J. Physiol. (1955) 130, 291–325

# THE ELECTRICAL PROPERTIES OF THE MOTONEURONE MEMBRANE

By J. S. COOMBS, J. C. ECCLES and P. FATT

*Department of Physiology, The Australian National University, Canberra, Australia*

(*Received* 28 *March* 1955)

Interest in the cells of the central nervous system has been directed mainly toward their synaptic function, since the outstanding feature of these cells is to receive and integrate synaptic influences from different sources. But in considering synaptic responses, it is helpful to have available data on the electrical and electro-chemical properties of the cell membrane upon which synaptic influences are exerted and in which propagated impulses are set up. In a first analysis the surface membrane of the cell body and dendrites of the central neurone may be taken to have properties similar to those of an unmyelinated nerve fibre. The membrane properties of such fibres have been investigated in considerable detail, using giant fibre preparations of invertebrates (Hodgkin, 1951). One might, however, expect that the membrane of the central neurone would have some distinctive characteristics, for it has long been known that tissue containing such material (i.e. cell bodies and dendrites) has a higher rate of metabolism than tissue, either central or peripheral, containing only the axons of cells (Holmes, 1930; Gerard, 1937). This provision of energy, even in the resting condition of the cells, makes it possible that there exists across the resting neuronal membrane an intense active transfer of material—much greater than could exist across the nerve fibre membrane. The rapid reduction of membrane potential during anoxia (van Harreveld, 1946) suggests that there is a continuous active transfer of ions which must be maintained in order that the electrical state of the resting membrane be preserved.

Recently experiments have been described in which electric potentials have been recorded with intracellular microelectrodes from the cell bodies of single spinal motoneurones, when at rest and when activated in different ways (Brock, Coombs & Eccles, 1952, 1953; Woodbury & Patton, 1952). In the present study electric potentials have been recorded in these cells in conjunction with two types of treatment which could be applied directly to the

particular cell under observation by means of the microelectrode: (*a*) externally controlled (i.e. extrinsic) currents were applied to the cell membrane, and (*b*) various species of ions were injected into the cell, thus changing the composition of the intracellular medium while the extracellular medium remained constant. By making full use of these techniques, it is possible to determine the state of the motoneuronal membrane in terms of its conductance toward different ions. In this paper only the resting condition and the action potential response (spike-potential plus after-potential) of the membrane are analysed in this way. Analysis of excitatory and inhibitory synaptic actions will be given in later papers.

## METHODS

Experiments were performed on the lower lumbar region of the cat's spinal cord under pentobarbital anaesthesia. The technique of micromanipulation was the same as that described by Eccles, Fatt, Landgren & Winsbury (1954).

*Microelectrodes.* Capillary microelectrodes for intracellular usage were drawn from hard glass tubing and filled with a concentrated aqueous solution of an electrolyte. Filling with the desired solution was accomplished by immersing the microelectrode in the boiling solution for a period up to ½ hr or, alternatively, by immersing it in boiling distilled water for this period and, after cooling, placing it in a solution from which the electrolyte would pass into the water-filled microelectrode. Connexion of the microelectrode with the electrical equipment was made through Ag–AgCl junctions. The indifferent lead from the cat was made through an Ag–AgCl junction and a saline-soaked cotton gauze pad which made a low resistance contact with the surface of the lumbar musculature.

In order to record potentials from the interior of a cell at the same time as a current was being applied, a double-barrelled microelectrode was devised. This was fabricated by placing a flat glass partition longitudinally within a glass tube. One end of the tube was sealed and two side arms were attached there, one leading from each side of the partition. The tube was then drawn into a microelectrode and filled in the usual manner (cf. sketch of electrode in Fig. 1). On examination under the microscope, using a water-immersion objective of n.a. 1·20, the separate barrels were seen to end together at the tip, which still had a circular or oval cross-section. The overall diameter of the tip was not different from that found on single electrodes, i.e. it ranged to below 0·5 $\mu$. The tips of these electrodes were more liable to break when subjected to mechanical stress than single-barrelled electrodes, and it was a common occurrence that the double-barrelled electrode was damaged on being driven through the spinal cord, as was inferred from alterations of its electrical properties. Because of electrical artifacts inherent in the technique to which the double-barrelled electrode was applied, it was not usually possible to use electrodes with a tip diameter of less than about 0·7 $\mu$. On microscopic examination after use, successful electrodes were seen to have tip diameters of 0·7–2·0 $\mu$, and occasionally one barrel would open a few microns short of the other.

*Electrical artifacts.* Both the recording of potential and the passage of current individually presented the usual technical difficulties, such as have been described in various papers where single-barrelled electrodes have been used for these two operations. In addition, special problems arise with the use of a double-barrelled electrode, due to electrical coupling between the barrels. Typical values of the relevant electrical characteristics are indicated in Fig. 1. The capacity between the barrels (of the order of 20 pF) caused such a large artifact to appear in the recording system that it was impossible to follow in detail potential changes occurring in a cell within 2–3 msec after a change in applied current. When a voltage step is applied to the free end of the 100 MΩ resistance represented in Fig. 1, a current develops through the microelectrode tip, rising toward a plateau with a time constant of 0·025 msec (5 pF coupled to 10, 10 and 100 MΩ in parallel). Initially, the current will be carried via both barrels because of the large capacity coupling the two. In the recording system, a large voltage pulse will appear, which is entirely an artifact, as it does not represent a potential change at the microelectrode tip. It rises with a time constant of 0·025 msec

toward a potential equal to $\frac{1}{21}$ of the voltage step produced by the current-generating device; and it decays with a time constant of 0·4 msec (20 pF discharging through 10 and 10 MΩ in series). Transient artifacts having this origin appear in Figs. 4, 5 and 13. It will be appreciated that there is little possibility of making accurate measurements until this artifact has very largely subsided.

The large resistance (100 MΩ) was placed between the current-applying device and the microelectrode in order to minimize distortion in the recording of potential changes that arise as the response of the cell. If the barrel for applying current were directly connected to the current-generating apparatus, which was of comparatively low impedance, the capacity between the barrels would cause an attenuation in the recording of rapid potential changes at the tip of the

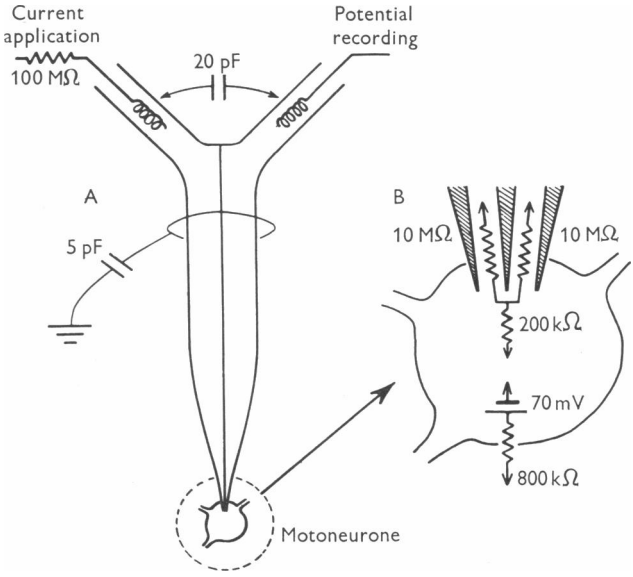


Fig. 1. A. Double-barrelled microelectrode and its immediate connexions. Typical values are given of the several electrical characteristics which are significant in the use of the electrode. B. Enlarged view of the microelectrode tip in the motoneurone. The motoneurone properties represented are the potential and resistance (ignoring the reactance) between the inside and outside of the inactive cell, as determined in this paper. For diagrammatic purposes the microelectrode tip is shown greatly magnified relative to the motoneurone.

microelectrode (the time constant of the circuit attenuating the high frequencies would be given by 20 pF × 10 MΩ). With the introduction of the 100 MΩ resistance, the whole of the microelectrode was free to follow transient potentials developed in the cell, while the steady polarizing currents were only slightly affected by these potentials.

A serious problem in the measurement of membrane potential changes produced by extrinsic current arises from the resistive coupling between the barrels. With the electrode dipping into physiological saline the application of a steady current through one barrel caused a potential change to be recorded from the other. This was due to the resistance immediately around the electrode tip shared by the current-applying and potential-recording systems. The greater the tip diameter the less was the coupling resistance. It was, furthermore, much reduced if the electrode was fractured so that one barrel opened a few microns short of the other. The coupling resistance was liable to fluctuate considerably on pushing the microelectrode through the spinal cord. It was therefore necessary to make frequent checks of its value. The values subtracted from the intracellular determinations were usually those obtained immediately after withdrawing from the cell,

this being based on the assumption that the coupling resistance exclusive of the cell membrane was the same inside the cell as outside. The changes in coupling resistance could be explained by breakage of the tip decreasing its value, or by joint blockage of the barrels by some material of high resistivity increasing its value. Since the resistance between the interior of a motoneurone cell body and the external conducting medium was, as eventually determined, of the order of 800 KΩ, reliance could not be placed on measurements made with electrodes with coupling resistance greater than 300 KΩ. It was this consideration that compelled the rejection of electrodes with tip diameters less than about 0·7 $\mu$. Usable electrodes had coupling resistance ranging between 60 and 300 KΩ.

*Injection of ions.* Another technique requiring description is the injection of ions from the microelectrode into the cell. Some passage of ions would ordinarily occur by diffusion. When the same species of ion is present in the microelectrode as in the cell, its movement would be mainly from the microelectrode to the cell, because of the much greater concentration within the microelectrode. The microelectrodes were filled with a near-saturated solution of the salt, the concentration of any particular species of ion being at least 1·2 equiv/l. A rough calculation made for an electrode filled with 3 M-KCl indicates that at the most $6 \times 10^{-14}$ equiv per sec of $K^+$ and $Cl^-$ ions will be leaving the electrode by diffusion (cf. Nastuk & Hodgkin, 1950).

Control over the rate of injection was obtained by applying to the microelectrode a potential of up to a few volts. The current through the electrode can then be used to estimate the rate of injection. Since the concentration of ions in the cell is much lower than in the electrode, most of the current will be carried by the movement of ions from the electrode into the cell. However, a problem arises from the circumstance that the current is a measure of the difference in the movement of cations and anions from the electrode into the cell. For example, a current from the electrode to the indifferent lead gives the rate at which the charge is carried by a preponderance of cations over anions, moving out of the electrode; the movement of anions is decreased from that prevailing when diffusion alone is operating, while the movement of cations is increased. Conversely, with a current in the opposite direction, the movement of anions out of the electrode is increased, while that of cations is decreased. This problem of calculating the rate of ionic movement will not be gone into further here. The data presented in this paper of the amount of ion injected into a cell are no more than semi-quantitative. More detailed consideration of ionic movement by diffusion and under the influence of electric fields is given in another paper (Coombs, Eccles & Fatt, 1955).

The ion species which have been injected into motoneurones are the following:

Cations: potassium, sodium, tetramethylammonium, choline.

Anions: chloride, bromide, nitrate, thiocyanate, bicarbonate, glutamate, sulphate, phosphate.

In electrodes used for investigating the effect of a given cation, the accompanying anion was usually sulphate. Correspondingly, for investigating the effects of a given anion, the accompanying cation was usually potassium.

## RESULTS

### *The resting motoneurone*

#### *Membrane potential*

In agreement with previously reported determinations (Brock *et al.* 1952; Woodbury & Patton, 1952) the resting potential measured with a KCl-filled electrode from within securely impaled motoneurones was about −70 mV, the negative sign being used to signify that the inside of the cell is negative with respect to the outside.

It is possible to establish that undiminished resting potentials of motoneurones were recorded in some cases. Any diminution of membrane potential due to an electrical leak would cause the responses of the cell to be altered in the way that is observed in experiments in which depolarizing

extrinsic currents were used. The agreement between the form of certain responses obtained with the present technique and those obtained by methods which are less direct and less likely to disturb the cell provides strong evidence that the cell is in its normal state, although impaled by the microelectrode. A case in point is the observation of the prolonged hyperpolarization occurring after the spike-potential. This after-potential is sensitive to changes in membrane potential: if the resting potential of the motoneurone was as much as 20 mV greater than the potential prevailing in impaled cells, the hyperpolarization could not occur (p. 313). Nevertheless, the existence of this hyperpolarization is indicated in experiments other than those employing microelectrodes (cf. Lloyd, 1951).

It should be noted that, even if damage to the cell can be discounted, there is still an uncertainty in the evaluation of membrane potential. This is due to the liquid junction potential existing between the electrolyte solution within the microelectrode tip and that of the surrounding fluid. No attempt was made to correct for this factor even though various electrolytes were used in the recording electrodes, because the experiments were mainly concerned with changes of membrane potential.

While the average resting potential, as recorded with a 3M-KCl-filled electrode, has been estimated to be $-70$ mV, it is reasonable to suppose that there is a relatively wide dispersion of resting potential within the population of motoneurones. The standard deviation may amount to $\pm 5$ mV or more. That the resting potential should have a greater dispersion in motoneurones than in excitable cells outside the central nervous system, e.g. in striated muscle fibres, is made probable by its greater dependence on metabolic processes.

*Effect of injected ions on resting potential.* The effect on resting potential of the injection into the cell of different species of ions would be expected to give an indication of the differential permeability of the membrane to ions. Studies on other tissues suggest that ions might be differentiated into two classes, depending upon how readily they penetrate the membrane. Into a class of readily penetrating ions would fall potassium, chloride, nitrate, bromide and other ion species where the hydrated ions are of similar small size. Into an opposite class of not readily penetrating ions, at least as far as passive penetration is concerned, would fall sodium, sulphate, phosphate, and other ions of comparatively large size (Boyle & Conway, 1941; Hodgkin, 1951). It may be predicted that the introduction into the neurone of readily penetrating anions would cause a reduction of the resting potential from which recovery would occur gradually as the anions diffused outwards across the neuronal membrane and the internal ionic composition returned to normal. Experimentally it was found that the injection of $Cl^-$, $Br^-$ or $NO_3^-$ produced a reduction of up to 10 mV in the resting potential. For example, after the injection of $Cl^-$ from a microelectrode at a rate of approximately 0·3 p-equiv/sec (1p-equiv $= 10^{-12}$ equiv) for 60 sec, the resting potential of a neurone was diminished by 8 mV. During the subsequent recovery the resting potential returned one-half of the way back to its initial level in less than 1 min. Similar findings were obtained in other cells with $Br^-$ and $NO_3^-$ injections. It should be noted that such potential changes, extending over a period of several minutes, were near the limits of accuracy of the recording technique. Hence it was not possible to make accurate determinations of the time course of

recovery of membrane potential. A possible consistent error in such investigations was an alteration of the liquid junction potential between the fluid in the microelectrode tip and that in the neurone, which would be expected to occur after an injection due to temporary changes in the ionic composition of the fluid within the microelectrode tip as well as within the neurone. It is, nevertheless, clear from the effects produced on the inhibitory post-synaptic potential, which will be considered in a later paper, that after an injection of $Cl^-$, $Br^-$ or $NO_3^-$ the injected ions proceeded to leave the neurone so that it was half-way back to its original condition in about 20 sec. The small depolarization produced by the injection of these anions suggests that the ionic currents contributed by the outward diffusion of such ions were small compared to the total steady-state ionic currents across the membrane, including that due to the active transfer of ions (Ussing, 1949; Grundfest, Kao & Altamirano, 1954). The injection of sulphate and phosphate in large amounts (order of 50 p-equiv) often appreciably reduced the resting potential and recovery was incomplete or absent, which may be attributed to an uptake of water and swelling of the cell as the total internal content of non-penetrating salts was increased.

Cations would be expected to react with the systems employed in the specific transfer of ions, i.e. with carriers and pumps, and their injection would therefore be likely to have specific effects, although the distinction between penetrating and non-penetrating ions could still have its significance for passive transfer. If potassium ions penetrate the cell membrane readily, and if current is used for their injection from the microelectrode, they would be carried out of the cell where this same current passed outward across the membrane. With the transference number (i.e. the fraction of current carried by a particular ion species) approaching unity at both the microelectrode tip and the cell membrane, no significant addition would be made to the amount of this species of ion within the cell. A fraction of the current across the membrane during the injection of potassium from the electrode would be accounted for by the outward passage of other cations such as sodium and by the inward passage of the penetrating anions in the external medium such as chloride. By the operation of diffusion from an electrode, potassium could be introduced into a cell in company with a selected anion. However, even by such means the internal concentration of potassium ions could not be increased much over that normally present. Assuming the motoneurone to be like other excitable cells, the osmotic pressure of the interior would be largely made up by potassium salts. The addition of more potassium salt would lead to an influx of water across the cell membrane, so returning the potassium concentration to near that normal for the cell. In accord with such reasoning, no significant effect was ordinarily observed from the intracellular injection of potassium by any method. On a few occasions a reduction of resting potential occurred on injecting potassium with an electric current, which

possibly could be due to chloride being carried across the membrane into the cell.

In contrast to potassium, the injection of sodium ions (20–50 p-equiv) by current from a sodium sulphate microelectrode nearly always diminished the resting potential to the extent of about 10 mV. The diminution can be explained by a displacement of potassium ions by the injected sodium, for the current would almost entirely be carried out of the electrode by sodium ions, while, as before, potassium ions would be largely responsible for carrying it outwards across the cell membrane. From the low resting potential occurring at the termination of injection, the cell proceeded to recover so that the resting potential returned one-half of the way to normal in 150–250 sec (Fig. 9), and had completely returned in about 500 sec. Approximately the same time course of recovery obtained for other alterations that the injection of sodium ions produced in the membrane properties of the cell, in particular, in the spike- and after-potential.

A usual occurrence was for the resting potential to increase beyond its original value. About 10–20 min from the injection a value was attained that was 10–15 mV in excess of the normal resting potential (e.g. Fig. 9). That this recorded potential change genuinely resided in the cell membrane was shown by changes in the responses of the cell, which resembled those occurring with a membrane potential raised by extrinsic current. Possibly the raised potential is to be attributed to a specific active extrusion of sodium ions carrying positive charges out of the cell. The effect of the sodium extrusion mechanism (or pump), tending to raise the resting potential, would be initially counteracted by the effect of the low intracellular potassium, tending to depress it, but, once the potassium was largely restored, the continued high activity of the sodium pump could produce the increased resting potential. The results of sodium injection, including the initial reduction and later raising of resting potential, could be elicited several times in the same cell by successive injections.

Tetramethylammonium ions were injected into some cells, and results were obtained which indicated that the membrane was relatively impermeable to the ions and not capable of actively extruding them. Thus when large amounts were injected, the resting potential was reduced as in the case of sodium injection, but subsequently recovery was very slow or absent. Such recovery as did occur may be attributed to slow passive diffusion out of the cell. Choline ions were also used and they appeared to have a chemically specific effect on the cell membrane. After an injection there was a slow progressive loss of resting potential.

*Membrane resistance*

The use of double-barrelled microelectrodes has made possible the evaluation of electric resistance between the inside and outside of motoneurones. It was

even more important than with the resting potential investigations to select determinations which were least upset by injury or by imperfections in the recording technique. Voltage-current curves, extending through wide ranges, were obtained with the use of currents which had already been applied for several seconds before each recording. An example is given in Fig. 2 of two series of uncorrected potential measurements obtained within a cell for various

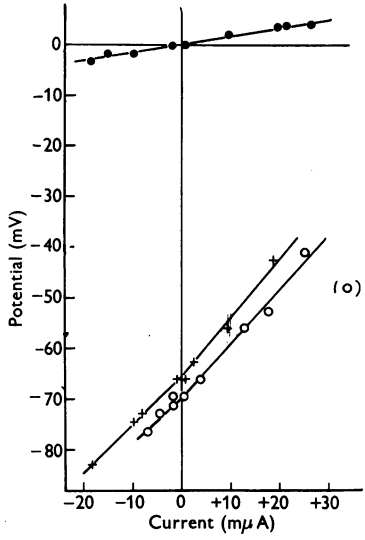


Fig. 2

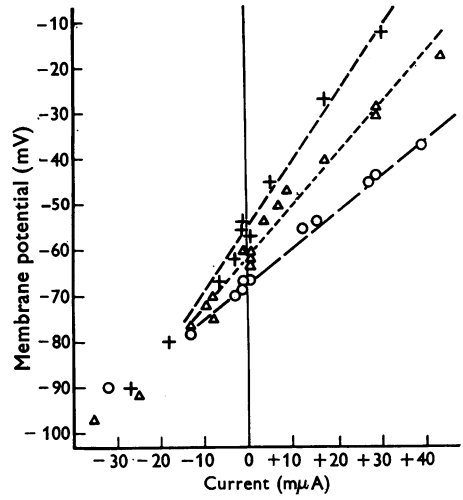


Fig. 3

Fig. 2. Directly recorded potentials plotted against current, using a $Na_2SO_4$-filled, double-barrelled microelectrode. Current is indicated to be positive when directed out of the microelectrode. Potential is that at the microelectrode tip with respect to a distant indifferent electrode. Points designated ○ and + were obtained with the electrode in a motoneurone; those designated ● were obtained immediately after withdrawing from the cell. After the first set of recordings ○, approx. 30 p-equiv of $Na^+$ (p-equiv $= 10^{-12}$ equiv) was injected from the microelectrode into the cell (by applying an outward current of $5 \times 10^{-8}$ A, lasting 60 sec), and the second set of recordings + were made in the subsequent 30 sec. A straight line has been fitted to the points ●, while two straight lines joining at zero current have been fitted to each of the sets obtained within the cell. The recording at the greatest outward current, marked (○), was affected by a continuous repetitive discharge of action potentials in the cell.

Fig. 3. Plots of potential against current, made in a different motoneurone but with the same microelectrode as used in the experiment of Fig. 2 and with the same convention for representing current. Potential measurements have been corrected to give membrane potential by subtraction of the potential appearing in the resistance to the surroundings shared by the two barrels of the microelectrode when the microelectrode was outside the cell, i.e. the potential is that inside the cell minus that outside. After obtaining points ○, approx. 45 p-equiv of $Na^+$ was injected into the cell (applying $5 \times 10^{-8}$ A for 90 sec) and the recordings + were made in the subsequent 30 sec. Following a gap of 60 sec, the set of recordings △ were made in a further period of 90 sec. A straight line has been fitted to the points in each set, except for the five points obtained with the largest inward (hyperpolarizing) currents.

currents, both inward and outward. In addition, potentials recorded under similar circumstances, but outside the cell, are plotted and fitted with a straight line. In order to obtain corrected membrane potentials, points on this line are subtracted from the internally recorded potentials for corresponding currents. Such corrected potential measurements obtained in other cells are plotted in Figs. 3 and 15 and are quoted in Figs. 7, 8 and 11. The slope of the curve of corrected membrane potential plotted against current at any point represents the dynamic (or differential) membrane resistance. Usual values of dynamic resistance in the vicinity of the resting potential were from 400 to 1300 KΩ.

As noted earlier, the double-barrelled electrodes were larger than the single ones used for accurate resting potential determinations and would be more likely to cause some damage of the motoneurones, with a consequent lowering of the voltage change produced by a given current. It may therefore be supposed that the larger values of resistance, those between 1000 kΩ and 1300 kΩ, represent more nearly the condition of an uninjured cell. An unexpected result was the close approximation to linearity of the voltage-current relation through the whole range of currents for which the technique could be satisfactorily applied—usually about $3 \times 10^{-8}$ A in each direction. There was, however, often a slight but significant degree of rectification. Thus in Fig. 2 a better fit of plotted measurements is obtained by two straight lines meeting at zero current, and assigning in the region of depolarization a dynamic resistance which is 20% greater than that in the region of hyperpolarization. A similar slight rectification is seen to apply for the plotted measurements in the lower part of Fig. 15, though the points have been fitted to single straight lines. No explanation can be offered for this rectification. It is in the reverse sense of that expected from potassium ion penetration with the known concentrations of these ions on the opposite sides of the membrane (see below).

A very large deviation from the normal voltage-current relation sometimes occurred with the largest hyperpolarizing currents. In such cases the dynamic resistance appeared to fall off sharply when the membrane potential was increased above a critical level. This was always in excess of 20 mV above the resting potential. On cessation of the current it was found that the potential did not return to its original resting level, but to a somewhat lower level. It is probable that the dielectric strength of the membrane had been exceeded by the large potential developed across it and that dielectric breakdown had occurred.

With the technique of applying steady currents and waiting several seconds before recording potentials, it was possible to plot values at all depolarizations except for a limited range within which rhythmically recurring spike-potentials appeared. With larger depolarizations, reasonable values could be obtained a few seconds after an initial burst of spike-potentials. Another procedure was to apply pulses of current lasting only 10–20 msec. As noted in the section on Methods, no significance could be attached to potentials recorded within 2–3 msec from the start of the pulse on account of electrical artifacts. Thereafter, when a voltage plateau had been nearly reached, the same results were obtained as with prolonged currents. However the brief-pulse technique had the drawback that larger depolarizing currents set up spike-potentials and no steady potential level was attained.

*Effect of injected ions on membrane resistance.* The investigation of the effect of injecting ions on membrane resistance was attended with considerable difficulty. Resistance determinations required the plotting of several potential-current recordings, and under the most favourable circumstances were accurate to only ±10%. Since a large number of potential recordings had to be made,

any rapid alteration of membrane resistance during recovery from the injection would upset the plotting, and only the direction of the change could be indicated. Furthermore, since the same electrode would be used for injecting ions as for the electrical measurements, care had to be taken that considerable quantities of ions were not inadvertently passed into the cell by the currents used for resistance measurement.

The injection of sodium ions provided the only situation in which a definite effect was obtained. Figs. 2 and 3 show the changes produced in two cells. In Fig. 2 the injection of 30 p-equiv of sodium has lowered the resting potential of the cell by 4 mV but has not had a noticeable effect on membrane resistance. In Fig. 3 the injection of 50 p-equiv of sodium into a different cell is shown to increase the membrane resistance considerably, followed by a return toward its original value. The resistance before the injection was 900 KΩ. During the 30 sec immediately following the injection the resistance was 1450 KΩ. From 90 sec after the injection until 210 sec further measurements were made, indicating a resistance of 1150 KΩ. The initial loss of resting potential and the later recovery appears to be part of a resistance alteration, since the plotted curves converge with inward (hyperpolarizing) current. The curves should not intersect. As the inward current increases, it tends to be carried preponderantly by external cations moving inward and the internal cation composition thus becomes inconsequential. It is apparent therefore that any unequal concentration of penetrating ions on the two sides of the membrane will provide some rectification, even where there is no specific permeability change (cf. Goldman, 1943). The raised membrane potential which sometimes appeared at 5–8 min after sodium injection was not reflected in any appreciable alteration of resistance.

Voltage-current curves, giving membrane resistance, were plotted in many cells, for such curves were required whenever the effect of membrane potential on responses was to be interpreted accurately. Thus many measurements were accumulated. On the whole, they are of interest only in giving the values typical for nearly normal conditions.

## *The spike* — *The activated motoneurone*

*The types of antidromic response.* A spike response may be set up in a motoneurone by three methods of stimulation: by the propagation of an antidromic impulse up the motor axon, by synaptic stimulation, and by direct electrical stimulation. The spikes so set up by these three methods are essentially similar in amplitude and duration (Fig. 5; Brock *et al.* 1952, 1953), but important differences occur in the initial depolarization from which the spike deflexion arises.

When an impulse is propagated antidromically along the motor axon, it would be expected to encounter a region of low safety factor at the junction

between the non-medullated axon and the soma. Action currents generated in the motor axon are here required to activate the expanded surface membrane of the cell body. Thus, even if the surface membrane had uniform properties over the whole of the non-medullated axon and the soma, a delay, or possibly failure, in conduction would be expected to occur at the junction between them. When complete invasion of the motoneurone does occur, a distinct step is seen on the rising phase of the recorded potential change at a level of about 30 mV depolarization. This has been attributed to delay at the axon-soma junction (Brock *et al.* 1952, 1953). In many motoneurones the complete action potential is not normally evoked and the response is then a simpler wave of depolarization of up to 30 mV in amplitude. It will be convenient to call this small spike the NM spike as heretofore, while the large spike which arises when presumably the soma and large dendrites are activated will be called the SD spike. When this NM–SD blockage was not normally present, it could be induced by repetitive stimulation (Brock *et al.* 1953). Blockage could also be produced by an underlying hyperpolarizing current (Brock & McIntyre, 1953; and bottom record of Fig. 8). On the other hand, if blockage normally occurred, it could be relieved by a depolarizing current (upper three records of Fig. 7). Such effects had earlier been suspected to occur from the results of experiments in which the antidromically elicited action potential was recorded extracellularly within a population of motoneurones, and conditioning of the cell was accomplished by excitatory and inhibitory synaptic action. From the increase produced in the externally recorded action potential by an excitatory volley it could be inferred that there were many motoneurones which were ordinarily not invaded completely by an antidromic impulse, but which could be invaded farther during a subthreshold excitatory action (Renshaw, 1942).

Another type of blockage has been studied by applying electric currents to the motoneurone. Typically a motoneurone at its resting potential would be fully invaded by an antidromic impulse, so giving an NM spike leading on to an SD spike. On increasing the membrane potential by a few millivolts the impulse would fail to invade the soma and the simpler NM spike would then be recorded. If now the membrane potential were increased further, even this NM spike would fail to appear (bottom three records of Fig. 7). The new type of blockage occurred just as abruptly and depended just as critically on the membrane potential as the axon-soma blockage. When the stimulus applied to the ventral root was critically adjusted for the threshold of the motor axon under investigation, it was possible still to identify a small spike-like deflexion being produced by stimulation of that axon (bottom record of Fig. 7 at increased amplification). This small spike had an amplitude varying from 1 to 5 mV with different motoneurones. Blockage was probably occurring in the ultimate section of motor axon approaching the soma, the effectiveness of the spread of activation there being affected by the level of membrane potential,

which was altered by extrinsic currents extending from the soma. The same type of blockage has been described previously to occur during a rapid repetitive series of antidromic impulses (Brock *et al.* 1952). It is convenient to use the term M spike to designate these small spike-like potential changes which are recorded intracellularly from the soma in response to an antidromic impulse which may only invade the medullated axon (Brock *et al.* 1953). Again, as with blockage between the NM and SD spikes, this blockage between the M and NM spikes has been found to occur normally with some motoneurones. When this was the case, the NM or the NM plus SD spike was obtained on depolarization of the cell by extrinsic current. In a motoneurone in which under normal conditions a complete action potential was very effectively set up by propagation of an antidromic impulse up the motor axon, it was frequently not possible to produce an NM–SD type of blockage, whatever hyperpolarizing currents were used. On hyperpolarizing, a point could be reached at which either the SD spike was set up or blockage occurred along the axon, giving an M spike, there being no intermediate stage of NM spike (bottom record of Fig. 5A).

Changes in latency of the SD spike on applying currents to the soma are attributable to alterations in the delay of propagation at each of the two sites of possible blockage. Thus on hyperpolarizing, there is an increase in the time spent in the transition from the NM to the SD spike. But in addition, as seen in Fig. 5A, a considerable delay can be introduced before the commencement of the NM spike.

Immediately following the injection of sodium ions (20–50 p-equiv), NM–SD blockage developed in cells which were normally completely activated (Fig. 9A). This often occurred in spite of a reduction of membrane resting potential which should have facilitated invasion. After a short time complete invasion could again be obtained, apparently as the result of the removal of some of the injected sodium ions. This result is consistent with the hypothesis of the involvement of sodium ions in the generation of the action potential (Hodgkin & Katz, 1949; Hodgkin, 1951).

Graded potential changes occurred whenever the NM spike was near threshold for setting up the SD spike, there being added a further slight depolarization and delayed decline. The deviation can be interpreted as a local response (or partial activation) of the membrane, such as has been demonstrated in other tissue to result from near-threshold depolarization. Thus with the cell of Fig. 7 initially at its resting potential (−80 mV), an antidromic impulse caused an NM spike to be set up which had an unusually broad summit and late decline. This response was converted to the more typical one by hyperpolarizing the cell by 2 mV. On the other hand, a depolarization of 2 mV caused an SD spike to appear intermittently. In the cell of Fig. 8, blockage of the SD spike did not occur unless it was hyperpolarized by at least 7 mV to a membrane

potential of −88 mV. At this level of potential the resulting NM spikes showed appreciable variability in their peak amplitude and time course of decay, although the rising phase was repeatable.

*The threshold for evoking a response.* The potential changes set up in the soma on antidromic activation would appear to indicate a threshold depolarization of almost 30 mV. On the other hand, the threshold depolarization for activation of the soma by the excitatory synaptic potential was much lower, usually only about 10 mV (Brock *et al.* 1952). From the study of other excitable tissues, including the junctional regions of cells, it is not clear why there should be this discrepancy. A factor which must complicate the problem in the motoneurone is the complex geometry of the cell. Either the apparent threshold obtained by antidromic activation is deceptively high on account of peculiarities of the local action currents at the axon-soma junction, or the apparent threshold obtained by excitatory synaptic action is deceptively low because activation by this means starts elsewhere than in the soma membrane under observation. An important additional factor may be the duration of the depolarization. Thus there may be a higher threshold level for brief pulses of depolarization, with the threshold decreasing as the depolarization is prolonged. Such a utilization phenomenon would help to explain the discrepancy of threshold.

The simplest way of determining the threshold of the soma membrane is by direct stimulation with a depolarizing current pulse. Using a pulse of several milliseconds duration, the threshold was found by this method to occur at a depolarization of about 10 mV (Figs. 4 and 5 A, C). The difference between the threshold height of the NM spike and the depolarization due to the direct application of a current that is just effective in initiating an action potential is obvious in the top records of Figs. 5 A and B. The top record of Fig. 5 B shows that, even when the membrane has already been depolarized almost to the extent necessary to initiate an action potential, the SD spike does not arise until the NM spike has attained a height of about 20 mV. On the other hand, it appears from Fig. 5 C that the thresholds for activating the cell with excitatory synaptic action and with depolarizing current are very nearly the same.

The approximate agreement between the threshold depolarizations obtained by excitatory synaptic action and by direct stimulation indicates that the actual threshold of the soma membrane is the one obtaining for the synaptic potential rather than the higher value derived from the NM spike. However, the time course of depolarization leading to activation is much longer in the case of the extrinsic current than in either of the other two cases. The SD spike arises 0·25–0·5 msec from the beginning of the depolarization of the NM spike, while activation by the excitatory synaptic potential occurs in 0·3 to 1·2 msec from its onset. On the other hand, activation by an extrinsic current pulse occurs at 3–8 msec, when it is near the minimum effective strength. More

intense currents set up spikes with less delay, and the threshold was then apparently at a larger depolarization. On comparing the second and third records in Fig. 4 A it is seen that the point of maximum curvature remained at an apparent depolarization of about 11 mV as the strength of the current pulse was increased and the duration of the preceding depolarization was shortened from 4 to 3 msec. In the top record of this series strengthening of the current reduced the time required to initiate the spike to 2 msec, but the apparent threshold was then increased to about 15 mV. It must be noted, however, that at such short intervals from the beginning of the current there were large artifacts in the recording. Besides the artifact due to coupling resistance, which is constant throughout the application of current, there is the transient artifact caused by the capacity between the two barrels of the

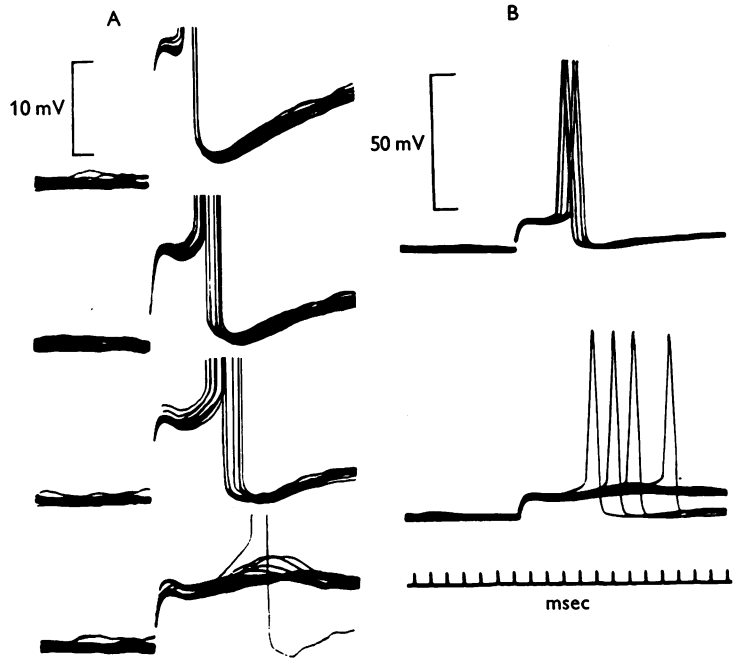


Fig. 4. Superimposed sweeps displaying membrane potential in a motoneurone on application of rectangular steps of outward current. Current commences about one-third of the way through each sweep and continues throughout the sweep. In both A and B, current increases from the bottom to the top record. Resting potential was −64 mV. Both here and in Fig. 5, the initial rapid rise of potential and the overshoot of plateau at the start of the current are artifacts due to capacitative coupling between the two barrels of the microelectrode and were not actually developed across the cell membrane. Furthermore, about one-sixth of the potential making up the plateau is also an artifact due to the resistance coupling the two barrels, and again is not developed across the cell membrane. This artifact sums with all potentials appearing in the cell during the current. Spikes have been retouched to restore losses in photography. Time scale applies to whole figure.

microelectrode. This artifact will diminish with a time constant of about 0·4 msec (Methods), as may be seen with the overshoot of the plateau potential at the beginning of the current pulses in Fig. 5A and C.

The possible complication of a utilization phenomenon can be overcome to some extent by superimposing the synaptic activity on the polarizing effects of extrinsic currents. Results of this kind were obtained from a few cells without, however, giving a conclusive solution to the problem. The experiment required the observation of small potential changes, since threshold depolarizations obtained by either extrinsic current or synaptic activity were small, and large hyperpolarizing currents could not be used, because under such conditions the synaptic potential was not large enough to activate the cell. For the cell illustrated in Fig. 5C the threshold appeared to remain very close to 10 mV as the relative proportion due to excitatory synaptic action and to

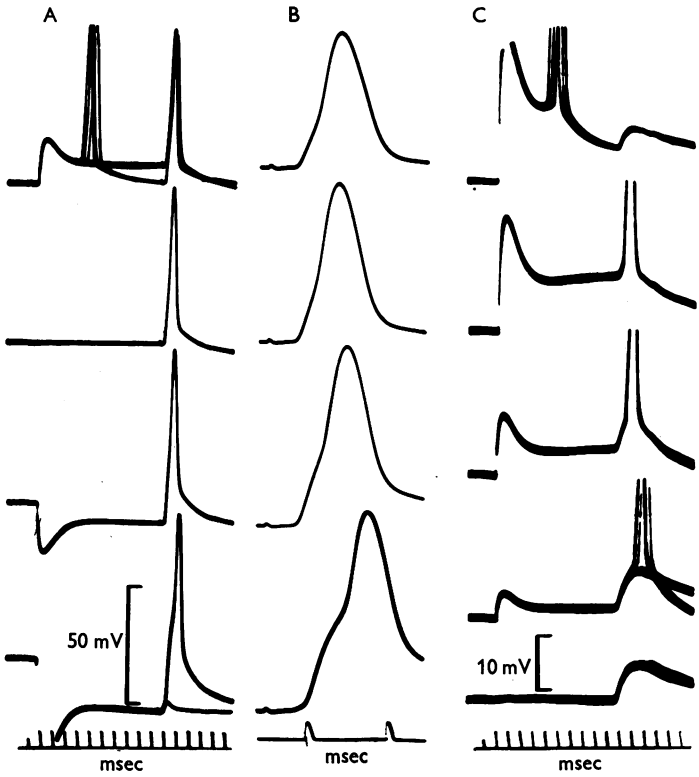


Fig. 5. Potential records in a biceps-semitendinosus motoneurone obtained on the application of steps of current, on activation by antidromic impulses and by excitatory synaptic action. In A an antidromic impulse is propagated into the motoneurone cell body about two-thirds of the way through the sweep. In C an excitatory synaptic potential, produced by a volley in group I*a* afferent fibres in biceps-semitendinosus nerve, is set up about two-thirds of the way through the sweep. In B more rapidly swept records of antidromically elicited spikes are shown, set up during a current as in the corresponding records of A. (Top record of B obtained in the presence of preceding activation by the depolarizing current and bottom record in the presence of blockage by the hyperpolarizing current.) Resting potential was −66 mV. Same voltage scale applies to A and B.

extrinsic current was varied. The slight deviation of threshold could be attributed entirely to the potential drop across the resistance shared by the two barrels of the microelectrode, independently of the cell membrane. In another cell there was appreciable deviation from a constant threshold. This deviation became particularly great when the excitatory synaptic potential was combined with a hyperpolarizing current. It is possible that during the prolonged hyperpolarization there had been accommodation of the membrane to the new higher potential, so that the transient depolarization required to activate approached that obtaining for the cell at its original resting potential.

When the NM spike approached close to threshold, local responses of the membrane were superimposed on it. Likewise local responses were superimposed on the membrane potential changes produced by depolarizing current pulses that were just below the threshold for initiating spikes. For example, in the lower records of Fig. 4 A and B, they appeared as a slow development of depolarization which continued for several milliseconds even when no spike was set up. On the other hand, local responses were not a conspicuous feature with synaptic excitatory action, though evidence has been presented to the effect that a small response normally appears during and after the summit of the excitatory post-synaptic potential (cf. Eccles, 1952). Possibly this relative insignificance is explicable by the wide dispersal of the excitatory synapses over the surface. If this dispersal were also uniform, the whole surface membrane of the soma and proximal dendrites would be uniformly depolarized by synaptic excitation, there being as a consequence little chance that an appreciable local activity would develop in some areas and not in others.

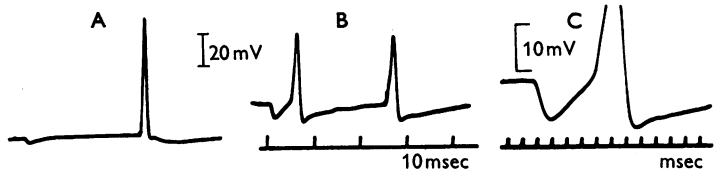


Fig. 6. Activation by recovery from the inhibitory synaptic potential in the depolarized motoneurone. From a resting value of −64 mV in A, the membrane potential had been reduced to −35 mV prior to recording B and C. In each record the inhibitory hyperpolarization commences about one-fifth of the way through the sweep. In A and B an action potential is set up antidromically about two-thirds of the way through the sweep. Common voltage and time scales are shown for A and B. C is taken at a higher amplification and faster sweep speed.

When the depolarizing current applied to a motoneurone was between 1 and 1½ times threshold, only a single spike was set up at about 4 msec from the beginning of the current, as has already been noted. Currents of greater intensity caused a repetitive series of spikes to be set up, which with currents several times threshold had a repetition rate of a few hundred per sec (cf. Brock & McIntyre, 1953). However, during the application of these strong currents, the repetition rate quickly fell and the cell usually became quiescent within a few seconds.

A striking property of the motoneurone was the rapid recovery of threshold that occurred on remission of a long-maintained depolarization which was initially supra-threshold. This effect is seen in Fig. 6, where an extrinsic current was used to produce a steady depolarization of about 30 mV. Several action potentials had been set up early during the depolarizing current, but the cell soon became quiescent again with its normal membrane resistance.

A partial recovery from the depolarization was then produced by the activation of inhibitory synapses, i.e. by the transient hyperpolarization of the inhibitory post-synaptic potential. It is seen in Fig. 6 that a spike-potential arose during the recovery phase of the inhibitory synaptic potential, i.e. while the motoneurone was being again depolarized. Under the conditions specified these spike-potentials were regularly obtained. When the inhibitory synaptic activity was weak, typical local responses appeared instead of spikes.

*Effect of variations in membrane potential on the spike.* The effect of changes of membrane potential with the present technique of applying current allowed little quantitative information to be obtained about membrane resistance during the spike.

The reason for this is that an initial shift of membrane potential from its resting level appeared to upset the activation of the sodium-carrier mechanism. In most cases the spike deflexion was considerably increased when the spike was set up on the hyperpolarized membrane (e.g. Fig. 5A). The increase in spike deflexion in some cells came close to compensating for the hyperpolarization of the resting membrane, which would indicate a low membrane resistance at the peak of the spike relative to that of the resting membrane. On the other hand, there were conditions under which hyperpolarizing the membrane reduced the amplitude of the antidromic spike. For example, in Fig. 8 the spike-potential was diminished from 78 to 71 mV when the membrane was hyperpolarized from −81 to −88 mV. The reduction of the spike peak was related to the late origin of the SD spike from the initial NM spike. It suggests that there is normally a degree of summation of the potential changes which separately constitute the NM and the SD spikes. In no case could the hyperpolarization be extended beyond 10–20 mV, and the cell still be completely invaded by the antidromic impulse. Blockage eventually occurred, either between the NM and SD spikes (bottom record of Fig. 8), or, somewhat less usually, between the M and NM spikes (bottom record of Fig. 5A).

The upper four records of Fig. 8 indicate the effect on the antidromically elicited action potential of an initial large depolarization with extrinsic current. A different procedure was employed here from that in the experiment illustrated in Fig. 5A. The initial depolarization was maintained for several seconds, and, after cessation of the series of spike-potentials evoked by this depolarization, antidromic action potentials were recorded. A complicating factor was that the amplitude of the antidromic spike sometimes decreased as the duration of the preceding depolarization increased. The upper four records in Fig. 8 were taken at intervals of 10–20 sec in order of increasing depolarization, and therefore the spikes in the uppermost records would be depressed by the prolonged depolarization. It is, however, clear qualitatively that the amplitude of the spike decreases with decreasing membrane potential. When the membrane was depolarized to the extent of 30 mV or more, it became impossible to set up a complete spike antidromically in the wake of the series of action potentials evoked by the depolarization itself. In this condition an antidromic impulse succeeded only in producing the diminished NM type of spike, as is illustrated by the most densely superimposed traces of the top record of Fig. 8.

Thus there is the same failure of invasion, both when the membrane is heavily depolarized and the activation mechanism is continuously partially engaged, and when the membrane is hyperpolarized and the axonal currents are insufficient to depolarize the membrane to the extent of setting off the activation mechanism. Furthermore, when blockage occurs by depolarization, local responses of graded intensity appear, increasing and prolonging the NM spike. Thus in several respects the types of responses appearing in the

bottom and in the top records of Fig. 8 are similar. There occur NM spikes, NM spikes with variable degrees of local response, and one or more delayed SD spikes.

It is evident from the records of Figs. 5B and 8 that the rate of rise of the NM spike also varies directly with membrane potential. In this respect the NM spike does not react like the displacement of membrane potential produced by a fixed current pulse, which on account of the negligible rectification would be the same regardless of the membrane potential at which it was set up. This

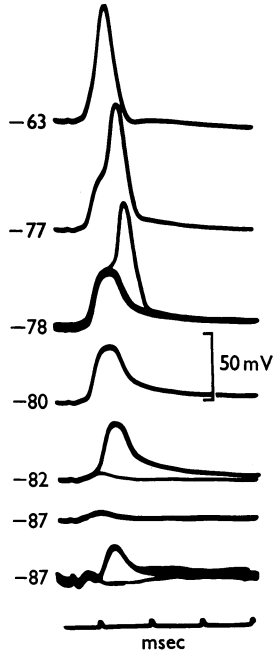


Fig. 7

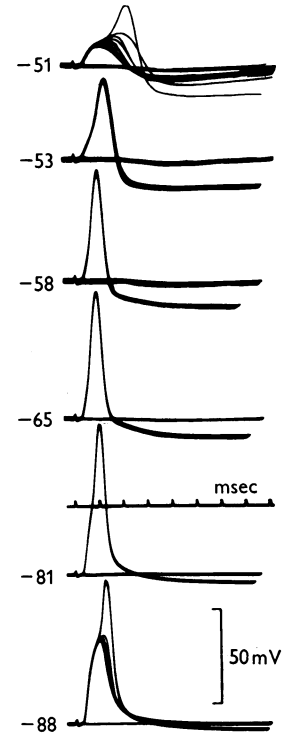


Fig. 8

Fig. 7. Stages of blockage of the antidromic spike in relation to the initial level of membrane potential. Initial membrane potential (indicated to the left of each record) was controlled by the application of extrinsic currents. Resting potential was at −80 mV. The lowest record was taken after the amplification had been increased and the stimulus had been decreased until it was just at threshold for exciting the axon of the motoneurone. Voltage scale applies to all but the lowest record, for which the amplification was increased 4·5 times.

Fig. 8. Action potentials in a motoneurone elicited by antidromic impulses at various levels of membrane potential. Membrane potential (indicated alongside each record) was controlled by extrinsic currents. Resting potential was at −81 mV. Records were taken in succession during depolarization at −65, −58, −53 and −51 mV. In the latter two there was probably some depression of the spike due to the preceding relatively prolonged depolarization.

indicates that the region where the NM response is generated is affected by currents applied to the soma in the same way as is the region of the SD spike. As will be shown below, the NM spike is also similarly affected by the injection of ions from the microelectrode into the soma.

*Effect of injected ions on the spike.* The effects of the intracellular injection of ions on the motoneuronal spike are consistent with the movements of sodium and potassium ions in the production of the spike, such as has been established to occur in other excitable cell membranes (cf. Hodgkin, 1951). When a depolarizing current was applied through a KCl or $K_2SO_4$ electrode, there was at most a small change in the spike from which recovery occurred within 10 sec. This is attributable to the ineffectiveness of this method of increasing the internal potassium concentration (cf. p. 296). The injection of sodium ions, on the other hand, produced large changes and recovery was much slower, taking at least 5 min. These effects are satisfactorily explained by the combined actions of an increase in the intracellular sodium concentration and a decrease, due to displacement, of the intracellular potassium concentration. Immediately after the injection of sodium there was usually a failure of the antidromic impulse to set up an SD type of spike. Following the recovery from this blockage, or immediately after the injection, when blockage did not occur, the SD spike had rates of rise and of fall which were considerably less than normal (Fig. 9A). The slowed rise may be explained by a reduction of the external/internal sodium concentration ratio and the slowed decline by a reduction of the internal/external potassium ratio.

It should be noticed that the NM spike has its size and rate of rise reduced by the injection of sodium ions just as does the SD spike and the two recover together. In fact the NM–SD blockage immediately after the injection appears to be due to a combination of two factors. The rate of rise and the size of the NM spike are reduced, while at the same time the threshold for evoking the SD spike is likely to be increased.

Two complications arise in picturing the ionic changes taking place with the injection by a depolarizing current of large amounts of sodium over long periods of time. First, as the intracellular potassium ions are progressively replaced by sodium ions, the inward movement of chloride ions must carry an increasing proportion of the current across the membrane. As the cell thus becomes filled with sodium chloride, it would swell on account of the osmotic inflow of water. Secondly, when the injecting current lasts for several minutes, an appreciable fraction of the injected sodium ions would escape from the cell by outward passage across the surface membrane. There is evidence to indicate that sodium ions are removed from a cell at such a rate that virtually all the excess is extruded in 5–8 min. Thus even for the injections of Fig. 9, lasting 2 and 2·5 min, an appreciable fraction of the sodium that has passed into the cell would have left again before the end of the injection.

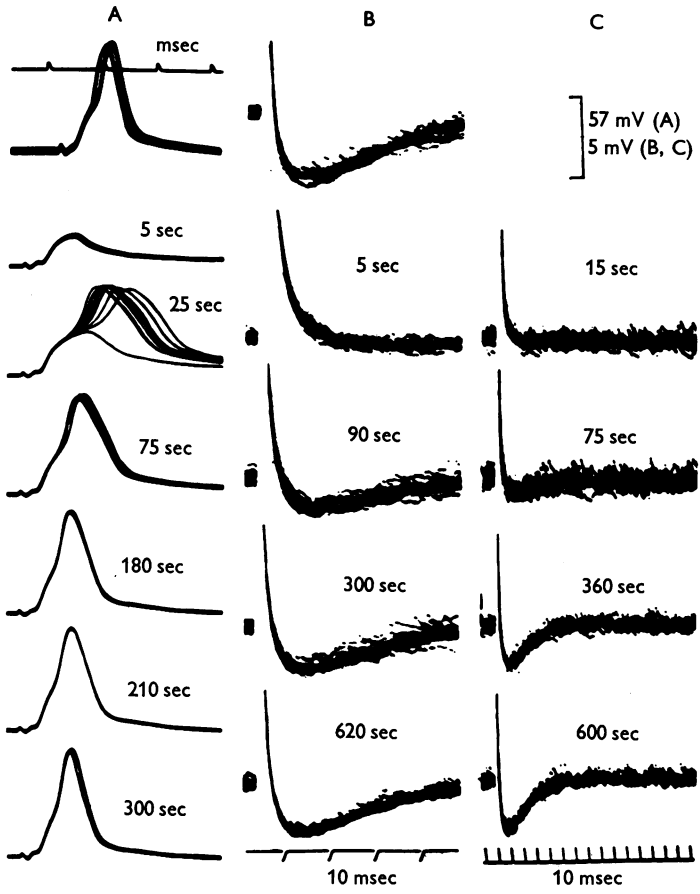


Fig. 9. Effect of the injection of $Na^+$ on the antidromic spike and after-potential of a motoneurone. After obtaining the top record in A, approx. 50 p-equiv of $Na^+$ (applying $4 \times 10^{-8}$ A for 120 sec) was injected into the cell and the further records in A were obtained at the approximate times indicated following the injection. Complete recovery had occurred by the time of the last record in A, and the top record of B was then taken. This was followed by the injection of approx. 75 p-equiv $Na^+$ (applying $5 \times 10^{-8}$ A for 150 sec) and the remaining records in B and C were taken at the indicated times after the injection. In A the resting potential was the same 5 sec after the injection as before, although it is probable that the resting potential was not steady by the time of the first record, taken soon after penetrating the motoneurone. From 5 to 300 sec the resting potential climbed from $-82$ to $-86$ mV and was at the latter value for the top record in B. For the subsequent four pairs of records in B and C the resting potentials were approx. $-74$ mV (5–15 sec), $-77$ mV (75–90 sec), $-87$ mV (300–360 sec) and $-93$ mV (600–620 sec.) In B and C a full action potential has been set up in each sweep, although the spike is not shown with the slow sweep and high amplification used to display the after-potential. Voltage scale applies to different parts of the figure as indicated.

The injection of tetramethylammonium ions allowed some observations to be made on the SD spike without the complications produced by rapid removal of the injected ions. Such an injection is far less effective than the sodium injection in producing NM–SD blockage, which may be attributed to the virtually unchanged sodium concentration gradient across the surface membrane. Furthermore, as long as the membrane potential is not greatly depressed, this species of ion does not affect the rate of rise of the spike to the extent that sodium does. The rate of decline of the spike is however greatly depressed, due apparently to a displacement of internal potassium, although it is possible that there is a specific chemical effect on the spike mechanism as well, which acts to delay the falling phase. In some cells, after the injection of tetramethylammonium ions, the rate of decline of the spike was reduced from 120 V/sec to only 25 V/sec. With more massive injections the resting potential was reduced and NM–SD blockage occurred. There was usually some recovery from the effects of the injection of tetramethylammonium ions, but it was very slow. Possibly the diffusion of the ions out of the electrode served to balance losses from the cell to the exterior.

Choline, when injected into cells, produced a drastic change in the time course of the spike, for which it is doubtful whether a sufficient explanation can be given in terms of the altered concentrations of ions traversing the membrane during activity. As appears in Fig. 10, after the injection of choline the spike declined slowly from its peak until a point was reached where the descent was suddenly accelerated. The delayed onset of the rapid decline indicates a separation of the processes responsible for the rise and the fall of the spike. It seems probable that the change is due to a specific effect of the internally applied choline on the spike mechanism. This is also suggested by the slow development of the effect subsequent to an injection and its seeming irreversibility. Thus the slow diffusion from a choline-filled electrode appeared to be sufficient to produce a gradual lengthening of the spike together with a reduction in the resting potential.

On a few occasions choline-filled electrodes were introduced into the axons of motoneurones rather than into somas, a situation which could be recognized from the typical nerve fibre action potentials obtained there. The injection of choline into motor axons had no discernible effect on their action potential.

*The after-potential*

*Description.* Under normal conditions the recovery phase of the SD spike merges into a much more slowly declining depolarization, which in turn reverses into a hyperpolarization having a maximum at 5–10 msec and a total duration of about 100 msec (Brock *et al.* 1952). These slower potential changes have hitherto been called the negative and positive after-potentials in accordance with the potential changes observed with extracellular recording. The present

investigation is restricted to the prolonged hyperpolarization, which will simply be referred to as the after-potential.

*Effect of changes of membrane potential on the after-potential.* The generation of this after-potential could be effectively investigated by the use of extrinsic

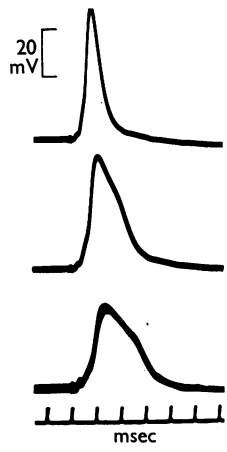


Fig. 10

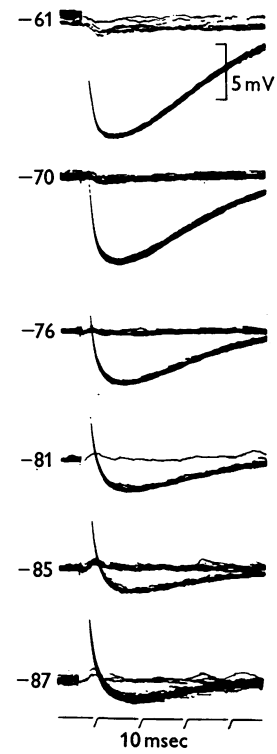


Fig. 11

Fig. 10. Action potentials in a motoneurone on dosage with choline ions. Top record was obtained soon after penetrating the cell and before any injection of ions by current. Resting potential was about −60 mV. Following this, approximately 10 p-equiv of choline$^+$ (applying $1{\cdot}5 \times 10^{-8}$ A for 60 sec) was injected into the cell, and the middle record was obtained within one minute of the end of the injection. The resting potential remained at −60 mV. After another few minutes the resting potential had fallen to −45 mV and the bottom record was then obtained.

Fig. 11. After-potentials of a motoneurone, occurring at various levels of membrane potential, as controlled by extrinsic current. For each record, the stimulus applied to the ventral root was adjusted to the critical strength at which the axon of the particular motoneurone was sometimes excited and other times it was not. The motoneurone was selected because it displayed little inhibitory effect of Renshaw cells when the stimulus was at the threshold for exciting its axon. The membrane potentials in mV at which the action potentials were evoked are given alongside each record. The resting potential varied from −76 to −79 mV. The spike component of the action potential does not appear in these records, the amplification being too high and the sweep too slow to display it satisfactorily.

currents, because, when once set into action, the underlying mechanism producing the membrane change was apparently not itself appreciably affected by the alterations of membrane potential. Decreasing the membrane potential, while it decreased the spike potential (p. 307), markedly increased the amplitude of the after-potential (cf. Figs. 8 and 11). On the other hand, increasing the membrane potential above its resting level reduced the after-potential. Depending upon the effectiveness of the NM spike in setting up the SD spike, the membrane potential might be increased to a level at which hardly any after-potential was developed. Further increase in membrane potential caused failure of the SD spike.

In order to investigate the effect of greater hyperpolarization the device was adopted of starting the hyperpolarizing current immediately after the spike had been set up and maintaining it during the period of the after-potential. An example of this appears in Fig. 13, where arrows are used to indicate corresponding times during the response with and without hyperpolarization. The arrows also indicate which of the two types of trace in each record is the after-potential following the spike, as distinct from control sweeps obtained when the motoneurone was not activated. The identity of these two types was satisfactorily established in other records in which they were obtained separately. It appears that with sufficient hyperpolarization the after-potential may be obtained in inverted polarity. Furthermore, it is shown that, if the hyperpolarizing current is terminated during the after-potential, the latter simply reverts to the voltage that it would have in the absence of a hyperpolarizing current.

The usual procedure in the above investigations (illustrated in Figs. 8, 11, 13 and 14) was to have the stimulus applied to the ventral root just at threshold strength for the axon of the particular motoneurone under observation. Since the motoneurone would not be activated every time, the superimposed sweeps would fall into one or other of two wave forms, the first being due to the stimulation of other axons and the second to the superposition on this background of the response of the motoneurone to stimulation of its axon. When the membrane potential of the cell was altered, it became necessary to readjust the stimulus strength on the ventral root in order to remain at the threshold for activation of the axon of the particular motoneurone. Apparently the current applied to the soma spread along the axon to the site of stimulation in the ventral root and consequently the excitability there was altered. This rather exacting procedure was adopted in order to determine and allow for the inhibitory synaptic potential occurring as the result of the excitation of Renshaw cells by motor-axon collaterals (Eccles, Fatt & Koketsu, 1954). This inhibitory synaptic activity is the cause of the deviations in the control sweeps in Figs. 8 and 13 beginning at about 2 msec from the first signs of electrical activity. The value of the present procedure depended upon the circumstance that the collaterals of a given motor axon do not specially produce an inhibition of that cell. Nevertheless, the quantitative study of the after-potential, as in Fig. 12, was possible only when Renshaw inhibitory activity was relatively small or absent. The resolution of inhibitory synaptic potential and after-potential would necessitate an analysis that at present rests upon an uncertain theoretical foundation.

The amplitude of the after-potential had an approximately linear relationship to the membrane potential at which it occurred. An example appears in

Fig. 12, where the peak amplitude of after-potential is plotted. This is obtained from an extensive series of records, a few of which are illustrated in Fig. 11. The relation of after-potential to membrane potential strongly suggests that, just as with the spike, the after-potential owes its existence to a permeability increase of the membrane toward some particular species of ions. The ions would move along their electrochemical gradient across the membrane, their rate of passage (or flux) being proportional to the displacement of the membrane potential from the level at which the ions on the opposite sides of the membrane

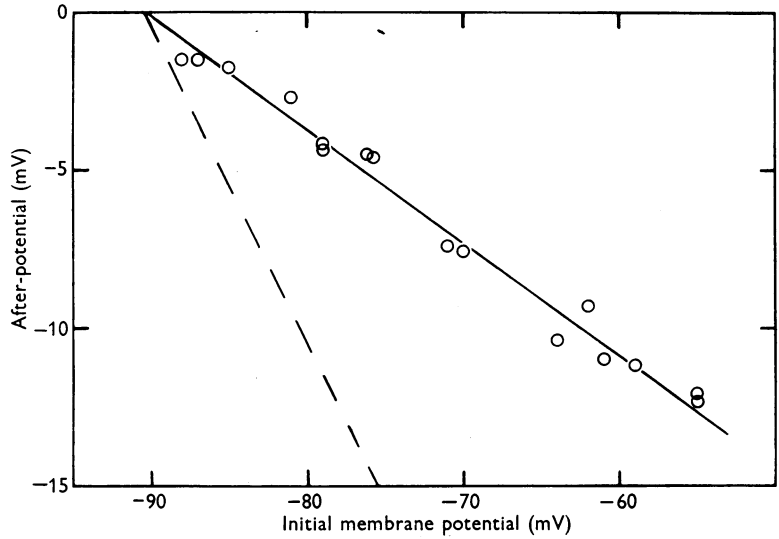


Fig. 12. Plot of the peak amplitude of after-potential against membrane potential. Part of the series of records from this motoneurone is illustrated in Fig. 11. A straight line has been fitted to the plotted points. Broken line indicates the relation that would obtain if there was complete compensation by the after-potential. Resting potential varied from −76 to −79 mV. Negative values of after-potential indicate increase of internal negativity.

would be in electrochemical equilibrium. Investigations on fourteen motoneurones indicated that the equilibrium level at which there was no after-potential, and hence no net flux of the critical ions, was in the range of −80 to −100 mV with a mean of −89 mV. In nearly all cases this equilibrium level was found by extrapolation as in Fig. 12. The slope of the plotted curves indicates the fractional displacement of potential toward the equilibrium level or, viewed in another way, the degree of compensation during the after-potential for any alteration in potential which has been produced by a fixed current. Values of this quantity at the peak of the after-potential ranged in different experiments from 15 to 40%. In the experiment of Fig. 12 the fractional displacement of potential was 37%.

When for any cause the SD spike failed to be set up by an antidromic impulse, the characteristic after-potential did not appear. Under normal conditions (i.e. with the cell initially at its resting potential), although the NM spike attained a height which was a considerable fraction of the SD spike, it was not followed by any detectable after-potential in the direction of a hyperpolarization. Where such an after-potential has been described as a prominent accompaniment of the NM spike (cf. Brock *et al.* 1953), it is probable that what was actually observed was an inhibitory hyperpolarization effected via Renshaw cells, which were activated by the collaterals of all the stimulated motor axons. Such a misinterpretation was avoided here by having the stimulus straddle the threshold of the axon of the particular motoneurone under observation. Although there was normally no identifiable after-potential following the NM spike, a transient hyperpolarization did occur after the NM spike when the membrane was initially depolarized with a large extrinsic current (cf. top record of Fig. 8). This must be attributed to the condition of the membrane in the region where the NM spike is generated. A phenomenon

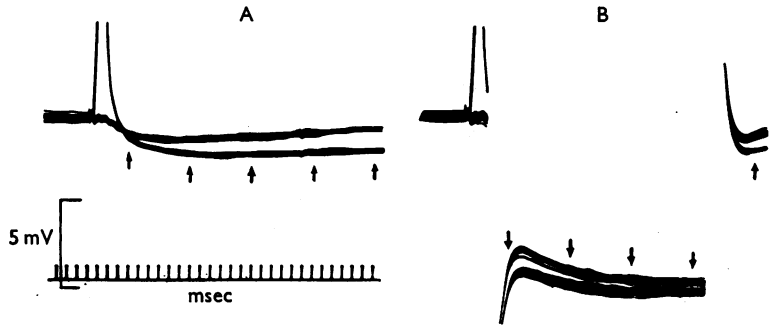


Fig. 13. Effect of a large hyperpolarization on the after-potential of a motoneurone. In A there is no extrinsic current; in B a pulse of hyperpolarizing current starts late on the falling phase of the spike and lasts about 20 msec, occupying the middle two-thirds of the sweep. Arrows in A and B indicate corresponding times with respect to the start of the spike, and designate which of the two types of trace in each record follows the spike.

of the same nature was recorded with the microelectrode in motor axons which were depolarized (p. 319). The hyperpolarization observed under these conditions was of much shorter duration (about 5 msec) than the after-potential of the SD spike. This is seen clearly in Fig. 14, where the transient hyperpolarizations are shown in a depolarized motoneurone following SD and NM types of spikes.

*The effect of injected ions on the after-potential.* The injection of different species of ions was a useful procedure for deciding the type of permeability change producing the after-potential. As in the case of the spike, no anion species was observed to have a direct effect, that is, an effect which was not secondary to a change in resting potential. The injection of potassium ions

also had no effect, but this is of no diagnostic value, since it would not be possible by this means to alter appreciably the internal potassium concentration (cf. p. 296). On the other hand, the injection of sodium ions gave definite indications of the involvement of cations in the generation of the potential. The injection of doses of about 50 p-equiv resulted in the after-potential being reduced considerably in amplitude, an effect which occurred simultaneously with the prolongation of the spike (cf. p. 309). This effect could not be due to a direct participation of a flux of sodium ions in the generation of the after-potential, since increasing the internal sodium concentration would then have had the opposite effect from that observed, i.e. there would have been an increased hyperpolarization if this cation species were to move from an in-

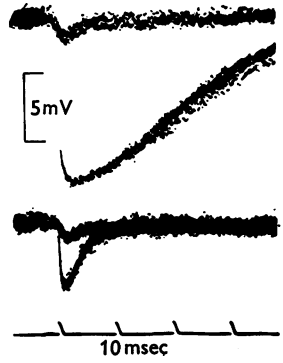


Fig. 14. Records of antidromic response elicited in the same cell as in Fig. 11–12 and again with the stimulus just at threshold for the axon. For both records the initial membrane potential was about −55 mV. Examination of the response with a lower amplification and faster sweep showed that during the upper record complete spikes were set up, while during the lower record, taken about 30 sec later, only NM spikes occurred. The high frequency electrical noise appearing in these records is an artifact consequent on passing large currents through the microelectrode.

creased internal concentration. Thus it may be concluded that the injection of sodium has operated by displacing the predominant species of internal cation, viz. the potassium ion, which itself moves across the membrane during the after-potential. Following an injection of sodium, recovery occurred from the depression of the after-potential over the same time-course that has already been described for the lowering of the resting potential and the prolongation of the spike. The whole sequence of changes following the injection of sodium is illustrated in Fig. 9. The lowering of the resting potential would by itself have increased the after-potential rather than have decreased it.

The effect of the injection of sodium ions was analysed further by making a few measurements at different membrane potentials (varied by means of extrinsic current) before and after injection. The effect of injecting sodium,

and thereby reducing the internal potassium concentration, was found to be a shift in the equilibrium position for the after-potential, i.e. the level of membrane potential at which there was zero after-potential. Qualitatively, the same dependence of after-potential on membrane potential was observed before and after injection, but the rapid recovery from the effect of the injection did not permit complete curves to be plotted. As with the effect on the spike, the injection could be repeated in the same cell and recovery would occur each time within a few minutes. With large injections, failure of the SD spike prevented after-potentials being obtained immediately after the injection. Also, when the resting potential had risen beyond its initial level, at about 8 min from the injection, NM–SD blockage sometimes occurred. If during this late increase of resting potential the SD spike did not fail, the after-potential was reduced just as would be predicted for the increase in membrane potential on the assumption that the cell was otherwise normal. This agrees with the suggestion that the high resting potential is due to a continued efflux of sodium ions and is not associated with any significant alteration in potassium ion concentration (cf. p. 297).

The injection of tetramethylammonium ions permitted more complete measurements to be made of after-potential versus membrane potential, because, following the injection, recovery was either very slow or absent. The results thus obtained point to the conclusion that has already been reached from the injection of sodium ions. The after-potential was reduced simultaneously with the reduction of the resting potential and the slowing of the rate of decline of the spike. The plotting of after-potential against membrane potential showed the linear relationship both before and after the injection. In Fig. 15 the principal effect of the injection is seen to be a shift of the equilibrium for the after-potential to a lower level of membrane potential. In addition there is a small, but significant, reduction of slope, which is in agreement with the idea that there has been a reduction in the concentration of the internal cation which moves during the after-potential.

*Membrane properties of structures other than the motoneuronal soma*

On probing the spinal cord with the microelectrode large negative potentials ($-50$ to $-80$ mV) were sometimes obtained which could not be related to the motoneuronal soma by the wave forms produced on testing with various nerve volleys. In some cases they could be identified as due to the penetration of afferent or efferent nerve fibres. With the techniques at hand, results obtained on the motor axon contrasted with those described for the motoneuronal soma. Resting potentials obtained from the motor axon were not reliable, because there was usually evidence of injury: there was an initial discharge of several action potentials set up by the penetration, and the resting potential fell gradually while the electrode remained in the fibre. On applying current with a double-barrelled electrode the resistance from the inside of the cell to the surroundings was found to be several times greater in the axon than in the soma. The resistance was of the order of 5 MΩ on hyperpolarizing, with a somewhat lower value of about 3–4 MΩ on depolarizing.

The axon spike had usually a simpler form than the soma spike; but when there was considerable injury, the spike might be divided into two distinct components, due evidently to the action of two

nodes of Ranvier. With progressive injury the slightly later component would fail, there being apparently a failure to excite the node nearest the point of penetration. In a relatively uninjured axon the amplitude of the spike deflexion could be greatly altered by shifting the membrane potential with extrinsic currents. On hyperpolarizing the membrane, the spike deflexion was increased. In the example of Fig. 16, at the peak of the spike there was a compensation of about 70% of the initial shift of membrane potential. Depolarization caused the spike to decrease, but there soon occurred an abrupt failure presumably at the nearest node of Ranvier.

In most axons from which potentials were recorded there was no after-potential, such as has been described for the motoneurone soma. However, in a few cases, a prolonged hyperpolarization of 1 to 3 mV followed the spike. In each such case, testing with afferent nerve volleys yielded synaptic potentials of up to a millivolt in amplitude. It was therefore concluded that in these cases the electrode was lodged in the motor axon not far from the cell body, and that the after-potential

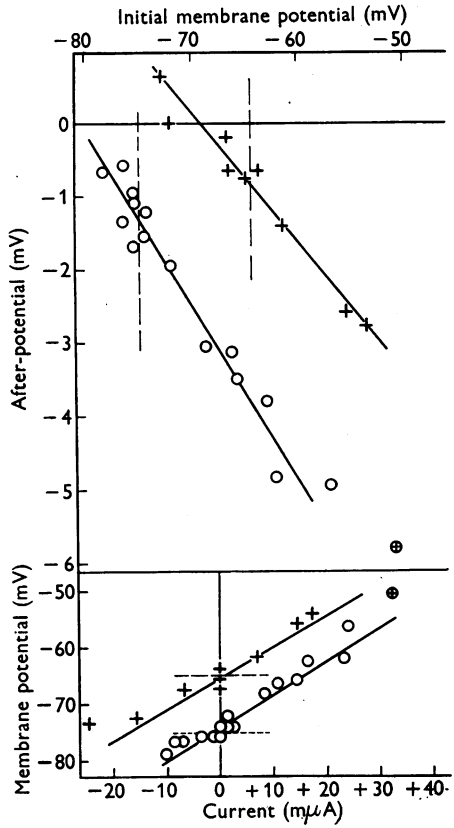


Fig. 15. Effect of injecting tetramethylammonium ions on the relationship of after-potential to initial membrane potential to extrinsic current. In upper part after-potential is plotted against initial membrane potential. In lower part membrane potential is plotted against extrinsic current. There are corresponding points in the two parts of the figure. Points ○ were obtained before, and points + after, the injection of approximately 20 p-equiv $(CH_3)_4N^+$ (applying $3 \times 10^{-8}$ A for 60 sec). Point ⊕ represents measurement made at the start of current used for injection. Each set of points is fitted with a straight line. Broken lines indicate approximate resting potentials before and after injection.

as well as the synaptic potential were recorded only after electrotonic spread to the position where potentials were led from the axon. Hyperpolarization of the axon membrane caused the appearance of a prolonged state of recovery from depolarization following the spike, i.e. a 'negative after-potential' (cf. Fig. 16). It is likely that this represents the normal response, since axons were usually depolarized to some extent by penetration with the electrode. The time course of the axonal action potential would thus agree with the description of Lloyd (1951), who used a less direct method of recording, but one which would not injure the axons. On depolarization, this late phase of the action potential did not appear in inverted form, i.e. as a transient hyperpolarization, such as would be expected if it were produced in a way similar to the after-potential recorded in the soma.

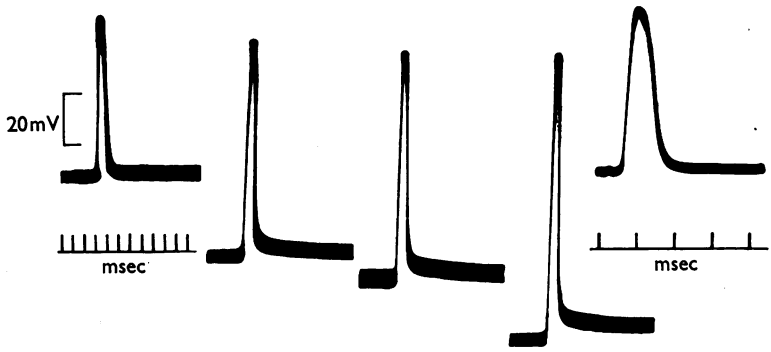


Fig. 16. Action potentials recorded within a motor axon on alteration of initial membrane potential with extrinsic currents. Records on extreme right and left obtained before applying extrinsic current with microelectrode, although there may already be some current due to injury of axon. Second, third and fourth from left obtained with increasing inward (hyperpolarizing) current. Vertical position throughout figure represents membrane potential. Voltage scale applies to all records. Time scale on left applies to all records except that on extreme right.

Under conditions of a depolarization, a transient hyperpolarization did follow the spike, but this had a duration of only 3–5 msec. It would appear probable that this latter effect was due to an extension of the mechanism which produces the rapid fall of the spike, i.e. the brief phase of high potassium conductance (Hodgkin, 1951).

Occasionally, in searching for cells within the spinal cord, large negative potentials (about −70 mV) appeared, from which no alteration could be elicited by testing nerve volleys. As the appearance of these potentials was abrupt and they could be recorded undisturbed over long periods, it was presumed that they represented the resting potentials of large cells. With the application of the double-barrelled electrode, it became possible to test the direct electrical excitability of these structures. It was found, surprisingly, that they could not be activated by depolarizing currents, although the depolarization could be made quite large since these structures displayed resistances of up to several megohms. It has been concluded that the electrode may in these cases have become lodged in non-neural cells, possibly large neuroglial cells.

## DISCUSSION

### *Evidence for active ionic fluxes*

The evidence may now be adduced which bears out the suggestion that there are large fluxes of ions across the membrane of resting neurones in the central nervous system. It has been shown that, after sodium ions have been injected

into a cell and the response of the cell has thereby been altered, complete recovery to the initial condition occurs within 5–8 min. In some cells thus treated there is a subsequent augmentation of membrane potential (i.e. increase of internal negativity), which is attributable to a direct effect of the sodium ion flux out of the cell. The efflux of sodium ions would be in opposition to the electrochemical potential difference for this species of ion. It must therefore depend upon the operation of an 'ion pump' consuming metabolic energy. In contrast, there was only very slow recovery from the effects of tetramethylammonium injection, which were in several respects similar to those produced by sodium. The ion pump would thus appear to extrude sodium ions selectively. What recovery did occur after tetramethylammonium injection is explicable by passive diffusion out of the cell, since there would be no significant concentration of tetramethylammonium in the external environment.

It is also necessary to postulate an ion pump in order to account for the after-potential, in the form of a hyperpolarization, occurring in the motoneurone under normal conditions of membrane potential. There is good evidence that this after-potential is due to the net outward flux of potassium ions consequent upon a specific permeability increase toward these ions, and that there is a potassium equilibrium at a membrane potential some 20 mV higher (internally more negative) than the resting potential. Expressed otherwise, at the resting potential the internal potassium ion concentration is too great to be retained within the cell in equilibrium with the external medium. An ion pump operated by metabolic energy must therefore carry potassium from the exterior into the cell in order to balance the loss by diffusion.

Metabolically driven pumps selectively transporting sodium ions outward and potassium ions inward have been postulated to exist in other cell membranes (cf. Hodgkin & Keynes, 1954). However the motoneuronal membrane may be distinctive in the intensity of these pumps. The relatively high conductance of the resting membrane would permit a large leakage of ions which would have to be balanced by pump activity. With pumps playing a prominent part in ionic movements, the membrane potential may not be determined solely by diffusion across the membrane of the various species of ions (cf. Grundfest, Kao & Altamirano, 1954). If the movement of different ions by the pump was so balanced that there was no net transfer of charge, the membrane potential would be determined by the concentrations of, and passive permeabilities toward, all the different species of ions on the two sides of the membrane. Calculations have been made for such a condition in the giant axon of the squid (Hodgkin & Katz, 1949). If, on the other hand, the pumps produce a net transfer of charge, the membrane potential may take on quite different values. The raised resting potential appearing some minutes after the injection of

sodium ions suggests that the latter condition applies here. It further suggests that the level of activity of the sodium pump is determined by the internal concentration of sodium ions.

*Membrane resistance of the motoneurone*

From the resistance measurement made with an electrode in the soma an approximate estimate may be made of the specific membrane resistance (i.e. resistance for unit area of membrane). Taking the cell to be a simple sphere of $70\mu$ diameter its surface area will be $1{\cdot}5 \times 10^{-4}$ cm$^2$. From the resistance between inside and outside of 1 M$\Omega$, the specific membrane resistance is found to be 150 $\Omega$ cm$^2$. The cell may be more accurately represented by adding on to this spherical cell body six dendrites of $5\mu$ diameter and indefinitely great length and by assuming uniform membrane resistance throughout this whole structure. Using an internal specific resistivity of 50 $\Omega$ cm, which is approximately that of Ringer solution at 38° C, the specific membrane resistance is calculated on the basis of cable theory to be 500 $\Omega$ cm$^2$. It can also be shown that, of the total conductance ($10^{-6}\,\Omega^{-1}$), only about 30 % would be due to the soma, the rest being contributed by the dendrites. The length constant within these dendrites would be about $350\mu$.

Given the value of the electric time constant, the specific membrane capacity can be calculated. On account of electrical artifacts (p. 292) it is not possible to determine the electric time constant from the time course of the potential change at the beginning or end of a current pulse. The later part of the simplest post-synaptic potentials, produced in monosynaptic excitation and direct inhibition, appears to be determined by a passive decay of charge, which has been placed on the membrane capacity during the brief initial phase of transmitter action. If it be assumed that synaptic activity is distributed uniformly over the surface membrane of the cell body and dendrites, which enter into the electrical system under observation, then the rate of decay of this potential should be exponential and give directly the electric time constant of the membrane. The decaying phase of the synaptic potential approximates to an exponential decay with a time constant of about 4 msec (Brock *et al.* 1952). Considered together with the estimated specific resistance, this would give a specific membrane capacity of $8\mu$F/cm$^2$. Although a specific membrane capacity of this order has been found in muscle fibres, it is much greater than the value of about $1\mu$F/cm$^2$ found in other nerve membranes.

*Intracellular concentrations produced by ion injections*

It has not been attempted in this paper to make the injection of ions strictly quantitative. Nevertheless, it might be enquired what amount of ion could reasonably be expected to produce an appreciable change in the internal composition. A spherical cell with a diameter of $70\mu$ would have a volume of

$1{\cdot}8 \times 10^{-7}$ cm$^3$. To this might be added the volume of six cylindrical dendrites, each 5 $\mu$ in diameter and 350 $\mu$ long, thus bringing the total effective volume to $2{\cdot}3 \times 10^{-7}$ cm$^3$ and making the total surface area $4{\cdot}8 \times 10^{-4}$ cm$^2$. At a concentration of 150 m-equiv/l., the quantity of cation contained in such a cell would be 35 p-equiv. Experimentally, effects were produced by the injection of sodium in amounts of 10 to 20 p-equiv, although several times this amount was used to produce the more striking effects illustrated in this paper. It suggests that the effective volume is being underestimated with the cell model used here. Taken together, all the estimates of cell properties based on the model, i.e. specific membrane resistance, specific membrane capacity and alteration of intracellular ionic concentration produced by a known injection, have unusual values, suggesting that there has been a considerable error in the specification of the model and that in reality the cell is larger than has been supposed. Reasonable results could be obtained in the above calculations if both the effective surface area and the effective volume of the model were increased by a factor of at least two. For the surface area and volume to be thus increased, the dendritic processes must contribute rather more than has thus far been allowed.

The results described in this paper are compatible with the idea that the rising phase of the spike is due to an influx of sodium ions which is quickly terminated and replaced by an efflux of potassium ions, such as has been described for other excitable structures. The movement of potassium ions in this scheme would be responsible for the rapid falling phase of the spike. The long after-potential which follows the spike would be due also to a movement of potassium ions consequent on a permeability increase toward this species of ion. It has been assumed that the potentials seen are those occurring in the cell body, because of the large size of the cell body and the consequent likelihood of penetrating it with a microelectrode. But there is still no direct evidence to show in precisely what regions of membrane the described ionic movements occur, as distinct from where potential changes occur.

## SUMMARY

1. The technique is described by which a double-barrelled intracellular microelectrode is used, in the anaesthetized cat, for recording potentials from a motoneurone that is being subjected to extrinsic electric currents. The further technique is described of injecting various species of ions into motoneurones. By these means it is possible to control the membrane potential and the concentration difference of various ions across the membrane, two factors which are of primary importance in the excitatory activity of the membrane.

2. The membrane potential was diminished only slightly (0–10 mV) by large increments in intracellular concentration of $Cl^-$, $Br^-$ and $NO_3^-$. It was diminished to the extent of about 10 mV by injections of $Na^+$ or $(CH_3)_4N^+$

of the order of 50 p-equiv. The latter effect is attributed to a greater permeability of the membrane toward the normally occurring internal $K^+$ than to the injected cations which replace it. Following an injection of $Na^+$, the membrane potential returned to its original level in 5–8 min and thereafter often increased beyond its original value.

3. The resistance measured between the inside and outside of a motoneurone during the application of a prolonged current amounted to 0·4–1·3 MΩ. In any given cell the relation of membrane potential to applied current was nearly linear over the range in which observations could be made. The resistance could be increased temporarily by the injection of $Na^+$.

4. With the application of outwardly directed pulses of current, action potentials were initiated in the motoneurone when the membrane had been depolarized to a critical extent of about 10 mV. When the pulse was just suprathreshold, action potentials were initiated 3–8 msec from the start of the depolarizing current. This threshold, obtained by direct stimulation, was nearly the same as that for the setting-up of an action potential by the excitatory post-synaptic potential.

5. If a motoneurone was normally completely invaded antidromically, a hyperpolarizing current could induce blockage at either of two stages in the invasion. In one type of blockage a spike-like deflexion of about 30 mV amplitude remained (the NM spike). In the other type a deflexion of only 1–5 mV amplitude remained (the M spike). In many motoneurones either one or the other type of blockage normally occurred. It was then possible to relieve this blockage by initially depolarizing with an applied current. When a strong depolarizing current was applied, a train of action potentials was initially evoked and following this it was possible to obtain only an NM spike as the response to antidromic stimulation although with less depolarization complete invasion was obtained (Fig. 8).

6. The amplitude and rate of rise both of the complete spike and of the NM spike diminished with increasing depolarization and with increasing duration of a steady depolarization. The injection of sodium ions decreased the amplitude of the spike and prolonged its rising and falling phases, recovery occurring in about 5 min. The injection of tetramethylammonium and choline ions also caused a decrease and prolongation of the spike, choline being particularly effective in delaying the recovery phase of the spike. Usually these effects were irreversible, though with the former species of ion there may be a very slow recovery.

7. When the membrane potential was altered by applied currents, the after-potential, which followed the spike and was normally in the form of a prolonged hyperpolarization, varied in the inverse sense. Reversal of the after-potential to the form of a depolarization occurred when the membrane potential was increased about 20 mV beyond the resting potential. During the

after-potential there was a displacement of potential toward this reversal potential by about 30%.

8. The injection of $Na^+$ or $(CH_3)_4N^+$ ions into the motoneurone diminished the after-potential for a given membrane potential and lowered the reversal potential. It was concluded that this effect was due to a displacement of intracellular $K^+$ by the injected cations and that the after-potential is generated by a movement of $K^+$ across the membrane, which has an increased permeability toward this ion species. Consequently, $K^+$ would be in equilibrium across the membrane when its potential is at the reversal potential for the after-potential, i.e. at about 20 mV above the normal resting potential.

9. The $K^+$ concentration within the cell is too high to be retained within it at the normal resting potential and an ion 'pump' must be present, expending metabolic energy in transporting $K^+$ ions inward as rapidly as they passively diffuse outward through the membrane. In addition, the presence of a $Na^+$ 'pump' is indicated by the recovery of membrane potential after an injection of this ion species, but not after $(CH_3)_4N^+$, and by the later increase of membrane potential above the resting level. The latter effect requires that in the operation of the $Na^+$ 'pump' there is a net transfer of charge across the membrane, that is, the outward transport of $Na^+$ is not balanced by the transfer in the appropriate direction of an equivalent charge of other ions by the 'pump' mechanism.

## REFERENCES


BOYLE, P. J. & CONWAY, E. J. (1941). Potassium accumulation in muscle and associated changes. *J. Physiol.* **100**, 1–63.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.* **117**, 431–460.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1953). Intracellular recording from antidromically activated motoneurones. *J. Physiol.* **122**, 429–461.

BROCK, L. G. & MCINTYRE, A. K. (1953). Responses of motoneurones to stimulation by internal microelectrodes. *Proc. Univ. Otago med. Sch.* **31**, 19–20.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955). The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol.* **130**, 326–373.

ECCLES, J. C. (1952). The electrophysiological properties of the motoneurone. *Cold Spr. Harb. Symp. quant. Biol.* **17**, 175–183.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

ECCLES, J. C., FATT, P., LANDGREN, S. & WINSBURY, G. J. (1954). Spinal cord potentials generated by volleys in the large muscle afferents. *J. Physiol.* **125**, 590–606.

GERARD, R. W. (1937). Brain metabolism and circulation. *Proc. Ass. Res. nerv. ment. Dis.* **18**, 316–345.

GOLDMAN, D. E. (1943). Potential, impedance and rectification in membranes. *J. gen. Physiol.* **27**, 37–60.

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

VAN HARREVELD, A. (1946). Asphyxial depolarization in the spinal cord. *Amer. J. Physiol.* **147**, 669–682.

HODGKIN, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**, 339–409.

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

HODGKIN, A. L. & KEYNES, R. D. (1954). Movements of cations during recovery in nerve. *Symp. Soc. exp. Biol.* **8**, 423–437.

HOLMES, E. G. (1930). Oxidation in central and peripheral nervous tissue. *Biochem. J.* **24**, 914–925.

LLOYD, D. P. C. (1951). After-currents, after-potentials, excitability and ventral root electrotonus in spinal motoneurones. *J. gen. Physiol.* **35**, 289–321.

NASTUK, W. L. & HODGKIN, A. L. (1950). The electrical activity of single muscle fibres. *J. cell. comp. Physiol.* **35**, 39–73.

RENSHAW, B. (1942). Effects of presynaptic volleys on spread of impulses over the soma of the motoneurone. *J. Neurophysiol.* **5**, 235–243.

USSING, H. H. (1949). Transport of ions across cellular membranes. *Physiol. Rev.* **29**, 127–155.

WOODBURY, J. W. & PATTON, H. D. (1952). Electrical activity of single spinal cord elements. *Cold Spr. Harb. Symp. quant. Biol.* **17**, 185–188.