

Preparation no.	ΔK (mM)	Temp. (° C)
3	2·9	22
11	2·8	26
12	3·6 (2·6–4·6)	26–27
13	4·1 (3·8–4·4)	22
Mean	3·35 (2·6–4·6)	24
S.E. of mean	0·3	

invertebrate non-myelinated fibres, e.g. in *Carcinus* 1·7 (Hodgkin & Huxley, 1947) and 2·4 (Keynes, 1951*a*), in *Loligo* 3·0 (Keynes & Lewis, 1951) and 3·7 (Shanes, 1954) and in *Sepia* 3·4 (Weidmann, 1951), 4·3 (Keynes, 1951*b*) and 3·6 pmole/cm^2 per impulse (Keynes & Lewis, 1951). A value not more than a few per cent higher is obtained when the volume of the mesaxon is also taken into account. This volume may come rapidly into diffusion equilibrium with the space around the fibre (see Appendix).

Effect of temperature. Lowering the temperature of the Locke's solution prolonged the negative after-potential. This is illustrated in Fig. 6. In three nerve bundles, in which the time constant was measured at different temperatures, an increase of about twofold was found for a 10° decrease in temperature in the range from 38 to 10° C. This is shown in Fig. 7. As these temperature coefficients were obtained from the compound after-potentials the question arose as to whether they were influenced by a change in temporal dispersion resulting from the effect of temperature on conduction velocity. This effect seems to be small in the conditions of our experiments, as shown by the following considerations: if the negative after-potential of all the fibres in the bundle had the same time constant, the time constant of the compound after-potential would be the same as that for the individual fibres and independent of temporal dispersion. We know, however, that this simple case almost certainly does not apply to our results, since the nerve bundles contained a few B fibres, which had a time constant about one third of that of the C fibres. Nevertheless, since the temporal dispersion was small in relation to the observed time constant, it could not have had a large effect on the measured Q_{10}. For a conduction distance of 6 mm which we used the temporal dispersion between

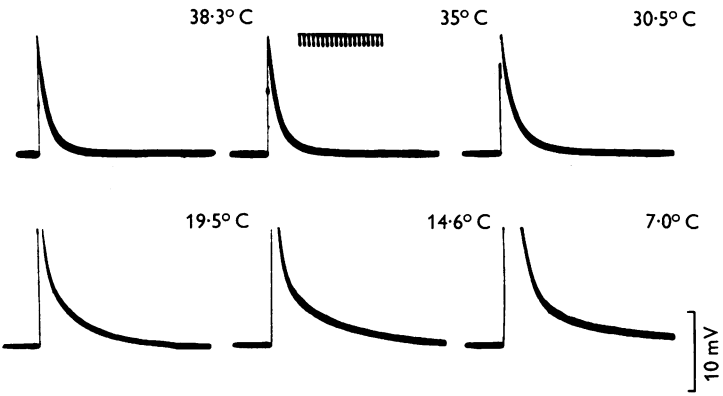

Fig. 6. The effect of temperature on the negative after-potential recorded from a bundle of C fibres from the rabbit's cervical sympathetic trunk kept in Locke's solution. Time 20 msec.

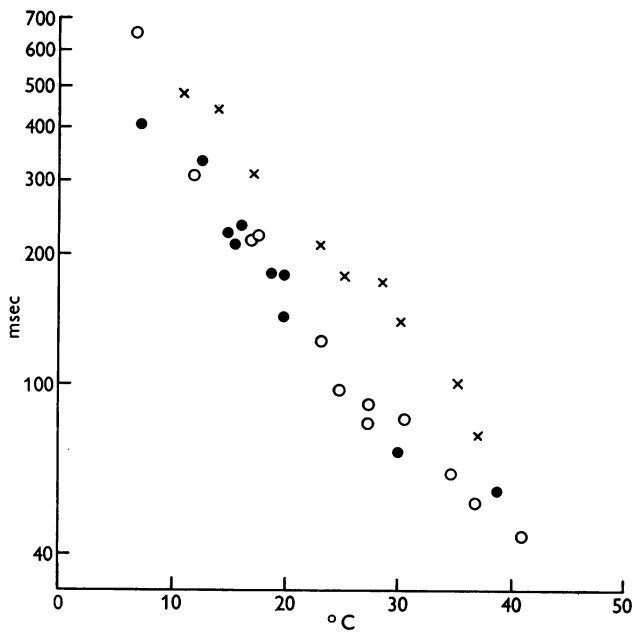

Fig. 7. The relation between the time constant of the negative after-potential and the temperature. The ordinate is the time in msec on a logarithmic scale, the abscissa is the temperature in ° C. The points represent the time constants of the decline of the negative after-potential measured at various temperatures in three bundles of C fibres from the rabbit's cervical sympathetic trunk. Lowering the temperature by 10° C corresponds to a twofold increase in the time constant.

the fastest B fibres and the slowest C fibres was about 10 msec at 37° C, calculated from the conduction velocity of 14 m/sec for the B fibres (Grundfest 1938), and 0·6 m/sec for the slowest C fibres (Grundfest & Gasser, 1938; Gasser, 1955). The bundles might have contained C fibres conducting even more slowly than 0·6 m/sec, since Gasser (1956) described C fibres which had a diameter of 0·1 μ. These fibres would have a conduction velocity of about 0·17 m/sec according to the relation $v = 1{\cdot}7d$ (Gasser, 1955), where v is the conduction velocity in m/sec and d the diameter in microns. The maximal dispersion would thus be about 35 msec at 37° C. In C fibres the conduction velocity around 37° C has a Q_{10} of 1·3 (Gasser, 1955), so that lowering the temperature by 10° C would at most increase the temporal dispersion by 11 msec. For the same change in temperature the time constant increased from 60 to 120 msec, and in this case, even with disadvantageous distribution of the time constants, the error in the time constant of the compound after-potential would not exceed 10%. This would cause a 10% error in the Q_{10}, which therefore is between 1·8 and 2·0. The temperature coefficient of the time constant of the decline of the after-potential in C fibres is thus definitely higher than the Q_{10} of 1·34 which was found in giant axons (Frankenhaeuser & Hodgkin, 1956).

The effect of a train of impulses. In giant fibres Frankenhaeuser & Hodgkin (1956) found that the time constant of the decline of the negative after-potential does not change during a train of impulses and that the negative after-potentials build up exponentially with about the same time constant as they decline.

In mammalian C fibres there is also a build up of a depolarization at the beginning of a train of impulses (Ritchie & Straub, 1957). We have studied this build-up process in more detail and found that in C fibres the addition of the negative after-potentials follows approximately the same pattern as in giant axons. In the experiment of Fig. 8, the bundle was stimulated with 6 shocks/sec and the negative after-potential of the successive impulses measured and plotted on a logarithmic scale against time. The figure shows that, as in giant fibres, the after-potentials add approximately linearly. Thus, the initial heights of the first three after-potentials, found by extrapolation as shown by the dotted lines, were 3·0, 4·7 and 5·0 mV respectively, and the calculated values for the second and the third after-potentials found by adding the initial height of the first after-potential to the after-potential remaining at the instant of the second or the third impulse, were 4·8 and 5·6 mV. The difference between the values found by these two methods is small and is probably due to a small error brought in by the extrapolation procedure. Measurements of the slopes in Fig. 8 show that the decline of the negative after-potential becomes faster during a train of impulses. This behaviour of the negative after-potentials in C fibres was also observed by

Grundfest & Gasser (1938). The increase in the rate of decline is probably due to an increased contribution of the factors giving rise to the positive after-potential; they also add during repetitive activity (Ritchie & Straub, 1957).

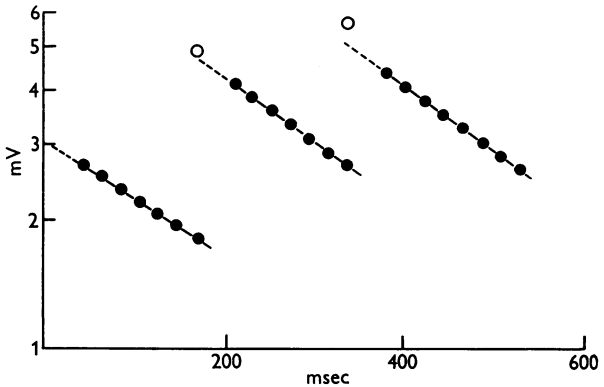

Fig. 8. The addition of the negative after-potentials in the beginning of a train of impulses. The ordinate represents the potential in mV on a logarithmic scale, the abscissa is the time in msec after the peak of the first spike. The closed circles represent the negative after-potentials of three successive impulses. The lines have been drawn through the points by eye. The rate of decline corresponds to a time constant of 316 msec for the first impulse and 290 msec for the second and third. The open circles represent the potential at the end of the second and third spikes obtained by adding the initial height of the first after-potential to the after-potential remaining at the instant of the second and third impulses. Temperature 25·0 °C.

The positive after-potential

The positive after-potential which follows the negative after-potential lasted several hundred milliseconds. In the experiment of Fig. 2, where the records were taken at 22° C, a peak of 0·6 mV over the resting potential was reached 800 msec after the onset of the spike. A similar positive after-potential was recorded by Grundfest & Gasser (1938); it reached its peak at 150 msec and lasted 1–2 sec at 37° C.

Changing the bathing Locke's solution to Li-Locke's solution greatly affected the positive after-potential. The lithium solution increased the resting potential by 3 mV, but did not appreciably affect the spike or the initial height of the negative after-potential. The positive after-potential, however, gradually diminished, as was shown when the nerve was stimulated at 1 min intervals; 5 min after changing to the lithium solution the positive after-potential disappeared. The effect was reversible provided the preparation was not left in the Li-Locke's solution for longer than 15 min. After changing back to normal Locke's solution within this period the positive after-potential re-appeared in 2–3 min; it became even slightly larger than before the Li treatment and its peak was reached later. This is illustrated in Fig. 9. When the preparation was left for more than 15 min in the lithium solution a small

positive after-potential reappeared whilst the nerve was still in the lithium solution, but there was no full recovery of spike height and after-potentials after changing back to normal Locke's solution.

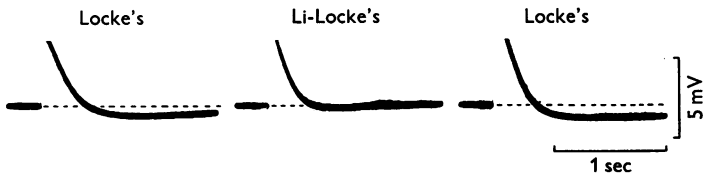

Fig. 9. The effect of lithium on the positive after-potential recorded from a bundle of C fibres from the rabbit's cervical sympathetic trunk. The record on the left was taken when the bundle was bathed in Locke's solution, the middle record after soaking for 9 min in a modified solution where the NaCl of the Locke's solution had been replaced by LiCl and the record on the right 5 min after the bundle had been restored to Locke's solution. All three records were mounted on the same base line; the increase in the resting potential produced by the lithium solution is not shown. Temperature 22° C.

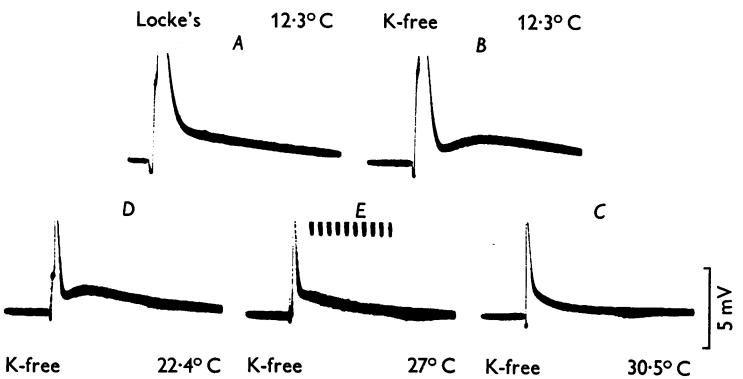

Fig. 10. The effect of potassium ions and temperature on the period of positivity of the negative after-potential. The top record on the left, *A*, was obtained from a bundle of C fibres from the rabbit's cervical sympathetic trunk whilst the bundle was bathed in Locke's solution; the top record on the right side, *B*, which shows a period of positivity, was obtained 3 min after changing to a potassium-free Locke's solution; for both records the temperature was maintained at 12·3° C. The bottom records were all obtained whilst the bundle was bathed in the potassium-free solution. They show that by increasing the temperature the period of positivity disappears. The sequence in which the records were taken was *A* (Locke's at 12·3° C), *B* (K-free solution at 12·3° C), *C* (K-free solution at 30·5° C), *D* (K-free solution at 22·4° C), *E* (K-free solution at 27° C). Time 20 msec.

The initial period of positivity

A period of positivity immediately following the spike in the compound action potential has been described in vagus C fibres (Bishop, 1934) and dorsal root C fibres (Gasser, 1950), but not in sympathetic C fibres (Grundfest & Gasser, 1938). In our experiments, too, no initial positivity was seen when the bundle of sympathetic C fibres was bathed in Locke's solution and kept at 37° C.

However, when the preparation was bathed in a potassium-free solution kept at a temperature below 27° C, there was a period of positivity immediately following the spike. The height of this positivity increased when the temperature was further lowered, and the period of positivity was reversibly abolished either by warming the potassium-free solution to about 30° C or by changing back to normal Locke's solution, containing 5·6 mM potassium, but maintaining the low temperature. This is shown in Fig. 10. When the temperature was sufficiently low, the positivity crossed the initial base line, as is seen in Fig. 3, which is from the same experiment.

Apart from this effect on the after-potential the potassium-free solution caused the well-known increase in the resting potential. This hyperpolarization of the resting membrane is unlikely to be the cause of the period of positivity, since in Li-Locke's solution, with normal potassium concentration, an almost equally high hyperpolarization of the resting membrane occurred but there was no sign of a period of positivity.

DISCUSSION

The negative after-potential

The present results on the negative after-potential in C fibres are consistent with the idea, put forward by Shanes (1949*a*, *b*, 1951) and by Frankenhaeuser & Hodgkin (1956), that the negative after-potential arises from a transient increase in the potassium concentration near the excitable membrane. From the results and the calculations of Shanes, Grundfest & Freygang (1953) and of Frankenhaeuser & Hodgkin (1956) it is clear that the potassium released during a single impulse can account for the height and duration of the negative after-potential in giant fibres only if the diffusion of the potassium ions away from the membrane is restrained by a diffusion barrier in the immediate vicinity of the excitable membrane. In giant fibres Frankenhaeuser & Hodgkin found evidence for a diffusion barrier near the membrane and suggested that it might be formed by the Schwann cell layer which surrounds the axon. For explaining the results of Ritchie & Straub (1957) and the present observations on the negative and the positive after-potential in C fibres some kind of diffusion barrier between excitable membrane and external solution is also required. In C fibres the relation between Schwann cell and nerve fibre is, however, different from that in giant axons: a giant axon is surrounded by a layer of Schwann cells (Geren & Schmitt, 1954), whereas a vertebrate non-myelinated fibre is contained in an invagination of the surface membrane of a Schwann cell, which closely invests the nerve fibre and forms the mesaxon (Nageotte, 1922; Gasser, 1955; Hess, 1956; Robertson, 1957*a*, *b*). Frankenhaeuser & Hodgkin (1956) suggested that in the giant fibres diffusion of potassium ions occurs through cracks in the Schwann cells and thus could account for the rate of decline of the negative after-potential, provided the

cracks contain an aqueous phase. If in C fibres the space between nerve fibre and Schwann cell and the mesaxon were to contain an aqueous phase and if we were to assume that the potassium ions diffuse through the mesaxon, the negative after-potential would have to decline with a time constant of the order of 1 msec or less (see Appendix). The observed time constant, however, was about 150 msec at 20° C. This discrepancy suggests that there is another barrier in addition to the Schwann cell. This additional barrier probably lies in the periaxonal space and the mesaxon, which has been found to contain a highly hydrated gel (Robertson, 1957*b*) which may well slow the diffusion of potassium ions; and a further barrier may be formed by the basement membrane which surrounds the Schwann cell.

To explain the effect of temperature on the rate of decline of the negative after-potential in C fibres we have to assume an active uptake of potassium during the negative after-potential. The Q_{10} for diffusion of KCl in aqueous solution is about 1·3, whereas the time constant of the negative after-potential had a Q_{10} of 1·8–2·0. As the potassium ions probably have to diffuse through the gel in the mesaxon and through the basement membrane, the conditions of free diffusion would not be fulfilled and a somewhat higher Q_{10} than 1·3 might be possible, but not as high as 1·8. One may explain the high temperature coefficient by assuming that a relatively large proportion of the released potassium ions returns into the fibres as a result of the relatively low permeability of the outer barrier. This assumption is supported by the fact that an increase in external potassium concentration increases both passive and active potassium uptake in a giant fibre (Hodgkin & Keynes, 1955*a*, *b*). In giant axons the active uptake has a Q_{10} of 3·3, the passive flux a Q_{10} of about 1·0. Thus the high Q_{10} of 2·0 might well result from an active potassium uptake during the negative after-potential. The following calculation supports the idea that the amount of potassium taken up actively during the negative after-potential is considerable and that it could explain the Q_{10} of 2·0. In giant axons the active extrusion of sodium is directly proportional to the external K^+ concentration in the range 0–10·5 mM (P. C. Caldwell & R. D. Keynes, personal communication) as presumably is the coupled active uptake of potassium. If the same were true for C fibres, then the increase in active uptake of potassium, in pmoles/cm^2, during the interval t_1 after an impulse would be

$$\int_0^{t_1} k_1 y \mathrm{d}t = \int_0^{t_1} k_1 y_0 \mathrm{e}^{-t/\tau} \mathrm{d}t = \tau k_1 y_0 [1 - \mathrm{e}^{-t_1/\tau}], \tag{1}$$

where τ is the time constant of the negative after-potential, k_1 is the constant of proportionality between the rate of active potassium uptake and the external potassium concentration, y, which, immediately after the impulse, is y_0.

The excess potassium in the space, however, is $M = M_0 \mathrm{e}^{-t/\tau}$ pmoles/cm^2

where M_0 is the value immediately after the impulse. At time t_1, the net quantity $M_0(1-e^{-t_1/\tau})$ has disappeared. Thus the fraction, f, of the excess disappearing by active uptake is

$$f=\frac{\tau k_1 y_0}{M_0}=\frac{100\tau k_1}{\theta}, \tag{2}$$

where θ is the thickness of the space in Ångstrom units.

For giant axons k_1 can be calculated from the data of Hodgkin & Keynes (1955*a*) to be 2·0 pmoles/cm².sec per mM external K^+ and if it is assumed that the same value is valid for C fibres, equation (2) becomes

$$f=\frac{200\tau}{\theta}. \tag{3}$$

For C fibres $\theta=140$ Å and at τ at 20° is 0·15 sec so that $f=0{\cdot}21$ i.e. 21 % of the disappearance of excess potassium has been by active uptake. With 21 % of the loss having a Q_{10} of 3·3 and 79 % having a Q_{10} of 1·3, a Q_{10} of 1·7 for the negative after-potential would be expected. This is in good agreement with the measured Q_{10} of 1·8–2·0.

In contrast, for giant fibres $\theta=250$ Å and at 20° $\tau=0{\cdot}05$ sec (Frankenhaeuser & Hodgkin, 1956) so that the active uptake would represent only 0·04 % of the total disappearance and thus a Q_{10} of 1·3 would be expected, which again is in agreement with the Q_{10} of 1·3 found for the negative after-potential (Frankenhaeuser & Hodgkin, 1956).

The positive after-potential

The finding that the positive after-potential is abolished when the sodium ions of the bathing solution are replaced by lithium ions enables us to explain the positive after-potential. Lithium ions are able to replace sodium ions in impulse conduction (Hodgkin & Katz, 1949; Gallego & Lorente de Nó, 1951), but on the other hand lithium ions which enter cells passively are pumped out at a much slower rate than are sodium ions (Harris & Maizels, 1951; Swan & Keynes, 1956). Thus the observation that the positive after-potential disappears in lithium solution and reappears upon addition of sodium ions links the positive after-potential with the active extrusion of the sodium ions which have entered the axon during the impulse.

Previous work on the hyperpolarization following a burst of activity showed that similarly the post-tetanic hyperpolarization is abolished in lithium solution and re-appears upon addition of sodium ions (Ritchie & Straub, 1957). The positive after-potential and the post-tetanic hyperpolarization can therefore be considered to be caused by the same mechanism. Ritchie & Straub (1957) calculated that a burst of activity is followed by a *large* increase of the sodium concentration inside a small nerve fibre, which could explain the post-

tetanic hyperpolarization. For the positive after-potential following a single impulse the following considerations apply: in giant fibres which have a diameter of 30–500 μ, the sodium influx of 4 pmoles/cm^2 during one impulse (Hodgkin, 1951) increases the internal sodium concentration by only 0·005–0·0003 mM or by 0·01–0·0006 % of the normal internal sodium concentration; accordingly the larger nerve fibres of *Sepia* or crustacea have no positive after-potential, unless tetanized at a high rate (Bugnard & Hill, 1935; Shanes, 1949*b*). For C fibres the sodium influx per impulse has not been measured, but we can assume that it is near 5 pmole/cm^2, since we found that the potassium efflux in these fibres which is approximately equal to the sodium influx was about 4·9 pmole/cm^2 per impulse. Thus the ion fluxes are approximately the same in large and in small non-myelinated fibres; on the other hand, the change in internal sodium concentration is inversely proportional to the fibre diameter. A single impulse would therefore increase the sodium concentration by 2 mM in the smallest fibres of 0·1 μ diameter (Gasser, 1955, 1956), and by 0·2 mM in the larger fibres which have a diameter of 1 μ. The normal sodium concentration in mammalian C fibres is not known, but in mammalian myelinated fibres, Krnjević (1955) found a sodium concentration of 40 mM. This value might be too high, but even with this value one impulse would still increase the sodium concentration by 5 % in the smallest and by 0·5 % in the largest C fibres. Further, Hodgkin & Keynes (1955*a*, 1956) found that the rate of sodium extrusion is proportional to the inside sodium concentration and that about 2/3 of the sodium extrusion is coupled with potassium uptake. In C fibres a single impulse would therefore be sufficient to cause a significant increase in the rate of sodium extrusion and potassium uptake. The positive after-potential can therefore be explained by the same mechanism which is responsible for the post-tetanic hyperpolarization (Ritchie & Straub, 1957), i.e. that the faster potassium absorption, coupled with the increased rate of sodium extrusion, decreases the potassium concentration in the vicinity of the membrane below the concentration maintained in the steady state before the impulse and thereby increases the membrane potential.

The initial period of positivity

A positivity following the spike, similar to that which we have found to occur in sympathetic C fibres, has been described in other types of non-myelinated fibres, e.g. in mammalian vagus fibres (Bishop, 1934) in mammalian dorsal root C fibres (Gasser, 1950, 1955) and in single, isolated cephalopod giant fibres (Hodgkin & Huxley, 1939; Curtis & Cole, 1942; Weidmann, 1951). The period of positivity observed in the three types of mammalian non-myelinated fibres and the positive phase in giant fibres can be considered to be the same phenomenon. This conclusion is based on the following observations, in which a similar behaviour of the period of positivity in the three types

of mammalian fibres and of the positive phase was found: (1) During the period of positivity and during the positive phase the fibres are in a refractory state. This was shown for dorsal root fibres by Gasser (1950) and for giant fibres by Hodgkin & Huxley (1952). (2) Both the period of positivity and the positive phase are dependent on the potassium concentration in the bathing fluid. In sympathetic C fibres we obtained a period of positivity only in potassium-free solution and the positivity disappeared when potassium was added to the bathing fluid; in giant axons the positive phase was found to be greatest in potassium-free solution and to disappear when the solution contained a high potassium concentration (Hodgkin & Katz, 1949; Hodgkin & Keynes, 1955*b*). (3) A train of impulses reduces the positivity: this was found in dorsal root C fibres by Gasser (1950) and in giant fibres by Frankenhaeuser & Hodgkin (1956). And (4) a rise in temperature reduces the positivity in the compound after-potential of a population of fibres. This was found by Gasser (1950) in dorsal root C fibres and in the present experiments on sympathetic C fibres.

In giant fibres the positive phase has been explained in terms of increased potassium permeability (Hodgkin & Huxley, 1952). There is evidence in these fibres that the potassium permeability increases during the second half of the spike and does not at once return to its resting value. The resting potential of an isolated axon is several millivolts below the equilibrium potential for potassium ions and therefore the persistence of the increased potassium permeability during the refractory period raises the membrane potential and generates the positive phase. In potassium-free solution the difference between the equilibrium potential for potassium and the resting potential is greatest and this explains why the positive phase is most pronounced in this condition. The period of positivity in mammalian C fibres is explained on similar lines, because it is also associated with a refractory period and because the relationship between resting potential and external potassium concentration (Ritchie & Straub, 1957) is similar to that in cephalopod axons (Curtis & Cole, 1942; Hodgkin & Keynes, 1955*b*).

The difference between sympathetic and dorsal root C fibres. We have concluded that there is no basic difference between the positive phase in an individual sympathetic C fibre and the positive phase in an individual dorsal root C fibre. It is clear, however, that the compound after-potentials recorded from a population of sympathetic C fibres or of dorsal root C fibres are different. Thus, at 37° C, in sympathetic C fibres the compound after-potential shows no period of positivity, even when the preparation is bathed in potassium-free solution, whereas, at the same temperature, in dorsal root C fibres the spike is followed by a large long-lasting positivity (Gasser, 1950, 1955, 1956). It has been suggested that the difference between these after-potentials is so important that it necessitates the division of the C fibres into two sub-groups, the

sympathetic C fibres (s.C) and the dorsal root C fibres (d.r.C) (Gasser, 1950, 1955). The relation between nerve fibre and Schwann cell cannot explain this difference, since the relation is the same (Gasser, 1955) in both types of fibres.

On the assumption that the positivity in an individual C fibre is caused by the same mechanism as the positive phase in a giant fibre the difference between these compound after-potentials can be explained by the difference in the duration of the refractory period. It is short, about 20 msec, in sympathetic C fibres, and unusually long, about 300 msec, in dorsal root fibres at 37° C (Grundfest & Gasser, 1938). Accordingly the positive phase in an individual sympathetic C fibre is brief, whereas in a dorsal root fibre it is long. Considering the temporal dispersion between the positive phase of the individual fibres this difference could produce the observed difference in the compound after-potentials. The long-lasting positivity in dorsal root fibres sums, as shown by Gasser (1950, 1955), and will therefore be revealed as a period of positivity in the compound after-potential. On the other hand, in sympathetic C fibres the brief positive phase of the individual fibres is masked by the long-lasting negative after-potential so that no positivity is seen in the compound after-potential. The observations on the effect of temperature on the period of positivity in the compound after-potential support this suggestion. Lowering the temperature decreases the rate of the permeability changes for potassium and sodium with a temperature coefficient of about 3·0 (Hodgkin, Huxley & Katz, 1952); lowering of temperature therefore greatly increases the duration of the positive phase and consequently tends to synchronize the positive phase of the individual fibres. The effect is seen in our experiments on sympathetic C fibres as well as in Gasser's experiments on dorsal root fibres by the increase in the height of the positivity which occurs on lowering the temperature. It might be argued that decreasing the temperature has the additional effect of decreasing conduction velocity and thereby increasing temporal dispersion. This effect, however, is small, as judged from the low Q_{10} of 1·3 for conduction velocity around 37° C (Gasser, 1950); furthermore, in C fibres lowering the temperature has a greater effect on the duration of the action potential than on the decrease in conduction velocity (Ritchie & Straub, 1956*b*).

Thus there seems to be no need for postulating a basic division between sympathetic and dorsal root C fibres, but the difference in the refractory periods ought to be kept in mind when other differences in the behaviour of these two types of C fibres are considered.

SUMMARY

1. Monophasic action potentials were recorded from desheathed bundles of mammalian sympathetic C fibres, kept in flowing saline solutions, with the sucrose-gap method.

2. The spike of the compound action potential was followed by a negative and a positive after-potential.

3. The negative after-potential declined approximately exponentially with a time constant of 150 msec at 20° C; the rate of decline was independent of the potassium concentration in the bathing fluid. The time constant of the decline had a Q_{10} of about 2·0.

4. In the beginning of a train of impulses there was an exponential build up of a depolarization which had approximately the same time constant as the decline of the negative after-potential.

5. It is suggested that in C fibres the negative after-potential is caused by the same mechanism as in giant fibres, i.e. by a transient increase in the potassium concentration in the vicinity of the excitable membrane.

6. An increase of about 3·3 mM in the potassium concentration near the membrane would account for the initial height of the negative after-potential. On the assumption of a uniform distribution of this increase in potassium concentration in the space between nerve fibre and Schwann cell, the net potassium efflux was estimated to be $4{\cdot}9 \times 10^{-12}$ moles/cm^2 per impulse.

7. The positive after-potential had a duration of several hundred milliseconds. It was reversibly abolished when the nerve fibres were bathed in a solution in which the sodium chloride had been replaced by lithium chloride.

8. It is suggested that the positive after-potential results from an increase in the rate of active potassium uptake following an impulse.

9. In potassium-free solution at a temperature below 27° C the spike was followed by a brief period of positivity preceding the negative after-potential. This brief period of positivity results from a period of positivity in the individual fibres which is suggested to be identical with the positive phase in giant axons.

10. The difference seen in the compound after-potential recorded from a bundle of sympathetic or dorsal root C fibres is explained by the difference in the duration of the refractory periods in the two types of C fibres.

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APPENDIX

Calculation of the time constant expected on the basis of free diffusion of K^+ ions away from a C fibre

Fig. 11 shows a schematic diagram of the axon (cf. Robertson, 1957*a*). B represents the boundary between the peri-axonal space and the mesaxon and B' that between the mesaxon and the bulk of the solution. For the calculation we assume a fibre of 1·5 μ circumference ($2L$), corresponding to a diameter of about 0·5 μ. For simplicity in solving the diffusion equation we

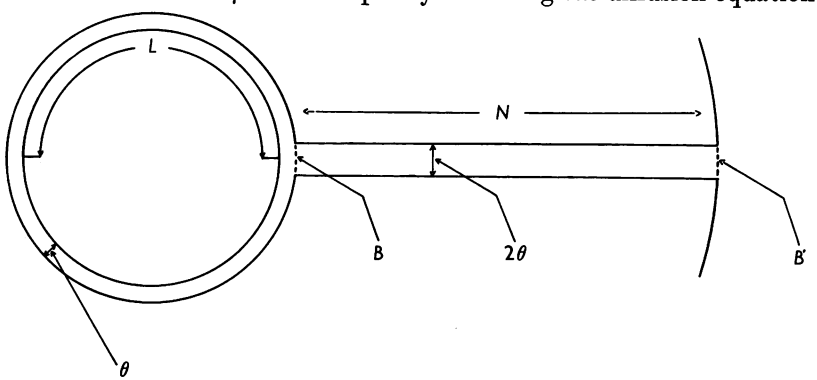

Fig. 11. Schematic diagram of the axon, peri-axonal space, mesaxon and Schwann cell of a C fibre. For clarity, the thicknesses of the peri-axonal space and mesaxon are drawn on a larger scale than is the axon.

assume the thickness, θ, of the space between axon and Schwann cell to be one half the thickness, 2θ, of the mesaxon; this assumption should not affect the order of magnitude of the results. It is further assumed that the negative after-potential is proportional to the average excess concentration of potassium in the peri-axonal space bounded by the surface B. Since the half circumference $L(=0{\cdot}75\ \mu)$ is much greater than $\theta(\cong 0{\cdot}014\ \mu)$, Fig. 11 is equivalent to the diffusion system shown in Fig. 12, where the sides and left-hand boundary are impermeable surfaces and where the peri-axonal space is represented by the length L and the mesaxon by the length N.

It is assumed that immediately after the impulse the excess concentration of K^+ is uniform in the peri-axonal space and zero in the mesaxon, and that at all times the space to the right of B', representing the bulk of the solution, is maintained at zero excess. From the solution to the diffusion equations with the boundary conditions stated above, the K^+ flux across the surface B can be calculated as a function of time and the total loss of K^+ is then given by integration with respect to time. These calculations were made for the three cases of mesaxons of length 0, L and infinity. The fraction, F, of the initial excess remaining at time t is then given by:

(a) For $N=0$

$$F=\sum_{n=0}^{\infty}\frac{8}{(2n+1)^2\pi^2}\exp\left(-[2n+1]^2\pi^2Dt/4L^2\right),$$

(b) For $N=L$

$$F=\sum_{n=0}^{\infty}\frac{8}{(2n+1)^2\pi^2}\exp\left(-[2n+1]^2\pi^2Dt/16L^2\right),$$

(c) For $N=\infty$

$$F=1-\sqrt{(Dt/L^2)}\,\{1/\sqrt{\pi}-ierfc\sqrt{(L^2/Dt)}\},$$

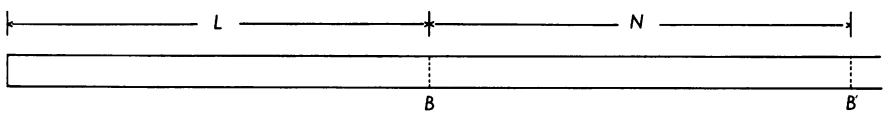

Fig. 12. Diffusion system equivalent to Fig. 11.

The equations for cases (a) and (b) are of the same form as equation 4.18 given by Crank (1956). Using the numerical solutions to these equations, illustrated in his Fig. 4.6, we have calculated that for case (a), F declines exponentially, for t greater than $0{\cdot}1\ L^2/D$, with a time constant of $0{\cdot}4\ L^2D$; and for case (b) F declines exponentially, for t greater than $0{\cdot}4\ L^2/D$, with a time constant of $1{\cdot}6\ L^2/D$. Taking $L=0{\cdot}75\times10^{-4}$ cm, as above, and using a value for the potassium diffusion coefficient, D, of $1{\cdot}5\times10^{-5}$ cm^2/sec (Landolt & Börnstein, 1935), the excess potassium in the peri-axonal space disappears with time constants of 0·15 and 0·60 msec respectively, whereas the experimental value was 150 msec. In case (c), the rate of disappearance of excess potassium never becomes exponential. Moreover, the rate is too rapid until almost all the excess potassium has disappeared. Thus, using the values of L and D given above, F decreases from 1·0 to 1·0/e in 0·75 msec and from 1/e to 1/e^2 in 5·4 msec.