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THE SPECIFIC IONIC CONDUCTANCES AND THE IONIC MOVEMENTS ACROSS THE MOTONEURONAL MEMBRANE THAT PRODUCE THE INHIBITORY POST-SYNAPTIC POTENTIAL

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In the first account of potentials recorded intracellularly from motoneurones by microelectrodes, it was reported that inhibitory synaptic action evoked a hyperpolarization of the neuronal membrane (Brock, Coombs & Eccles, 1952), which is the reverse potential change from that produced by excitatory synaptic action. This inhibitory post-synaptic potential (i.p.s.p.) of motoneurones appears to be analogous to the hyperpolarizing responses that have been observed during inhibitory action on other tissues, for example, during inhibition of cardiac muscle (Gaskell, 1887; Monnier & Dubuisson, 1934; Burgen & Terroux, 1953) and of crustacean muscle when the membrane potential is abnormally low (Fatt & Katz, 1953).

There is now much evidence that the excitatory responses of nerve and muscle cells are caused by specific increases in the permeability of the surface membrane to ions and the consequent ionic fluxes, and not, for example, by changes in the membrane capacity (cf. Cole & Curtis, 1939; Hodgkin, 1951). On this basis the electrical responses of giant nerve fibres and of muscle fibres have been satisfactorily explained by the movements of Na^+ and K^+ ions across the surface membrane (cf. Hodgkin, 1951; Hodgkin & Huxley, 1952*c*). Also the excitatory response at the neuromuscular junction (the end-plate potential) has been satisfactorily explained by the postulate that the neuromuscular transmitter causes a large transient increase in the permeability of the end-plate membrane to all ions (Fatt & Katz, 1951; Castillo & Katz, 1954). However, there has as yet been no complete explanation of the inhibitory responses of crustacean muscle (Fatt & Katz, 1953) or cardiac muscle (Burgen & Terroux, 1953). There is evidence that an increased permeability to K^+ ions occurs at both junctional regions, but at least with crustacean muscle it had

to be postulated that there was also an increased permeability to some other ion species which like K^+ ions would normally be close to electrochemical equilibrium.

The present paper gives an account of investigations designed to discover the ionic movements that are responsible for producing the i.p.s.p. of motoneurones. It has been found that identical ionic mechanisms are concerned in five types of inhibitory synaptic action that are exerted on motoneurones in the spinal cord: direct inhibition of antagonist motoneurones by group I*a* impulses from the annulo-spiral endings of muscle spindles (Lloyd, 1941, 1946; Laporte & Lloyd, 1952; Bradley, Easton & Eccles, 1953); the disynaptic inhibition by group I*b* impulses from Golgi tendon organs (cf. Granit, 1950; Laporte & Lloyd, 1952); the polysynaptic inhibition by group III muscle impulses (Lloyd, 1943); the polysynaptic inhibition by cutaneous impulses (Renshaw, 1942; Hagbarth, 1952); and the disynaptic inhibition by impulses in the motor axon collaterals, which will henceforth be called antidromic inhibition because it can be evoked by antidromic volleys (Renshaw, 1941; Eccles, Fatt & Koketsu, 1954). However, most of the investigation has been concerned with the first and the last of the above series, which give the most satisfactory i.p.s.p.'s for experimental investigation, because with the simple techniques of direct nerve stimulation they may be obtained uncomplicated by excitatory synaptic action. The relation of the results presented here to the inhibitory suppression of the discharges of motoneurones will be considered in a later paper.

A preliminary account of some of these investigations has been published (Coombs, Eccles & Fatt, 1953). The experimental procedures have already been fully described: the operative, and electrical procedures by Brock *et al.* (1952) and by Coombs, Eccles & Fatt (1955*a*); the micromanipulator technique by Eccles, Fatt, Landgren & Winsbury (1954); and the extrinsic current and intracellular injection procedures by Coombs *et al.* (1955*a*). Throughout the investigations all photographic records have been formed by the superposition of many faint traces (usually about 40) in order to reject random noise. This 'noise' was often considerable when large currents were being passed through the microelectrode.

RESULTS

A. *Effect of alterations in membrane potential on the i.p.s.p.*

By passing a direct current through one barrel of a double microelectrode that has been inserted into a motoneurone, the membrane potential may be altered to any desired value within a wide range, as has been fully described in the previous paper (Coombs *et al.* 1955*a*). The actual change in the membrane potential has been calculated as the potential alteration measured through the other barrel of the microelectrode less the potential drop that the current would cause in the coupling resistance between the two barrels of the microelectrode (cf. Coombs *et al.* 1955*a*). All potential measurements designated membrane potentials have been so corrected.

In Fig. 1 (A–G) it is seen that the brief hyperpolarization (the i.p.s.p.) produced in a biceps-semitendinosus motoneurone by a volley in group I*a* quadriceps afferent fibres at the normal membrane potential of −74 mV (D) was greatly modified by relatively small changes in the membrane potential, being increased by depolarizations (A–C) and diminished or even reversed (E–G) by hyperpolarizations. There is much evidence that this volley from the antagonist muscle exerts a purely inhibitory action on the motoneurone (Lloyd, 1946; Laporte & Lloyd, 1952; Bradley *et al.* 1953), hence it appears that an increase in the membrane potential above the resting level actually reversed the sign of the i.p.s.p., i.e. it converted the normal hyperpolarizing i.p.s.p. into a depolarizing i.p.s.p. This is similar to the alteration of inhibitory junctional potentials with change in membrane potential that occurs in the crustacean muscle, although in that case the reversal normally occurs at the resting potential (Fatt & Katz, 1953).

When plotted against the membrane potential, the corresponding peak voltages of the i.p.s.p. are seen to lie on a curve that crosses through zero at a membrane potential which may be called the 'reversal-potential' for the i.p.s.p. and which is approximately −80 mV in Fig. 2A, i.e. with the interior of the motoneurone 80 mV negative to the external indifferent electrode. The simplest explanation of this reversal is that the i.p.s.p. is generated by the net flux of some specific type of ion (or ions) through the post-synaptic membrane, this flux being consequent on a specific increase of the membrane permeability. The 'reversal-potential' would thus represent the membrane potential at which there was equality of the electric charges carried by the fluxes in the two directions across the membrane. With the normal resting membrane potential there must be a net outward passage of charge in order to produce the observed hyperpolarizing i.p.s.p. (cf. Fig. 1D). Hence, for example if the specific ions are anions, the ionic flux must be preponderantly inward, and if cations, it must be outward. A more complex situation will exist if, as seems likely, both anions and cations are included in the specific group (cf. Discussion, § A). The upward convexity of the curve in Fig. 2 is characteristic of our most reliable experiments. The theoretical significance will be considered in the Discussion (§ D).

As shown in Figs. 1H to N, the i.p.s.p. set up by an antidromic volley by means of the axon collaterals and repetitive interneuronal discharges (Eccles *et al.*, 1954) was almost identically affected by changing the membrane potential. This similarity both of reversal-potential and curvature is illustrated in Figs. 2A and B, and in Table 1 the reversal-potentials are seen to be almost the same in the four neurones where both were determined. Furthermore, the mean reversal-potential was about 11·5 mV (extremes 5 to 26) more negative than the mean resting potential in the eight motoneurones where curves similar to that of Fig. 2 were drawn for the i.p.s.p. of

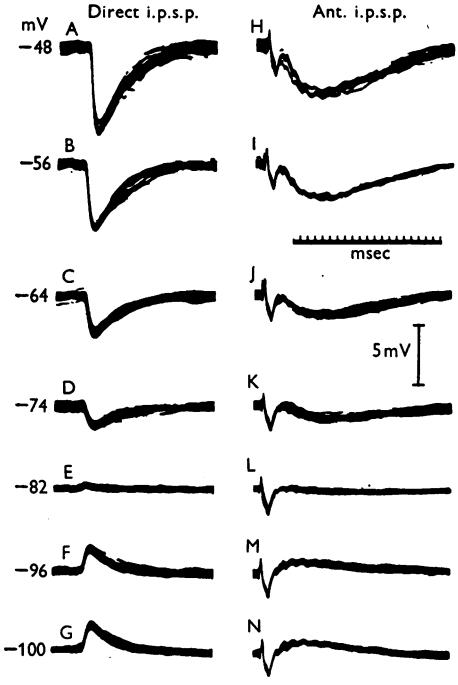

Fig. 1

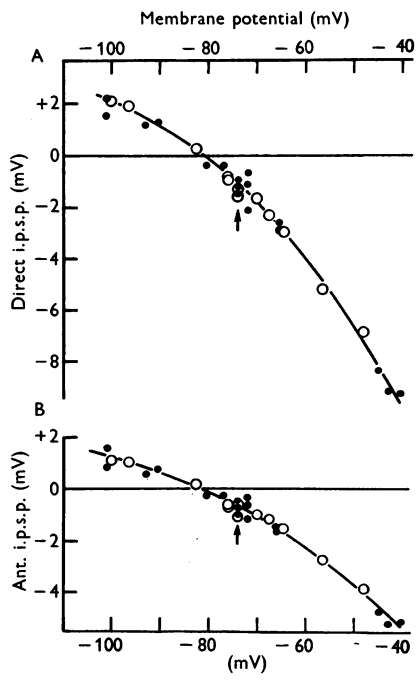

Fig. 2

Fig. 1. Potentials recorded intracellularly from a biceps-semitendinosus motoneurone by means of a double-barrelled microelectrode filled with 0·6 M-Na_2SO_4. All records are formed by the superposition of about forty faint traces. A to G show potentials set up by the direct inhibitory action of a group I*a* quadriceps afferent volley, while with H to N the inhibitory action is exerted by an antidromic volley set up in L_7 and S_1 ventral roots by a stimulus just below threshold for the axon of the motoneurone. The initial diphasic spike is the characteristic field potential produced by antidromic invasion of adjacent motoneurones. By means of a steady background current through one barrel of the double microelectrode, the membrane potential has been preset at the voltage indicated on each record. In the absence of such current the membrane potential was −74 mV. Potential and time scales apply to all records. Positivity of the microelectrode relative to the indifferent external electrode is recorded upwards. All voltages are given relative to this external reference electrode, hence hyperpolarization of the motoneurone is registered by an increasing negativity, i.e. by a downward deflexion, while the diphasic spike in the antidromic record (due to the external field from adjacent motoneurones) appears with an initial upward deflexion, which is the inverse of the usual convention.

Fig. 2. Maximum voltages of the i.p.s.p.'s of the series partly shown in Fig. 1 are plotted as ordinates against the respective membrane potentials as abscissae, A being for the direct i.p.s.p.'s and B for the antidromic i.p.s.p.'s. According to the convention adopted throughout this paper, hyperpolarizing and depolarizing i.p.s.p.'s are plotted respectively as negative and positive voltages. Points designated ● were obtained before, and those designated ○ some 9 min after, a depolarizing current of 5×10^{-8} A for 90 sec. There was no significant change in the resting potential, the mean value being indicated by the arrows.

direct inhibition, and about 14 mV (extremes 7 to 25) more negative in the seven motoneurones where antidromic i.p.s.p. curves were drawn.

The i.p.s.p.'s produced by three other types of inhibitory action have not been so systematically examined, but in all cases they have been similarly affected by variations in membrane potential, i.e. inhibitory actions by volleys in group I*b* muscle afferents, in group III muscle afferents, and in cutaneous afferents exhibit the same behaviour.

TABLE 1. Membrane potentials of motoneurones and the reversal-potentials for their hyperpolarizing responses

All potentials are those indicated by an intracellular electrode relative to the external reference electrode with due allowance for the potential drop in the coupling resistance. BST, FDL and GS signify biceps-semitendinosus, flexor digitorum longus and gastrocnemius-soleus motoneurones respectively.

			Reversal-potentials (mV)		
Neurone type	Salt in electrode	Resting potential (mV)	Direct i.p.s.p.	Antidromic i.p.s.p.	Positive after-potential
BST	Na_2SO_4	-70	-80	—	-95
FDL	K_2SO_4	-60	-65	—	-95
BST	K_2SO_4	-56	-68	-66	-81
BST	K_2SO_4	-68	-77	-77	-81
BST	Na_2SO_4	-74	-80	-81	—
BST	Na_2SO_4	-75	-90	—	—
?	$((CH_3)_4N)_2SO_4$	-70	—	-88	-94
BST	$((CH_3)_4N)_2SO_4$	-76	-81	—	-84
GS	K_2SO_4	-57	—	-82	—
BST	K_2SO_4	-54	-80	-78	—
?	K_2SO_4	-68	—	-88	-90
Mean values	—	-66	-77·5	-80	-88·5

Discussion. The similarity of the reversal-potentials indicates that all types of i.p.s.p. are generated by essentially the same ionic mechanisms. In contrast, the e.p.s.p. (excitatory post-synaptic potential) was reversed, i.e. changed to a hyperpolarizing potential, only when the membrane potential was itself reversed, i.e. changed to internal positivity (cf. Coombs *et al.* 1953 and unpublished observations). Even when the i.p.s.p. has been changed to the depolarizing type, the effect of varying the membrane potential readily distinguished it from the e.p.s.p. (cf. Figs. 3C, D; 9C, D; 13A, B, C, K, L, M) because of the difference in the values of the reversal-potentials, hence a discrimination was still possible when the recorded potential was a mixture of i.p.s.p. and e.p.s.p. Thus, in respect of its reversal-potential the e.p.s.p. resembles the end-plate potential of the neuromuscular junction (Fatt & Katz, 1951; Castillo & Katz, 1954), whereas the i.p.s.p. is similar to the inhibitory potential in crustacean muscle (Fatt & Katz, 1953).

This investigation into the effect of varying the membrane potential leads immediately on to the question: What species of ions are concerned in generating the i.p.s.p.? Or, alternatively, the question could be formulated: What are the species of ions to which the post-synaptic membrane becomes

more permeable when it is acted on by inhibitory impulses? In an attempt to answer the question the most direct experimental procedure would be to investigate the changes produced in the i.p.s.p. by altering ionic concentrations on one or other side of the post-synaptic membrane. For example the effect of varying the external potassium concentration has been studied in attempts to discover the ionic mechanisms producing the inhibitory potentials of crustacean muscle (Fatt & Katz, 1953) and mammalian heart (Burgen & Terroux, 1953). Analogous experiments on mammalian motoneurones *in situ* are technically difficult, and hitherto only a few preliminary attempts have been made. On the other hand, on account of the relatively small volume, large changes can readily be produced in the internal ionic composition of the motoneurone. As described in the subsequent sections these changes have been brought about either by diffusion of various salts out of the intracellular microelectrode or by the ionic movements that are produced when a current is passed through the motoneurone either from the indifferent electrode to the microelectrode, or in the opposite direction. Such currents would have respectively a hyperpolarizing and a depolarizing action on the motoneuronal membrane, and they have been designated accordingly.

B. *Effect on the i.p.s.p. of diffusion of ions out of the microelectrode*

When a microelectrode filled with a concentrated chloride solution was inserted into a motoneurone, the i.p.s.p of that motoneurone usually showed a gradual change from a hyperpolarizing to a depolarizing response (Fig. 3 A–C). With a microelectrode having a resistance as low as 5 MΩ, this inversion of the i.p.s.p. occurred very rapidly and after a few minutes a fairly steady state was generally maintained. This effect of diminution and eventual inversion of the i.p.s.p. was observed whether potassium, sodium or choline was the cation in the microelectrode, but did not occur when such anions as sulphate, phosphate or bicarbonate replaced the chloride. However the same effect was observed when the anion was bromide, nitrate (cf. Fig. 9 A–C) or thiocyanate.

Careful inspection shows that the inverted potentials such as those of Fig. 3 B, C are not mirror images of the initial hyperpolarizing i.p.s.p. They are much briefer, particularly on the declining phase, which often passes over into a low hyperpolarization (cf. Fig. 9 E). It may further be noted in passing that i.p.s.p.'s in the form of a depolarization have already been observed with chloride-filled electrodes, though they were not recognized as such (cf. Brock *et al.* 1952, fig. 6 A).

Since the membrane potential did not increase in Fig. 3 A–C, the change in the i.p.s.p. was not comparable with that seen in Fig. 1 D–G. However a diminution of membrane potential by an extrinsically applied current caused the i.p.s.p. to revert to the hyperpolarizing type (Fig. 3 D; cf. also Fig. 9 D). When the curve relating membrane potential to i.p.s.p. was determined as in

Fig. 2, it was found to be shifted to the right, having, for example, a reversal potential of only about −50 mV (curve through ● points in Fig. 4). Thus essentially the effect produced by a chloride-containing electrode was a displacement of the i.p.s.p./membrane potential curve to the right (and also upwards) so that the reversal potential occurred at a lower membrane potential. On account of this effect, microelectrodes filled with salts having such indifferent anions as sulphate or phosphate (cf. Table 1) must be used in determining the normal reversal potential for the i.p.s.p. On the other hand, no significant difference was observed between the three cations (Na^+, K^+ and $(CH_3)_4N^+$) used in the series of Table 1.

Discussion. The simplest explanation of the change in the i.p.s.p. from Fig. 3 A to C would postulate that it is due to the diffusion of chloride ions (or anions of like action, cf. Fig. 9 A–C) out of the microelectrode into the neurone with a consequent increase in the ratio of internal to external chloride concentration across the motoneuronal membrane. There would be the further provisional postulate that such anions are especially concerned in the ionic fluxes that are causally related to the i.p.s.p., i.e. that under the influence of inhibitory impulses, areas of the motoneuronal membrane become highly permeable to these anions, whereas they remain relatively impermeable to the ineffective anions. The change to a depolarizing i.p.s.p. would thus be caused by the change from a net inward to a net outward flux of the effective anions resulting from the increased intracellular concentration. This explanation receives support from an approximate calculation of the concentrations and fluxes obtaining for chloride ions under such experimental conditions.

The normal intracellular concentration of chloride has been estimated to be about $9\,\mu\text{equiv} \times \text{cm}^{-3}$ (cf. Discussion, § C). Taking the volume of the motoneurone to be $2{\cdot}3 \times 10^{-7}$ cm^3 (Coombs *et al.* 1955*a*), its total chloride content would be about 2 p-equiv. It has been calculated (cf. Appendix, § A) that there would be a chloride diffusion of about 0·06 p-equiv/sec from a 3 M-KCl-filled electrode that has a resistance of 5 MΩ when immersed in 0·15 M-KCl. Assuming an exponential time constant of 30 sec for the attainment of chloride equilibrium between a motoneurone and its environment (cf. Discussion, § B), this steady chloride injection from the microelectrode at a rate of 0·06 p-equiv/sec would cause the chloride content of the neurone to increase, with an exponential time constant of 30 sec, to a new steady level which is 1·8 p-equiv ($30 \times 0{\cdot}06$) higher than the initial level. Hence in a few minutes after insertion of a 5 MΩ electrode filled with 3 M-KCl, diffusion of chloride would produce a new steady state with about double the normal intracellular concentration, which is a relative increase of the order required for the above explanation of the observed change in the i.p.s.p. After insertion of the microelectrode the observed rate of change of the i.p.s.p. has often been much slower than the expected rate (time constant of about 30 sec), possibly on account of temporary

obstruction of the electrode orifice during the insertion. However, in a later section (D, 2*a*) an indirect method will be described for determining the rate at which the diffusional equilibrium is attained and the comparison will then be made (Discussion, § B).

The briefer time course already noted for the depolarizing type of i.p.s.p. produced by ionic diffusion (cf. Figs. 3B, C and 9E) can be explained as follows. The ions injected from the electrode would preponderantly affect the responses of those inhibitory post-synaptic areas of the membrane close to the microelectrode which would consequently give the depolarizing i.p.s.p., while more remote areas would be less affected and still give the hyperpolarizing i.p.s.p. The delay produced by electrotonic spread would cause this hyperpolarization largely to follow the depolarization, hence the diphasic character of the i.p.s.p. An alternative explanation would regard the diphasic type of i.p.s.p. as indicative of a dual composition, being attributable to two independent transmitter and ionic mechanisms. However, by varying the strength of stimulus setting up the inhibitory volley, it has not been possible to obtain a threshold separation between afferent fibres that give one or other type of response. Thus presumably the diphasic type of i.p.s.p. in Fig. 3B is given by a homogeneous group of afferent impulses (group I*a*) and, after further diffusion it is converted to a pure depolarizing i.p.s.p. (Fig. 3C, Q–T), which runs a time course virtually identical with the e.p.s.p. (cf. Fig. 16F, G), except that the latent period is almost 1 msec longer when measured from the time of entry of the respective volleys into the spinal cord (cf. Brock *et al.* 1952; Eccles, Fatt & Landgren, 1954). When fully developed, the depolarizing i.p.s.p. is a mirror image of the hyperpolarizing i.p.s.p. (Figs. 3A, C and 9). This mirror-image relationship has already been observed for the i.p.s.p.'s recorded when the neuronal membrane was set at potentials far on either side of the normal resting potential (cf. Fig. 1).

This technique of injecting ions by diffusion out of the microelectrode is unsatisfactory because there is no control of the rate of injection out of any given electrode. For example the rate at which cations can be injected by diffusion is too low to give significant changes. If the probable value of 150 mM is assumed for the potassium concentration of the interior of the motoneurone, the total potassium content would be over 30 p-equiv. Moreover, the time constant for attainment of potassium equilibrium across the membrane would probably be briefer than the value of 30 sec determined for chloride ions. Hence the diffusional rate of potassium out of a microelectrode of 5 MΩ resistance, 0·06 p-equiv/sec, would not significantly affect the potassium concentration of a motoneurone. Diffusion experiments are thus valueless as a test of a possible participation of potassium ions in the generation of the i.p.s.p. As described in the two following sections, effective control of the rate of ionic injection from a microelectrode has been achieved by applying a voltage to it so that current passes between the microelectrode and the cell either hyperpolarizing or depolarizing it.

C. *Effect produced on the i.p.s.p. by a hyperpolarizing current*

(1) *Chloride-filled microelectrode*

The membrane potential and the i.p.s.p. responses before, during and after a prolonged hyperpolarizing current ($3{\cdot}2 \times 10^{-8}$ A for 1 min) are shown in the

d.c. amplifier records of Fig. 3 E–L, which were obtained after the preliminary diffusional injection of chloride (cf. Fig. 3 A–C). The vertical positions of the records are significant in giving the level of membrane potential. The hyperpolarizing current caused an immediate large increase of the membrane potential, from −59 to −125 mV, and at the same time, as described in § A, there was an immediate large increase in the depolarization produced by the i.p.s.p., 3·6–14 mV (Fig. 3 F). However, these effects did not remain constant during the passage of the hyperpolarizing current, nor did they vary in the same sense. The membrane potential progressively declined down to −111 mV, while the i.p.s.p. response progressively increased to 25·5 mV (Fig. 3 G, H).

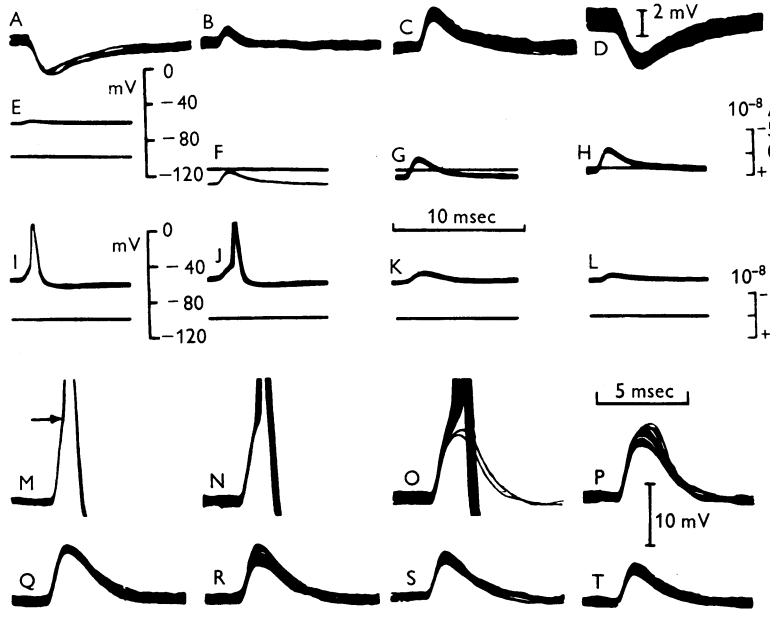

Fig. 3. Intracellular recording through a double-barrelled microelectrode filled with 3 M-KCl, of i.p.s.p.'s generated in a biceps-semitendinosus motoneurone by a quadriceps group I*a* afferent volley. A, B and C show effect of diffusion of Cl^- ions out of the microelectrode in changing the i.p.s.p. recorded at the resting membrane potential (−59 mV), while D shows restoration of the hyperpolarizing i.p.s.p. at a lower membrane potential (−27 mV). E–L show records both of current through one barrel of the electrode (straight lines) and of simultaneous i.p.s.p.'s obtained with a d.c. amplifier. The successive records are so placed that the scales on each side (potential on left, current on right) obtain right across the series, giving the actual levels of membrane potential and current respectively. E is before, F, G, H during and I, J, K, L after the application of $3{\cdot}2 \times 10^{-8}$ A for 60 sec. C and E are the same responses recorded at high and low amplification (note respective potential scales), while M–T cover same range of recovery as I–L, but again at a high amplification, though less than for C (note potential scales). Two time scales are shown, one for E–L, the other for the remaining records. All records formed by superposition of about forty traces. Note effect of wide variation in spike latency in N and O. Spikes in M, N and O are truncated.

On cessation of the current the membrane potential immediately fell to −51 mV, which was well below the initial level, and at the same time the i.p.s.p. was diminished in size as shown by the less steep rising phase (Fig. 3I), though to a value much larger than that initially existing before the hyperpolarization (cf. Fig. 3E with I, and C with M). At this membrane potential the depolarization of the i.p.s.p. was more than adequate to generate an impulse, as may be seen in the early records of the subsequent recovery series taken both at low (Fig. 3J–L) and at high (Fig. 3N–T) amplification. During

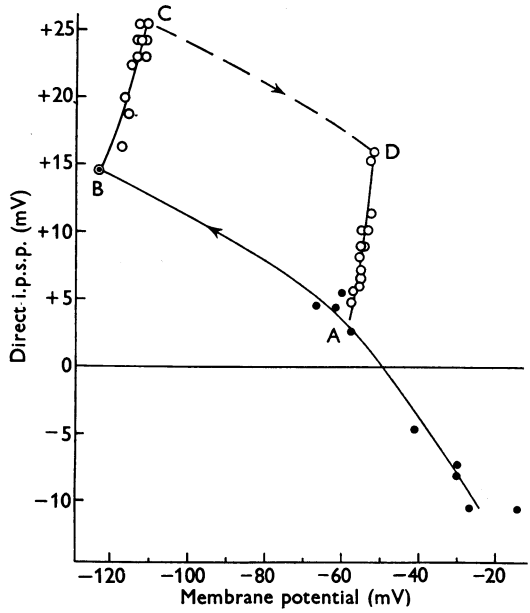

Fig. 4. Points designated ● are from initial records of i.p.s.p.'s at various membrane potentials as in Fig. 2, two such records being shown in Fig. 3C and D. ○ points plotted from B to C were obtained during the passage of the hyperpolarizing current ($3{\cdot}2 \times 10^{-8}$ A, cf. records F to H of Fig. 3, F being shown by ⊙ in Fig. 4), while ○ points from D to A were obtained after cessation of this current (records I to T of Fig. 3). Further description in text.

this recovery, the membrane potential gradually returned towards the initial resting level and the i.p.s.p. response declined so that eventually it resembled the relatively small depolarizing response observed before the hyperpolarization (cf. Fig. 3T with C).

The full course of the changes during and after the hyperpolarizing current of Fig. 3 is plotted in Fig. 4 on the co-ordinates used for the i.p.s.p./membrane potential curve which was determined by preliminary records as in Fig. 2. On hyperpolarization there was an immediate change from the initial condition obtaining at the resting potential, point A (Fig. 3E) to point B (Fig. 3F).

Thereafter, during the hyperpolarization, the gradually changing conditions were defined by the series of points lying approximately along the line BC, there being a progressive decrease in the rate of change. On cessation of the hyperpolarization, there was an immediate change from C to D (Fig. 3I, M) and then again a gradual change back to the original condition, as defined by the series of points lying approximately along the line DA. The generation of spike potentials prevented the direct measurement of the points near to D

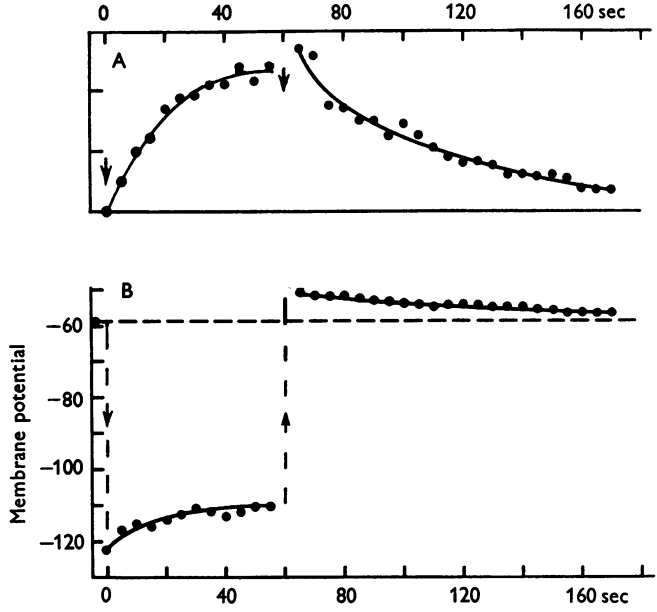

Fig. 5. A. Plot of time course of displacement of the i.p.s.p. responses along the line BC of Fig. 4 during the current and along the line DA after its cessation, as described in text. Ordinates are in arbitrary units measured along the lines BC and DA of Fig. 4. Arrows mark onset and cessation of current. B. Plot of the membrane potentials on same time scale as in A in order to show the similar time courses of the changes during and after the current. Arrows mark onset and cessation of current.

(cf. Fig. 3I, J, M, N). However, the summit-heights of the i.p.s.p.'s were calculated from the slopes of their rising phases on the assumption that similar time courses prevailed throughout.

It would appear that the hyperpolarization has caused the i.p.s.p./membrane potential curve to be displaced progressively upwards and to the right, and during recovery there was the reverse displacement. The time course of these changes may be determined approximately by plotting the displacements along the lines BC and DA against the times of the successive responses. As shown in Fig. 5A, the time-course is approximately exponential with a half time of about 15 sec during the hyperpolarization and about 30 sec after its

cessation. Fig. 5B also shows typically that the changes in membrane potential followed approximately the same time course. Usually the membrane potential has been depressed immediately after a hyperpolarizing current as in Fig. 5B, but sometimes there was no change, and a slight increase has even been observed (cf. arrows of Fig. 6).

On cessation of the hyperpolarization the time course of recovery with a chloride electrode has been so rapid that it has not been possible with the present technique to determine a satisfactory curve for i.p.s.p./membrane potential. For example, in Fig. 6A the ● points give the curve as determined before the hyperpolarization, while the + points give the curve at 10–35 sec after the end of a hyperpolarization (6×10^{-8} A for 60 sec). This curve appears to join the initial curve at its lower end, but this effect is at least partly attributable to the progressive recovery during the observations. The upper three

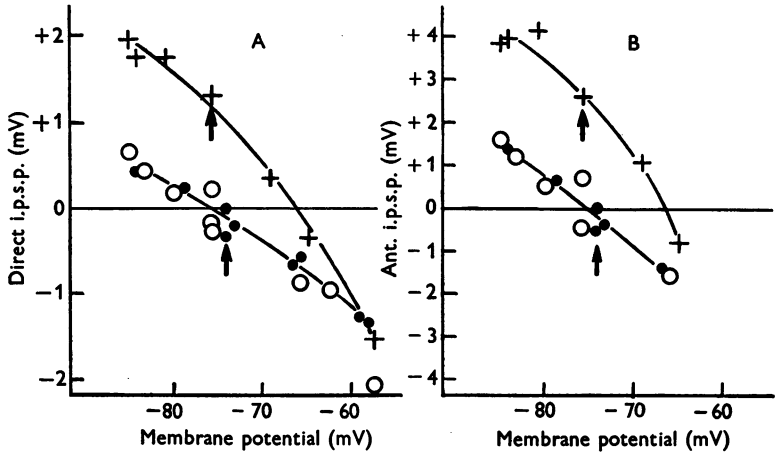

Fig. 6. A. Plot as in Fig. 2 of direct i.p.s.p.'s against membrane potential, but with a double microelectrode filled with 3M-KCl. Points designated ● were obtained before and + at 10–35 sec after passage of a hyperpolarizing current of 6×10^{-8} A for 60 sec. Finally, ○ points plot records at 2–2½ min after the current. Arrows indicate mean resting potentials. B. As in A, but for antidromic i.p.s.p.'s determined simultaneously.

+ points were determined first and the remaining four in serial order therefrom. The points indicated by ○ were obtained at 2–2·5 min after the cessation of the hyperpolarization and agree closely with the initial curve, showing that the recovery was then complete. Fig. 6B gives the simultaneously determined effects on the antidromic i.p.s.p. The series illustrated in Figs. 4 and 6 show typically that, immediately after the passage of a hyperpolarizing current through an electrode filled with chloride, the i.p.s.p./membrane potential curve was displaced upwards and to the right so that the reversal-potential for the i.p.s.p. was lowered, for example from −76 to −66·5 mV in Fig. 6 (cf. also Fig. 17C).

The effects of varying the duration of a hyperpolarizing current are illustrated in Fig. 7, in which the size of the i.p.s.p. is plotted before, during and after the currents. The small changes that occurred in the resting potential have been neglected in plotting the curves, but Fig. 4 B, C shows that the time courses of change of the i.p.s.p. would not be significantly distorted thereby. During the longest hyperpolarizing current (55 sec), the time course resembles that of Fig. 5 A, the curve flattening to a plateau in its later stages. On cessation of the current the consequent fall in membrane potential caused a sudden decrease in the i.p.s.p. (cf. Fig. 4 C, D) and thereafter there was the slow exponential decline as in Fig. 5 A. Similar features were observed for the briefer hyperpolarizing currents, but there was not then time for the full change in the i.p.s.p.

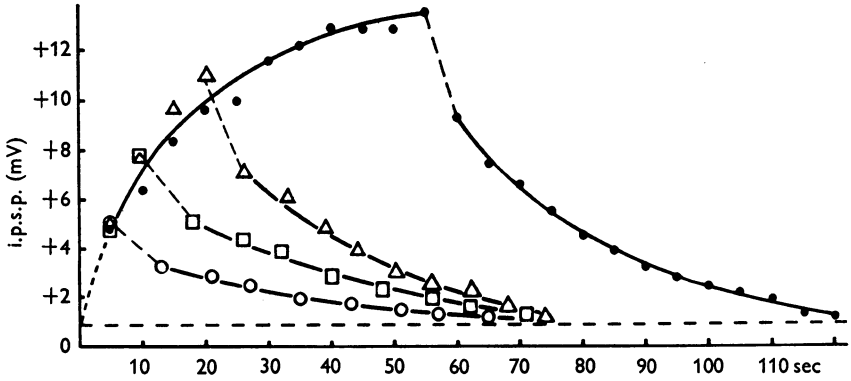

Fig. 7. Double microelectrode filled with 3 M-KCl inserted into gastrocnemius motoneurone. I.p.s.p.'s are plotted as ordinates during and after hyperpolarizing currents of 5×10^{-8} A for 5, 10, 20 and 55 sec duration, as shown by the respective symbols ○, □, △ and ●. Note the increase in the depolarizing i.p.s.p. during the current as in Fig. 5 A, and the approximately exponential decline thereafter. At onset and cessation of the currents the curve is shown as a broken line because it is partly due to the effect of the changed membrane potential on the i.p.s.p. The horizontal broken line corresponds to the small initial depolarizing i.p.s.p.

All types of i.p.s.p. have been similarly affected when the chloride content of the motoneurone was increased. For example, comparison of Fig. 6 A and B shows that there was virtually the same change in the inhibitory potentials produced either by direct inhibition or by antidromic inhibition. The results plotted in Fig. 7 were obtained with a polysynaptic inhibitory potential that was produced in a gastrocnemius motoneurone by an afferent volley in quadriceps nerve that included group I*b* and group II impulses.

The rapid and complete reversibility of the effects illustrated in Figs. 3–7 has not been observed with all motoneurones. In the earliest experiments (cf. Coombs *et al.* 1953) reversibility was rarely observed, there being generally a cumulative effect of successive hyperpolarizing currents, and often, too, a continuous drift to more depolarizing i.p.s.p. responses even when no current was

applied. In our more recent experiments such effects were observed only with badly injured cells that were rapidly deteriorating. One can be certain that the cells giving the reversible reactions of Figs. 3–7 were in a much better physiological condition than cells whose reactions to a comparable disturbance were irreversible. It seems likely that the improved physiological condition of the cells in our more recent experiments is attributable to the technical improvements which have reduced mechanical damage (Eccles *et al.* 1954).

Discussion. It would appear that the chloride which was injected into the cell by the hyperpolarizing current has caused the i.p.s.p./membrane potential curve to be displaced so that the reversal potential was at a lower level of membrane potential, though its more general features were retained. This result resembles that produced by diffusion, but may be made much larger and can be graded in intensity (cf. Fig. 7). In general, these effects may be explained in the same way as the comparable effects that have been attributed to diffusion of chloride ions. However, it is necessary to determine the effects which other ions have on the i.p.s.p. before attempting to develop any quantitative explanations of the changes produced in the i.p.s.p. by increased Cl^- ion concentrations. Nevertheless, the time course of the changes occurring during and after the passage of a hyperpolarizing current (Figs. 5 and 7) may profitably be considered at this stage, though a full treatment must await the final Discussion (§ B).

During the passage of the hyperpolarizing current it has been shown in Appendix B that the intracellular Cl^- concentration will increase exponentially to a level which would be much higher than the initial level. For example, a hyperpolarizing current of 5×10^{-8} A would be expected to increase the concentration by about 20 mM, the increase occurring with a time constant probably briefer than 20 sec. Changes of this magnitude and time course would account satisfactorily for the observed changes in the i.p.s.p. in Figs. 5 and 7. Likewise, on cessation of the hyperpolarizing current, the recovery towards the initial i.p.s.p. response may be explained by considerations of ion flux. The high intracellular concentration of Cl^- ion would be expected to cause a net outward flux of Cl^- ions across the membrane. Provided that the rate of diffusion out of the microelectrode has remained constant, and that the cell has remained in an unchanged condition, there should be a complete recovery to the initial i.p.s.p. response, as is indicated in Figs. 5 and 7.

(2) *Sulphate- or phosphate-filled microelectrode*

When even a large hyperpolarizing current was applied through a sulphate-filled electrode, there was no significant change in the i.p.s.p. (Fig. 8A, C). The e.p.s.p. was also virtually unaffected (Fig. 8B, D), while the resting membrane potential was a little depressed (-84 to -75 mV) and later recovered. A more complete test is illustrated in Fig. 8E, where the i.p.s.p. was determined over a wide range of membrane potentials before and after a

hyperpolarizing current of 5×10^{-8} A for 90 sec. Points ⊕ determined as early as 5 and 10 sec after the end of the hyperpolarizing current are seen to lie close to the curve determined before the current. If there was any change, it was in the reverse direction from that produced by the monovalent anions considered above. The membrane potential fell from −64 to −61 mV, and there was no recovery. A similar absence of significant change in the i.p.s.p. has been observed after each of the eleven hyperpolarizing currents that have been passed into six different cells. Currents of 4 to 8×10^{-8} A have usually

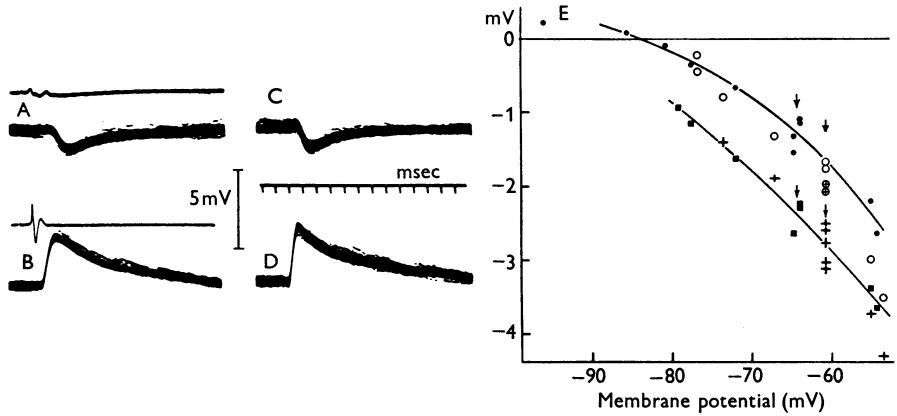

Fig. 8. A and B show respectively the direct i.p.s.p. and the monosynaptic e.p.s.p. set up in a biceps-semitendinosus motoneurone by the appropriate afferent volleys. Surface records from L7 dorsal root are also shown, negativity being downwards. C and D show the respective responses after passage of a hyperpolarizing current (12×10^{-8} A for 90 sec) through an electrode filled with 1·6 M-$[(CH_3)_4N]_2SO_4$. A small spike-like local response is superimposed on the e.p.s.p. of record D, being produced probably on account of the fall in resting potential. E. Points designated ● show the i.p.s.p.'s at various membrane potentials as in Fig. 2A, but with a double microelectrode filled with 0·6 M-K_2SO_4. Points designated ○ show that there was little change after the passage of a hyperpolarizing current (5×10^{-8} A for 90 sec). The first two records after the current are designated ⊕. Similarly, there was little change in the positive after-potential, points designated ■ being before and + after the same hyperpolarizing current. Arrows indicate mean resting potentials which were 3 mV lower after current. Curves are drawn through the initial series of records, ● and ■ respectively.

been applied for 1–3 min. Usually there has been a small fall in resting potential as in Fig. 8, and sometimes recovery occurred. It has been immaterial whether the cation in the electrode has been K^+ as in Fig. 8E or $(CH_3)_4N^+$ as in Fig. 8A, C. Fig. 8E also shows that after the current there was no change in the hyperpolarizing after-potential over a wide range of membrane potentials.

Similarly, when hyperpolarizing currents have been applied through an electrode filled with equimolar concentrations of K_2HPO_4 and KH_2PO_4 (seven applications of current to four cells), there has been subsequently no significant change in the i.p.s.p. (cf. Coombs *et al.* 1953, fig. 1I, J), or in the

resting potential and the e.p.s.p. Currents of 2 to 10×10^{-8} A have usually been applied for 1 min.

Discussion. This invariable failure of these polyvalent anions to affect the i.p.s.p. when injected by a hyperpolarizing current is paralleled by the absence of any spontaneous change in the i.p.s.p. immediately after the insertion of the microelectrode. For this reason sulphate (or phosphate) electrodes are preferred, and are essential when determining the reversal-potentials for the i.p.s.p. (Figs. 1, 2), and comparing them with the resting membrane potentials and the reversal-potentials for the hyperpolarizing after-potential as in Table 1. The absence of any effect by a hyperpolarizing current applied through an electrode filled with sulphate or phosphate is further of importance because it shows that the changes in the i.p.s.p. described in § C 1 are not consequential on the hyperpolarizing current itself or on osmotic swelling (cf. Appendix, § B), but presumably are due to the injection of chloride.

(3) *Electrodes filled with salts of various monovalent anions*

Among the several anions that have been tested, it has been found that, when hyperpolarizing currents were applied through electrodes filled with potassium nitrate, bromide or thiocyanate, the subsequent changes in the i.p.s.p. were indistinguishable from those observed with a chloride-filled electrode. Though the observations with chloride were of more significance because it is present in high concentration in the environment of the neurone and so might be expected to take part normally in the production of the i.p.s.p., tests with a number of other anion species were of interest in an attempt to investigate further the postulated permeability change in the inhibitory post-synaptic membrane. Instead of reporting the intensity and duration of the hyperpolarizing currents, it will be convenient henceforth to report merely the quantity of anions so injected (6 p-equiv being injected by 10^{-8} A flowing for 60 sec), for it is now justifiable to assume that hyperpolarizing currents produce the observed changes in the i.p.s.p. by means of anion injection. With the readily diffusible anions such as Cl^-, Br^-, NO_3^-, SCN^-, much of the quantity injected leaks out across the surface membrane: so that, after currents have flowed for 60 sec, they do little more than maintain the raised intracellular concentration (cf. Appendix, § B). This complication seriously limits the significance of the specification of the quantity injected.

As shown typically in Fig. 9 E, F, after the injection of about 18 p-equiv of NO_3^- ions by passage of a hyperpolarizing current through a KNO_3-filled electrode, the i.p.s.p. was transformed from a small depolarizing to a large depolarizing response which evoked the discharge of an impulse at just the same threshold depolarization (15 mV) as the e.p.s.p. (compare Fig. 9 F and G with L). It may be noted in passing that the injection caused no significant change in the e.p.s.p. (Fig. 9 L), or in the threshold at which it set up an impulse.

The series from F to K shows the partial recovery of the i.p.s.p. towards the initial response (compare Fig. 9K with E). The resting membrane potential showed the characteristic depression immediately after the hyperpolarizing current and a slow recovery thereafter, much as in Fig. 5B. The time course of the recovery from the large depolarizing response (Fig. 10A) closely resembled that observed for chloride (Fig. 5A). Altogether there were fifteen injections of NO_3^- ions into five motoneurones, and virtually the same result was observed in all. The usual injection rates varied from 18 to 42 p-equiv a minute and were

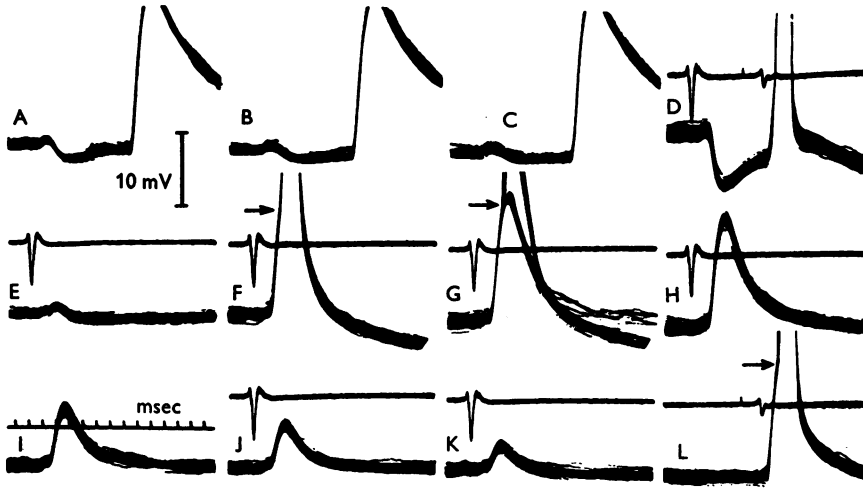

Fig. 9. Intracellularly recorded potentials from biceps-semitendinosus motoneurone with a single microelectrode filled with 3 M-KNO_3. In A to D a quadriceps group Ia afferent volley set up an i.p.s.p. and a later biceps-semitendinosus volley set up an e.p.s.p. Note in D–H and J–L the surface-recorded potentials from the L6 dorsal root, negativity being downwards. With D the membrane potential was diminished from 79 to 60 mV, resulting in a large hyperpolarizing i.p.s.p., and the generation of a spike by the e.p.s.p. All spikes are truncated. E shows the i.p.s.p. before and F immediately after the passage of a hyperpolarizing current of 3×10^{-8} A for 60 sec. Note that the large depolarizing i.p.s.p. sets up a spike in F and G, the threshold shown by arrow being about the same as the threshold for the e.p.s.p. (arrow in L). Records G–K show progressive recovery of the i.p.s.p. towards the initial low depolarizing response (E). The potential and time scales apply to all records.

continued for 1–2 min. The half times of recovery varied from 23 to 35 sec (cf. Fig. 10A), and the resting membrane potential was always temporarily depressed, usually by about 5 mV.

Fig. 10B shows typically the similar effects produced by an injection of about 24 p-equiv of Br^- ions by a hyperpolarizing current. The large depolarizing i.p.s.p. returned virtually to the initial value, while the membrane potential was slightly depressed from -70 to -69 mV after the current and then recovered to -75 mV. In contrast there was no appreciable change in the

e.p.s.p. The recovery closely followed an exponential curve with a half-time of about 14 sec. Altogether the effect of Br^- ions on the i.p.s.p. was tested with nine injections into four cells, usually at a rate of 18–24 p-equiv per min for 1–1½ min. With the exception of one cell, into which two injections were made, the effect of injection was reversible as in Fig. 10B, the half-times of recovery varying from 10 to 20 sec.

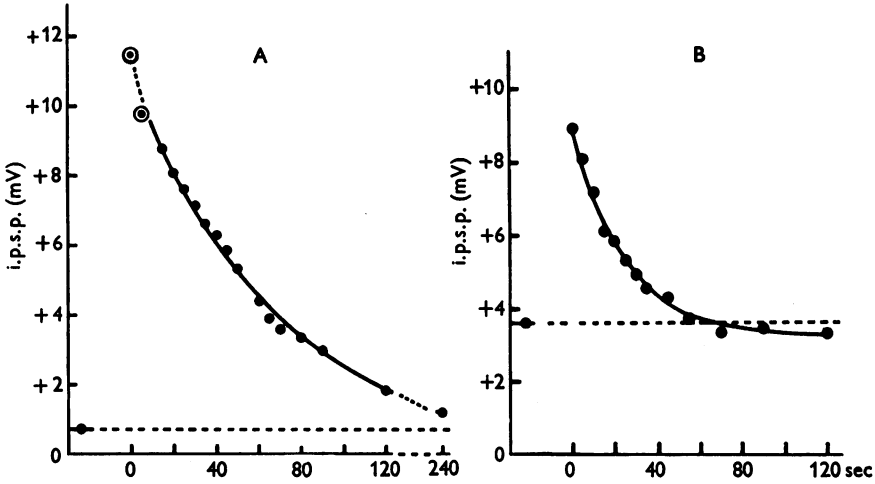

Fig. 10. A. Plot of heights of i.p.s.p.'s, showing time course of recovery after a hyperpolarizing current for series partly shown in Fig. 9. The two points designated ⊙ are the first two responses (cf. Fig. 9F) where the spike obscured the summit, the points being calculated from the slopes of the rising phases. The initial point on the horizontal broken line gives the initial response (Fig. 9E). B. Plot of i.p.s.p.'s as in A, but during recovery after a hyperpolarizing current (4×10^{-8} A for 60 sec) had been applied through an electrode filled with 4M-KBr.

The injection of SCN^- ions also produced similar changes in the i.p.s.p. Altogether ten injections were made into six cells, and a reversible change to the depolarizing i.p.s.p. was observed in all but one cell into which three injections were made. The membrane potential was always depressed by the injection, and recovery occurred with the five cells showing recovery of the i.p.s.p. The half-times of the recovery of the i.p.s.p. fell within the range of 15–20 sec.

As shown in Fig. 11, the injection of even a large quantity of HCO_3^- ions from a $KHCO_3$-filled electrode into a motoneurone did not change the i.p.s.p. to the depolarizing type of response. There was in Fig. 11B, after injection of about 30 p-equiv, a large increase in both types of i.p.s.p. in the hyperpolarizing direction, but this could have been caused by the depression of the resting potential (−66 to −59 mV), which was the usual effect of injections of HCO_3^- ions. As the resting potential recovered, the i.p.s.p.'s returned towards their initial size (cf. Fig. 11C and D with A). The complicating effects of changes in resting

potential were minimal after a second injection of HCO_3^- ions (60 p-equiv given in 1 min) into this cell. The resting potential was even slightly increased, an unusual finding, and correspondingly there was a diminution of both i.p.s.p.'s (cf. Fig. 11 E, F with D). Altogether there were twelve injections of HCO_3^- ions (ranging from 15 to 60 p-equiv) into six motoneurones, and in every case the changes produced in the i.p.s.p. were negligible when allowance was made for the effect of the depression of resting potential. However, our experiments could not exclude the possibility that HCO_3^- ions have a slight action on the i.p.s.p. of the order of one-tenth of that produced by Cl^- ions.

The initial effect of an injection of CH_3COO^- ions from a potassium acetate electrode closely resembled that of HCO_3^- ions, there being always a fall in the

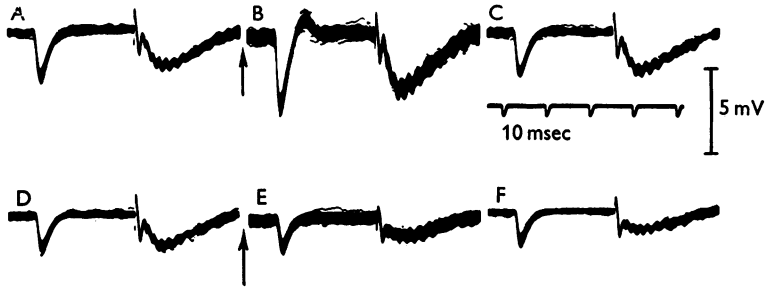

Fig. 11. A shows responses of biceps-semitendinosus motoneurone elicited as in Fig. 1, a direct i.p.s.p. being followed by an antidromic i.p.s.p. B shows increase in the hyperpolarization of the i.p.s.p.'s after the passage of a hyperpolarizing current (5×10^{-8} A for 60 sec) through a microelectrode filled with 2 M-$KHCO_3$. C shows recovery about 1 min later. Later still the responses of D were slightly diminished after the passage of a hyperpolarizing current of 10×10^{-8} A for 60 sec (responses E and F). Injections are indicated by arrows.

resting potential and an increase in the hyperpolarization of the i.p.s.p. as in Fig. 11 B. Membrane potential/i.p.s.p. curves were determined before and after several injections. Fig. 12 shows typically that immediately after the injection of about 60 p-equiv of CH_3COO^- ion there was no significant displacement from the curve obtaining before injection (the two points $\oplus$). However, with the later progressive fall in the resting potential there was also a displacement of the curve upwards and to the right, i.e. in the same direction as was observed after an injection of Cl^- ions. Discrimination is easy, for the acetate displacement developed late and was irreversible, being apparently associated with the progressive fall in resting potential, which indicated a specific injurious action either of acetate ions or of acetic acid. A further injurious action is shown by the blockage of axon-soma transmission that invariably developed after injection of acetate ions and from which recovery was never observed. Altogether eight injections of acetate ions have been made into five cells, and all have conformed to the above pattern, recovery never being observed. It seems that acetate ions do not have any appreciable

action on the i.p.s.p., other than that attributable to a change in membrane potential or to a slowly developing irreversible injury.

In contrast to acetate, motoneurones tolerated injections of glutamate ions very well, the microelectrode being filled with 4 M-mono-potassium glutamate. The resting potential was changed little if at all after any of our seven injections into four cells, for example being depressed from −83 to −80 mV after injection of about 100 p-equiv. Also axon-soma transmission of antidromic impulses and the e.p.s.p. were not affected even by this large dosage. There was always a small and irreversible diminution of the i.p.s.p. by each dosage of glutamate ions. Since the resting potential was usually unchanged, this effect would appear to resemble that of Cl^- ions, but even with a large dosage (100 p-equiv) it was not possible to halve the hyperpolarization of the

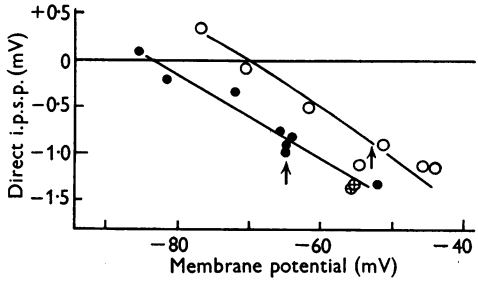

Fig. 12. Points designated ● plot i.p.s.p.'s against membrane potential with a double microelectrode filled with 5 M-potassium acetate. Points designated ○ give records after the passage of a hyperpolarizing current of 4×10^{-8} A for 90 sec. The two records immediately after the current are shown by ⊕. Mean resting potentials before and after the current are shown by the arrows.

i.p.s.p. It may be concluded that the glutamate ion probably shares to a very slight extent the property of changing the i.p.s.p. in the manner of Cl^-, Br^-, NO_3^- and SCN^- ions.

Discussion. Some generalizations may be made at this stage concerning the physico-chemical features which discriminate the anions which affect the i.p.s.p./membrane potential curve from those that do not. Chloride, bromide nitrate and thiocyanate apparently belong to the former group. In fact they are indistinguishable even quantitatively in their effects on the i.p.s.p., though comparison is not precise because it can be made only between the effects produced by injecting different ion species into different motoneurones. These ions are comparable in being monovalent and in having similar mobilities in aqueous solution, which presumably means that the hydrated ions are of similar size. In contrast polyvalent anions, SO_4^{2-} and HPO_4^{2-}, do not have any effect on the i.p.s.p. The individual ions in solution, besides carrying more than one negative charge, are also larger than those in the former group. When anions are injected from the potassium phosphate electrode with a

hyperpolarizing current, they will consist in part of singly charged $H_2PO_4^-$ ions. Nevertheless, no effect was obtained from such an injection. Furthermore, injections of HCO_3^-, CH_3COO^- and glutamate$^-$ were also without effect or had only a very slight effect. As judged by their mobilities these anions are distinct from the Cl^-, Br^-, NO_3^-, SCN^- group in having considerably greater hydrated size. Thus a common criterion determining whether a given type of anion will modify the i.p.s.p. appears to be ion size (cf. Discussion, § A). Before discussing further the significance of ion size, it is necessary to discover if any cations are also specifically concerned in producing the i.p.s.p. Injections of cations into motoneurones have been effected by depolarizing currents.

D. *Effects produced on the i.p.s.p. by a depolarizing current*

(1) *Effects attributable to cations*

Initially it will be convenient to describe the effects of depolarizing current flow on the i.p.s.p. when the microelectrode is filled with a salt solution containing an indifferent anion, e.g. sulphate, because the effect of the current on the background diffusion of anions out of the electrode will not then have a complicating effect on the i.p.s.p. (cf. § C 2). Since the principal interest of this investigation is to discover if any cations are specifically concerned in the production of the i.p.s.p., it is expedient to describe initially experiments in which large changes were produced in the cationic content of the motoneurone, i.e. when the depolarizing current was passed through electrodes filled with sodium sulphate or tetramethylammonium sulphate. Little cationic change can be expected when the depolarizing current is passed through an electrode filled with potassium sulphate (cf. Appendix C). However, the results obtained with such an electrode are important since they provide a control for the effects produced by the ionic flux carrying the current outwards across the neuronal membrane. Necessarily, with our present technique, these important control observations are very imperfect because they are made with different electrodes inserted into different cells.

(*a*) *Electrode filled with sodium sulphate.* Comparison of Fig. 13 with Fig. 1 shows the change produced in both the direct and antidromic i.p.s.p.'s when a depolarizing current of 5×10^{-8} A was passed for 90 sec out of a microelectrode filled with 0·6 M-Na_2SO_4. The resting membrane potential was lowered from -74 to -57 mV, and at the same time the i.p.s.p. was converted from a hyperpolarizing into a depolarizing type (compare Fig. 1 D with Fig. 13 D), i.e. there was, as shown by the plotted curve (+ points, Fig. 14 A), a large displacement upwards and to the right of the i.p.s.p./membrane potential curve, the reversal-potential changing from -80 mV to -35 mV. The effects produced by the depolarizing current thus resembled those arising from injections of Cl^- ions (compare Fig. 14 A with Fig. 6 A). The change in i.p.s.p. after a depolarizing

current was always transient, regression to the initial condition occurring within a few minutes. For example, the family of curves of Fig. 14A (cf. the i.p.s.p. records of Fig. 13A–E and K–O) and the plotted reversal potentials (▲ points of Fig. 14D) show the approximate time course of this regression, complete recovery having occurred in about 9 min. The antidromic i.p.s.p. was similarly affected and recovered similarly (Figs. 13F–J and P–T; 14B). After a much longer depolarizing current (5×10^{-8} A for 300 sec), there was a much larger change in the i.p.s.p., and regression followed a slower time course; yet,

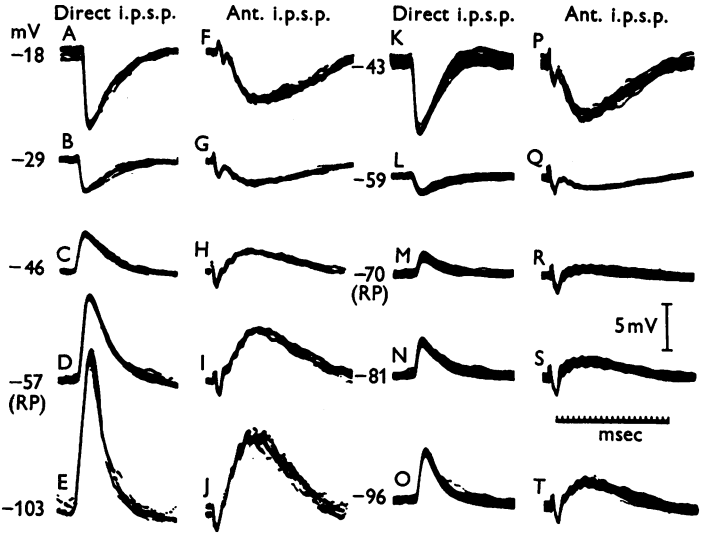

Fig. 13. A–E show records at the indicated membrane potentials of the same i.p.s.p. response in the same motoneurone as in Fig. 1A–G, but immediately (5–40 sec) after the passage of a depolarizing current, 5×10^{-8} A for 90 sec, the resting potential being −57 mV. Records F–J show simultaneously recorded antidromic i.p.s.p.'s (cf. Fig. 1H–N). K–O and P–T as in A–E and F–J respectively, but at different membrane potentials as indicated, and showing partial recovery at 180–230 sec, the resting potential being then −70 mV. The potential and time scales apply to all records. Electrode filled with 0·6 M-Na_2SO_4.

nevertheless, there was again complete recovery within 15 min, as revealed by the family of curves of Fig. 14C, and the plotted reversal-potentials (○ points of Fig. 14D). Similar behaviour was observed with all of the other eight neurones into which a depolarizing current was passed from a Na_2SO_4-filled microelectrode, there being in all seventeen applications of depolarizing current through this type of electrode. Half-recovery times have varied from about 100 to 250 sec for moderate doses of current as in Fig. 14A and B.

The depression of the resting membrane potential after such depolarizing currents is satisfactorily explained by the reduction which would occur in the intracellular potassium (Coombs *et al.* 1955*a*). The recovery of resting potential

followed much the same time course as the reversal-potential for the i.p.s.p. (■ and ● points, Fig. 14D). However this reversal-potential was diminished more than the resting potential; hence, immediately after cessation of the depolarizing current (Fig. 13D) there was a depolarizing i.p.s.p. at the resting

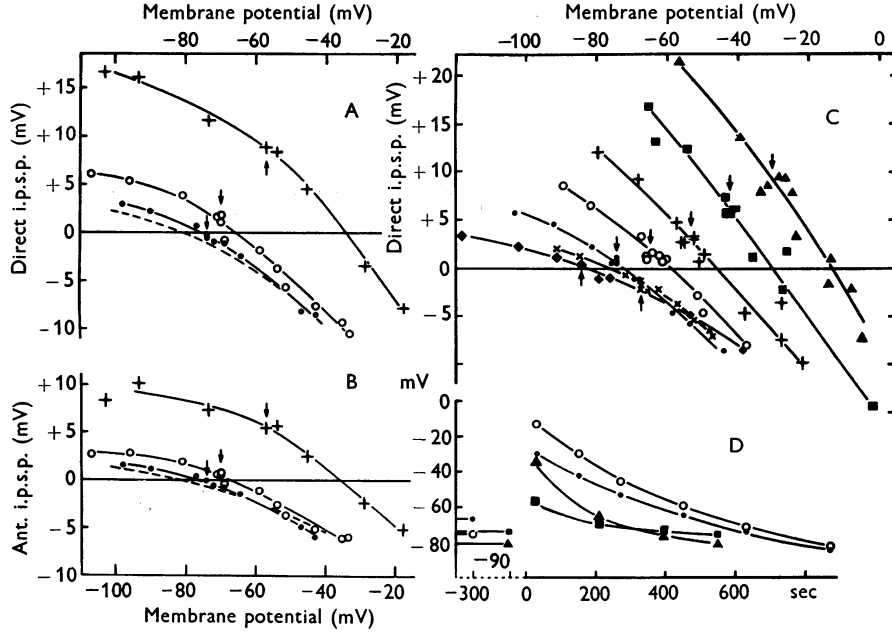

Fig. 14. A. Points designated + and ○ plot the series partly shown in Fig. 13 A–E and K–O, respectively, on the same co-ordinates as Fig. 2A. Points ● show a further stage of recovery at 360 to 430 sec after the passage of the depolarizing current. The broken line shows the curve of Fig. 2A which was obtained for this motoneurone both before the depolarizing current and after complete recovery at 510–580 sec. Note that compared with Fig. 2A the ordinate scale is halved relative to the abscissal scale. Arrows indicate respective resting potentials. B. As in Fig. 14A, but for antidromic inhibitory potentials that are partly illustrated in Fig. 13F–J and P–T. The broken line shows curve of Fig. 2B, but at half the ordinate scale as in Fig. 14A. C. Same motoneurone and i.p.s.p.'s as in Fig. 14A, but showing recovery after the passage of a much longer depolarizing current (5×10^{-8} A for 300 sec). The curve through the × points is the control curve before the current with the arrow showing resting potential of −67 mV, while immediately after the current (5–60 sec) the curve indicated by ▲ points was obtained. Progressive recovery is shown by the successive groups of records at 120–180 (■), 240–300 (+), 420–480 (○), 600–660 (●) and 840–900 (◆) sec respectively. Note that as shown by the arrows the resting potential had increased at 14–15 min to a value (−79 mV) considerably in excess of the initial value (−67 mV). D. Plot of recovery after depolarizing currents of responses of Fig. 14A and C. Zero on abscissal time scale gives instant of cessation of the current, the points to the left thereof giving the initial control values before the currents of 90 and 300 sec duration (note scale readings of −90 and −300 sec). Points indicated by ▲ and ■ plot respectively, the reversal potentials and the resting potentials for the series of Fig. 14A after a 90 sec current, while ○ and ● give the corresponding values for the series of Fig. 14C after a 300 sec current.

membrane potential then obtaining. After 10 min the resting potential had recovered to a value considerably above its initial level (Fig. 14C, D), as already described by Coombs *et al.* (1955*a*); hence the i.p.s.p. at the resting potential continued to be a depolarizing response even after 15 min (cf. Fig. 14C).

(*b*) *Electrode filled with tetramethylammonium sulphate.* With tetramethylammonium sulphate electrodes we have observed that a depolarizing current caused the same change in the i.p.s.p. as with Na_2SO_4-filled electrodes, as is shown by the family of curves plotted in Fig. 15A. The curves have been shifted to the right and become steeper with each successive current, and the magnitude of the shift has been of the same order as for Na_2SO_4-filled electrodes.

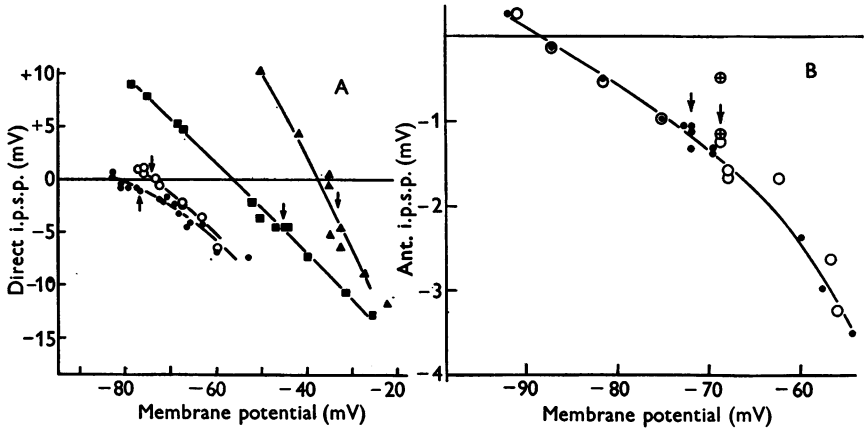

Fig. 15. A. A double microelectrode filled with 1·6M-tetramethylammonium sulphate was inserted into a biceps-semitendinosus motoneurone. Points shown as ● plot direct i.p.s.p. against membrane potential as in Fig. 2A. After a depolarizing current of 3×10^{-8} A for 60 sec the points are shown as ○, while ■ points give records after a repetition of this current and ▲ after a still further repetition. Arrows indicate mean resting potentials. B. A double microelectrode filled with 0·6M-K_2SO_4 was inserted into an unidentified motoneurone. Points shown as ● plot antidromic i.p.s.p. against membrane potential as in Fig. 2B. After a depolarizing current of 10×10^{-8} A for 90 sec the records are plotted as ○. The two records immediately following the current are shown by ⊕, the first being high above the control curve and the second showing a considerable recovery at about 15 sec. Arrows marking the resting potentials show that it was depressed by about 3 mV after the current.

However, an important difference has been that recovery towards the initial condition either failed altogether or at best was slow and incomplete. It has already been reported that the membrane potential has been depressed after each depolarizing current with a tetramethylammonium electrode and has recovered very slowly, if at all, the effects of successive currents being cumulative as shown by the arrows in Fig. 15A (Coombs *et al.* 1955*a*). This was attributed to the difficulty of eliminating the injected $(CH_3)_4N^+$ ion, and hence of

recovering the lost potassium. With the tetramethylammonium injection the neurone also finds it difficult to reverse the ionic shifts across the membrane that displace the i.p.s.p. response in the depolarizing direction. Thus it seems likely that both effects are attributable to the same ionic shifts.

(*c*) *Electrode filled with potassium sulphate.* When the microelectrode was securely implanted in the cell, the passage of a depolarizing current through a potassium sulphate electrode caused little or no change in the resting potential (cf. Coombs *et al.* 1955*a*). As a rule there was a brief displacement of the i.p.s.p. in the depolarizing direction, but this displacement was much smaller than with the sodium sulphate electrode and the time course of recovery was much faster, the half-time being usually less than 20 sec (cf. Fig. 15B). As a further example, a relatively large current of 7×10^{-8} A for 90 sec only shifted the reversal-potential for the i.p.s.p. from -88 to -82 mV. With repetition of this current, the reversal-potential was further depressed to -76 mV. In another experiment depolarizing currents were passed five times into one cell and caused no appreciable change in the i.p.s.p. even when they were as large as 15×10^{-8} A for 2 min.

(*d*) *Discussion.* The question now arises: Why does a depolarizing current have a very much larger and more prolonged effect on the i.p.s.p. when it is applied through a Na_2SO_4- or $((CH_3)_4N)_2SO_4$-filled electrode than when applied through a K_2SO_4-filled electrode? One can immediately eliminate any suggestion that it is a direct result of the injected cations, Na^+ or $(CH_3)_4N^+$. If the i.p.s.p. were caused in part by the increased movement of such cations across the neuronal membrane, a raised intracellular concentration would cause an increased outward flux during the i.p.s.p.; hence there would be an increased hyperpolarization, not the diminution or reversal actually observed. On the contrary the observed change in the i.p.s.p. could be caused by diminution of the concentration of a cation species that was specially concerned in the generation of the i.p.s.p. Thus the effect of the Na^+ and $(CH_3)_4N^+$ injections on the i.p.s.p. would be due not to any specific effect of these ions, but rather to the diminution of K^+ ions, which is an inevitable consequence of such injections (cf. Appendix, § C). It is therefore postulated that, under the influence of inhibitory impulses, the inhibitory post-synaptic membrane becomes highly permeable to K^+ ions as well as to the anions of similar small size (cf. Results, § C).

In part, the postulated permeability change of the membrane resembles that postulated to occur during the positive after-potential that follows a spike (cf. Coombs *et al.* 1955*a*). However, in this latter condition the high permeability was restricted to K^+ ions, whereas in the former it would be shared with the small anions. As a consequence of this complication, careful controls have to be made with regard to the effect on the i.p.s.p. that would be produced by the influx of Cl^- ions across the neuronal membrane. A considerable

part (up to 40%) of the depolarizing current used to inject the cations from the microelectrode is probably carried across the neuronal membrane by the influx of Cl^- ions. Experiments in which a depolarizing current is passed through a K_2SO_4-filled electrode provide a partial control for the effect of this Cl^- influx. With such an electrode a depolarizing current will also produce a considerable increase in the intracellular Cl^- ion concentration, but there will be virtually no change in the intracellular K^+ ion concentration (cf. Appendix, § C). However, the control is defective in that the Cl^- increase is likely to be much smaller than with the passage of the same current through Na_2SO_4- or $((CH_3)_4N)_2SO_4$-filled electrodes, which causes the readily penetrating K^+ ions to be replaced by less penetrating cations, with the consequence that a greater proportion of the current through the membrane is carried by the inward passage of Cl^- ions (cf. Appendix, § C).

On the basis of these expected differences it is possible to account for the respective changes observed in the i.p.s.p. in terms of the hypothesis that the i.p.s.p. is caused by the ionic fluxes consequent on a greatly increased permeability of the membrane to K^+ ions as well as to Cl^- ions. A hyperpolarizing i.p.s.p. would be produced by an inward movement of Cl^- ions and/or an outward movement of K^+ ions, while the inverse movements would give a depolarizing i.p.s.p. With the depolarizing current applied through Na_2SO_4- and $((CH_3)_4N)_2SO_4$-filled electrodes, both the increase in Cl^- and the decrease in K^+ ion concentration would contribute to the displacement of the i.p.s.p. in the depolarizing direction (Figs. 14, 15 A). On the other hand, with the K_2SO_4-filled electrode the displacement would be much smaller (cf. Fig. 15 B) because there would be less increase in the Cl^- and virtually no change in the K^+ ion concentration. With the Na_2SO_4-filled electrode the recovery to the initial condition would be attributable both to the outward pumping of Na^+ and consequent replenishment of K^+ ions, which in part at least is coupled with the Na^+ efflux (cf. Hodgkin & Keynes, 1954; Keynes, 1954), and to the net loss of Cl^- ions by diffusion outwards along their electro-chemical gradient. The slow and incomplete recovery with the $((CH_3)_4N)_2SO_4$-filled electrode presumably would be attributable to the difficulty in eliminating the $(CH_3)_4N^+$ ion from the cell interior and hence in regaining the lost K^+ ions. Some recovery would be expected while the raised Cl^- ion concentration was being lowered by outward leakage across the membrane. In contrast, the recovery of the i.p.s.p. after passage of a depolarizing current through a K_2SO_4-filled electrode was very rapid (cf. Fig. 15 B), which is to be expected because it involves only the outward diffusion of Cl^- ions.

Despite the sufficiency of these explanations the results on cation replacement are not so convincing as those involving anion injection. To a considerable extent the difficulty in investigating this problem arises from the fact that initially there is a high internal K^+ concentration. It is thus much easier

to produce a *relative* change in chloride concentration than a relative change in potassium concentration, and furthermore the osmotic influx of water frustrates any attempt to increase the intracellular potassium concentration (Appendix, § C). In principle, a better approach would be to change the extracellular, rather than the intracellular, potassium concentration, as has been done for the isolated crustacean neuromuscular and mammalian cardiac muscle preparations, where inhibition also occurs (Fatt & Katz, 1953; Burgen & Terroux, 1953). However, on account of technical difficulties, the few preliminary experiments have not yielded any significant results.

(2) *Effects attributable to anions*

When a depolarizing current was passed through a microelectrode filled with a potassium salt, there would be virtually no change in the cation content of the motoneurone (Appendix, § C). Hence any change in the i.p.s.p. would presumably be caused by changes in the anions. Experiments with the three potassium salts, KCl, KBr, and KNO_3 gave indistinguishable results, hence they will be considered together. The effects of depolarizing currents have not been investigated with a KSCN-filled electrode.

(*a*) *Electrodes filled with potassium chloride, nitrate and bromide.* In most of our experiments with these salts (sixteen injections into six cells) the i.p.s.p. had already been transformed into the depolarizing type by diffusion (cf. § B). After the passage of the depolarizing current there was then invariably a large diminution in the depolarizing i.p.s.p. and even a momentary reversal to a hyperpolarizing type (Fig. 16 A, B), but the effect was always transient, recovery rapidly occurring in the direction of the initial depolarizing i.p.s.p. (Fig. 16 C–F). The e.p.s.p. was unaffected (Fig. 16 G, H). As shown in Figs. 16 I, 17 A, B, the recovery curves for the i.p.s.p. following depolarizing currents were exponential in type and approximately mirror images of the recovery curves already described following hyperpolarizing currents (cf. Figs. 7, 10). The half-times of recovery have varied from 10 to 26 sec. At times there has been a small 'overshooting' of the recovery curve (cf. Fig. 16 I, 17 A), i.e. the i.p.s.p. was finally displaced further in the depolarizing direction than it had been initially. The resting potential was usually increased just after the current. It was never decreased. The maximum increase was 10 mV, but usually it was much less, about 2 mV. It returned with much the same time course as the change in i.p.s.p. Thus, for a constant membrane potential, the change in the i.p.s.p. would have been somewhat greater than appears in Figs. 16 and 17.

When diffusion out of the microelectrode was so small that the i.p.s.p. was not transformed into the depolarizing type, a depolarizing current invariably caused very little change in the i.p.s.p. (five injections into three cells). For example, there was possibly a slight shift in the hyperpolarizing direction with

the records of Fig. 17 C (○ points) immediately after a depolarizing current of 6×10^{-8} A for 60 sec, but recovery had occurred in a few seconds, as may also be seen with the ● points of Fig. 17 D. This contrasts with the effect seen after passing a hyperpolarizing current through the electrode (points designated ■ in Fig. 17 C and ○ in Fig. 17 D). The displacement of the i.p.s.p. in the hyperpolarizing direction is seen to be negligible when compared with the opposite displacement by a hyperpolarizing current of equal strength and duration. Fig. 17 C and D thus contrast with Figs. 16 I and 17 A, B. Furthermore, in the experiments illustrated by Fig. 17 C and D the resting potential was increased very little, if at all, just after the depolarizing current.

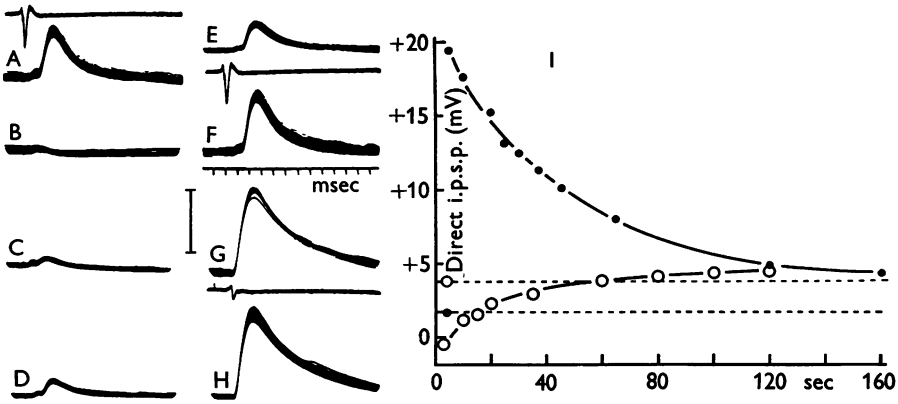

Fig. 16. A–H. A single microelectrode filled with 3 M-KNO_3 was inserted into a biceps-semitendinosus motoneurone. A shows the depolarizing i.p.s.p. set up by a quadriceps group I*a* volley. After the passage of a depolarizing current (4×10^{-8} A for 90 sec) there was a momentary reversal of the i.p.s.p. to the hyperpolarizing type (B) followed by recovery to the original response (C–F). G and H show e.p.s.p.'s set up by monosynaptic excitation before and immediately after the same depolarizing current. Records of the quadriceps afferent volley in L 6 dorsal root are shown in A and F, while the biceps-semitendinosus afferent volley similarly recorded is seen between G and H. The resting potential was −79 mV before passage of current and −82 mV immediately after. Potential scale gives 5 mV for records A and F, and 10 mV for all other records. Time scale applies to all records. I. Points shown as ○ plot recovery of i.p.s.p.'s for series partly illustrated in Fig. 16 B–F, the initial depolarizing i.p.s.p. (Fig. 16 A) being shown by ○ on the broken line, which thus represents a base-line. Time is measured from cessation of current. Points shown as ● represent recovery series for the i.p.s.p. after the earlier passage of a hyperpolarizing current (3×10^{-8} A for 90 sec), the initial value for the i.p.s.p. again having a base-line drawn through it (cf. Fig. 10).

(*b*) *Electrodes filled with sodium chloride.* With a NaCl-filled microelectrode a depolarizing current has always produced the same changes in the i.p.s.p., and the resting potential, as with a Na_2SO_4-filled electrode. There was a reversal of the i.p.s.p. to the depolarizing type, if it had not already been reversed by the diffusion of Cl^- ions out of the electrode (§ B), or, in the event

of prior reversal, a further increase in the size of the depolarizing i.p.s.p. After the passage of the current there has often been recovery towards the initial condition.

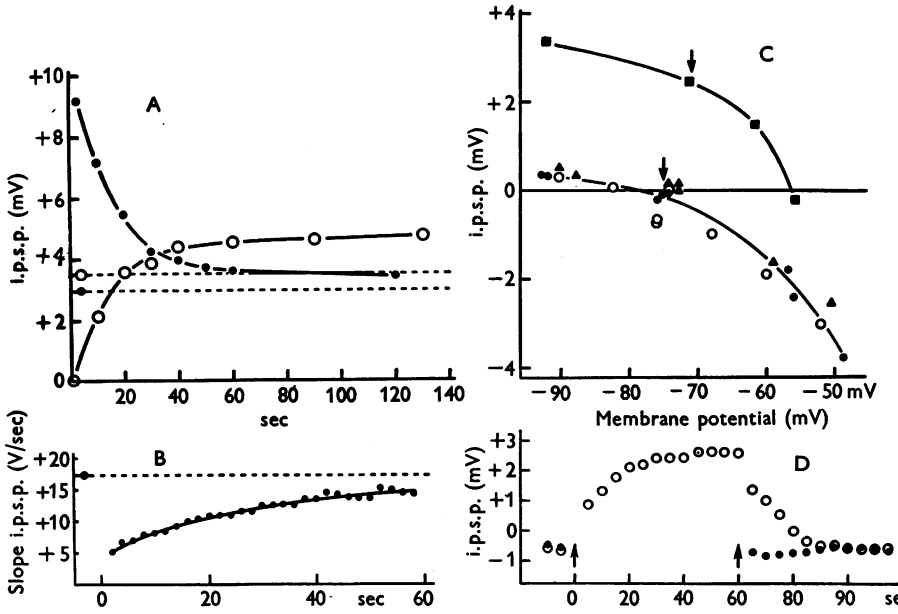

Fig. 17. A. Plotted curves as in Fig. 16I, but for a single microelectrode filled with 4M-KBr in a biceps-semitendinosus motoneurone. Points shown as ● and ○ plot respectively the recovery of i.p.s.p.'s after a hyperpolarizing and a depolarizing current (both 4×10^{-8} A for 60 sec). The initial responses and base-lines are shown as in Fig. 16I. B. Plotted curve as in Fig. 16I, but for a double microelectrode filled with 3M-KCl and inserted into a biceps-semitendinosus motoneurone. Zero time corresponds to cessation of a depolarizing current of 6×10^{-8} A for 60 sec. On the ordinate scale are plotted the steepest slopes of rising phases of the direct i.p.s.p.'s. C. A double microelectrode filled with 3M-KCl was inserted into a gastrocnemius motoneurone. Points shown as ● plot the antidromic i.p.s.p.'s against the membrane potentials as in Fig. 2B. ■ points plot four records obtained within 15 sec after cessation of a hyperpolarizing current (6×10^{-8} A for 60 sec). The arrows indicate the mean resting potential and show a small depression after the current. Points designated ▲ show that recovery was almost complete at about 60 sec after the current. After a further 60 sec, in order to ensure complete recovery, a depolarizing current (6×10^{-8} A for 60 sec) was passed through the electrode and the points designated as ○ plot the records just after this current. There was little change in the curve and no detectable change in the resting potential. D. A double microelectrode filled with 3M-KCl was inserted into a biceps-semitendinosus motoneurone. Points designated ○ plot the antidromic i.p.s.p.'s before, during and after a hyperpolarizing current of 5×10^{-8} A for 60 sec plotted as in Fig. 7, but for an i.p.s.p. that was initially of hyperpolarizing type. Points designated ● give the antidromic i.p.s.p.'s before and after a depolarizing current of 5×10^{-8} A for 60 sec. No records were possible during this current because the antidromic impulse then invaded the motoneurone. Arrows mark the onset and cessation of the currents.

(*c*) *Discussion.* The effects produced when a depolarizing current is passed through an electrode filled with KCl, KBr or KNO_3 solution can be satisfactorily explained by the diminution or suppression of the anionic diffusion out of the electrode. A steady state will prevail before the application of the current, because the anion concentration in the cell has been raised until the diffusion rate outwards across the membrane equals the steady diffusion rate from the electrode into the cell (cf. § B). During the flow of the depolarizing current there will be diminution or suppression of the diffusion of anions out of the electrode (cf. Appendix, § C); hence the anion concentration of the cell will decline towards its normal level. On cessation of the current there would be an immediate restoration of the anion diffusion out of the electrode, consequently the anion concentration of the cell would increase again towards the steady-state concentration that prevailed before the current. Thus the changes occurring after cessation of the depolarizing current should resemble those occurring initially on insertion of the microelectrode into the cell.

In this manner a satisfactory explanation is provided for the general observation that the faster the diffusion rate out of the electrode (as revealed by the magnitude of the displacement of the i.p.s.p. to the depolarizing type), the larger is the restorative effect produced during a depolarizing current (Figs. 16, 17). Furthermore, it leads to the expectation that, on cessation of the current, recovery to the initial steady state, i.e. towards the depolarizing type of i.p.s.p., would occur with approximately the same exponential time constant that obtains for recovery in the reverse direction after a hyperpolarizing current (cf. Figs. 7, 10, 16, 17). The above explanation takes no account of the Cl^- influx which would occur across the membrane during the depolarizing current (cf. Appendix, § C). Presumably in our experiments this influx has never been larger than the depression of the anion diffusion out of the electrode. When the depolarizing current is passed through an electrode filled with a potassium salt, there will be no significant change in the intracellular potassium concentration. However, with a NaCl-filled electrode, this current will cause, in addition to the suppression of Cl^- diffusion out of the electrode, a depletion of intracellular potassium, and also a larger Cl^- influx across the membrane (cf. Appendix, § C). The observed displacement of the i.p.s.p. in the depolarizing direction indicates that the latter two effects are predominant.

DISCUSSION

A. *Ionic permeabilities of the membrane during the inhibitory post-synaptic potential*

Experimental investigation of the i.p.s.p. at varying membrane potentials in § A led to the assumption that the i.p.s.p. is due to a great increase in the flux of some particular ion or ions across the post-synaptic membrane. The normal

level of the reversal-potential for the i.p.s.p. shows that the total ionic flux conveys no net charge across a membrane which is about 10 mV more hyperpolarized than the resting membrane (Table 1). This means that, if the particular ion species is an anion, its concentration in the neurone will be lower than the concentration giving equilibrium with the external concentration at the resting membrane potential, while, if a cation, it will be higher than this equilibrium concentration. Finally, if more than one ion species is involved, the i.p.s.p. will be the integrated result of the various ionic fluxes across the membrane, and the ratio of extracellular to intracellular concentration for any one species will probably give an equilibrium potential different from that derived for the i.p.s.p. Since the equilibrium potential for the i.p.s.p.

TABLE 2. Ion diameters in aqueous solution as derived from limiting ion conductances and expressed relative to $K^+ = 1{\cdot}00$. Values derived from Landolt-Börnstein (1936).

The horizontal broken line gives the division between the small ions that pass readily through the post-synaptic inhibitory membrane and the larger ions that pass with much greater difficulty or not at all.

Cations		Anions	
K^+	1·00	Br^-	0·94
		Cl^-	0·96
		NO_3^-	1·03
		SCN^-	1·11
Na^+	1·47	HCO_3^-	1·65
$N(CH_3)_4^+$	1·60	$CH_3CO_2^-$	1·80
		SO_4^{2-}	1·84
		$H_2PO_4^-$	2·04
		HPO_4^{2-}	2·58

is at a high level of internal negativity (about -80 mV), the ion species concerned must be so distributed that if they are anions there is a high ratio of extracellular to intracellular concentration, while if cations there would be an inverse distribution.

In order to explain the changes produced in the i.p.s.p. by the injection of various ions, it has been postulated (§§ C1, C3, D1*d*) that, when an impulse reaches an inhibitory synapse, it acts on the post-synaptic membrane (presumably by an inhibitory transmitter substance) and greatly increases its permeability to the monovalent anions, Cl^-, Br^-, NO_3^-, SCN^-, and also to the cation, K^+. On the other hand, there must be little or no change in the permeability to the anions SO_4^{2-}, HPO_4^{2-}, $H_2PO_4^-$, HCO_3^-, $CH_3CO_2^-$ and glutamate, and to the cations, Na^+ and $(CH_3)_4N^+$. In Table 2 these anions and cations have been arranged in order of their diameter in aqueous solution, as calculated according to Stokes's law from the limiting ion conductances (cf. Boyle & Conway, 1941). It will be seen that a greatly increased permeability has been postulated for all ions having diameters not exceeding 1·11 times that of K^+ ions, irrespective of their charge, while little or no increase in permeability has been postulated for the larger ions ranging from Na^+ (1·47 times the

diameter of K^+ ions) upwards. Thus the production of the i.p.s.p. may simply be due to the inhibitory transmitter substance acting on the post-synaptic membrane to make it a very selective ionic sieve, all ions smaller than a critical size passing through readily, i.e. those above the horizontal line in Table 2, while larger ions virtually fail to pass. In particular the hyperpolarization of the i.p.s.p. could be produced only if there is a very effective blocking of Na^+ ions. Further experiments with ions of relative diameters ranging from 1·1 to 1·6 are necessary in order to find the critical size.

This postulated sieve-like action of the cell membrane towards ions in aqueous solution resembles that proposed by Boyle & Conway (1941) for the surface membrane of muscle fibres, in that the permeability is determined by the size of the hydrated ion and not by its charge. As stated in § D 1*d* the evidence for high potassium permeability during the i.p.s.p. is not so convincing as for the high anion permeability; however, the close analogy with Boyle & Conway's sieve theory supports our interpretation of the experimental evidence. Further indirect support is provided by the evidence that there is a selective increase in the membrane permeability to K^+ ions during inhibitory responses of crustacean muscle (Fatt & Katz, 1953) and of mammalian cardiac muscle (Burgen & Terroux, 1953). It is probable that during these inhibitory responses there is also an increase in chloride permeability, because, at least with crustacean muscle, the flux of some ion other than potassium must have been responsible for the depolarizing inhibitory potentials that could still be evoked in a potassium-free medium (Fatt & Katz, 1953).

B. *Ionic equilibrium between a motoneurone and its environment*

It has been shown in §§ C 1 and C 3 that the injection of the order of 20–50 p-equiv of Cl^- (or of the comparable anions NO_3^-, Br^- or SCN^-) caused the i.p.s.p./membrane potential curve to shift so that for a given membrane potential the i.p.s.p. was changed to a smaller hyperpolarization or greater depolarization (Figs. 3, 4, 6, 7, 9 and 17 C). Subsequently, the i.p.s.p. curve returned toward its original position with a half-time of about 20 sec (range from 10 to 35 sec for the whole group of anions, Figs. 5, 7, 10, 16 I and 17 A). This regression is attributable to a fall of the Cl^- (or other anion) concentration immediately under the surface membrane of the motoneurone. The most likely explanation of this falling concentration is that the high anion concentration built up within the cell by the injection is reduced as the ion diffuses outward across the membrane.

However an alternative explanation would be that the hyperpolarizing current used for the injection has caused a gradient of Cl^- (or other anion) concentration to be set up within the cell with the concentration highest immediately under the membrane, and that the decrease in concentration indicated by the change of the i.p.s.p. would be due secondarily to diffusion from this region under the membrane to re-establish a uniform concentration within the neurone. This

postulated excess of anion concentration immediately under the membrane is likely to occur, since the transference number for the anion (the fraction of current due to a movement of this ion) will probably be less in the membrane than in the intracellular space. Nevertheless, the changes that were observed to last some tens of seconds cannot be due to this factor. Thus for a sphere of 70 μ diameter and a substance with a diffusion coefficient of $1{\cdot}2 \times 10^{-5}$ cm^2/sec, which are probable values for this case, it can be shown that any initial concentration difference between the surface layer and the remainder of the interior will rapidly diminish, so that within 0·3 sec there is a variation of no more than 10% of the initial difference (cf. Carslaw & Jaeger, 1947, for similar problems on the conduction of heat in solids).

Similar considerations apply to the situation following a depolarizing current as described in § D 2*a*. The i.p.s.p. was temporarily converted towards the hyperpolarizing type from which recovery to the initial condition occurred with approximately the same time constant as recovery in the reverse direction after a hyperpolarizing current (cf. Figs. 16 I, 17 A, B). Thus it appears that, after an applied current has caused either an increase or decrease in the intracellular concentration of Cl^-, NO_3^-, or Br^- ions, diffusion processes across the surface membrane cause a restoration of the initial concentration. Because of the high ionic concentration in the microelectrode there would be in the absence of current a steady output of anions (and cations) by diffusion. The initial steady-state condition would be restored as the net outward anionic flux across the surface membrane falls or rises to equal the output from the electrode. Thus it would be expected that the time constant of recovery, whose mean value is about 30 sec (derived from the mean half-time of about 20 sec), is dependent on the properties of the cell and not of the electrode, and that it would be the same in both directions, as is actually observed (Figs. 16 I, 17 A, B). For present purposes, no serious error is introduced by assuming that, when the microelectrode is filled with a potassium salt, these curves for the i.p.s.p.'s also express changes in the intracellular concentration of the anion species under investigation.

One may now profitably develop theoretical equations to describe the changes in intracellular concentration of such an anion species after the passage of a current through a microelectrode containing the appropriate potassium salt. During the initial steady state before the passage of the current, the anionic flux out of the microelectrode will equal the net outward flux across the surface membrane of the neurone. Suppose now that a hyperpolarizing or depolarizing current is passed through the microelectrode, so increasing or decreasing the quantity of that anion species within the cell. After this current the rate of change of the amount of anion within the cell may be expressed as

$$v\frac{dC}{dt} = -kA\,(C - C_i), \tag{1}$$

where v is the volume of the cell, A is the area of its surface membrane and k is the proportionality constant that at the resting membrane potential relates

the ionic efflux across the membrane to the intracellular concentration, C, the initial steady-state value of which is C_i. k is not a true permeability constant, in that it includes the effect of the electrical potential gradient across the membrane as well as the membrane permeability as strictly defined.

Integrating and rearranging eqn. (1):

$$C = C_i + (C_0 - C_i)\, e^{-kAt/v}, \tag{2}$$

where C_0 is the concentration at zero time, i.e. at the cessation of the hyperpolarizing or depolarizing current, t being measured from this instant. This expression shows that the concentration will fall or rise from C_0 to C_i with an exponential time constant of v/kA, which is the time constant that has a duration of about 30 sec in our experiments.

A more complex situation exists *during* injection of anions by a hyperpolarizing current, because the membrane potential is then displaced far from the initial resting value, and as a consequence there is a large alteration in the value of k, when the conditions are expressed as in eqn. (1). For example, when the interior of the cell is made more negative by a hyperpolarizing current, there will be a large increase in the value of k because there is an increase in the membrane potential, and hence in the driving force for the outward movements of anions. Thus, though still expressed as v/kA, the time constant for establishment of equilibrium during the passage of a hyperpolarizing current would be expected to be much briefer than the time constant for recovery after the current. The expected difference may be seen between the rising and falling phases of the curve in Fig. 5A, where the respective time constants are about 20 and 40 sec. On the other hand, no large error has been introduced by assuming a constant value for k after cessation of the applied current, because the change in membrane potential has been small throughout the recovery process (cf. Fig. 5B).

Having determined the value for k (as above defined), it is possible to calculate the total Cl^- ion efflux from the motoneurone under normal steady-state conditions. Thus, if M_{Cl} denotes the outward flux of Cl^- ions, the total efflux will be given by two expressions, which may therefore be equated:

$$AM_{Cl} = -kA[Cl]_i, \tag{3}$$

where $[Cl]_i$ is the normal intracellular concentration of Cl^- ions. Since at the resting membrane potential the time constant, v/kA, has been found to be about 30 sec for Cl^- ions,

$$AM_{Cl} = \frac{-v[Cl]_i}{30} = -0{\cdot}07 \text{ pmole/sec}$$

if $[Cl]_i = 9$ mM (cf. § C, below) and $v = 2{\cdot}3 \times 10^{-7}$ cm^3 (Coombs *et al.* 1955*a*). Since this chloride efflux occurs at the normal steady state, and since it has not been necessary to postulate a chloride pump (cf. § C, below), there will be

the same diffusional influx. It thus is possible to calculate the membrane conductance for chloride ions, G_{Cl}, from the formula derived by Hodgkin (1951),

$$G_{Cl}=\frac{F^2}{RT}M_{Cl},$$

where F, R and T have the usual connotation. The value so calculated for AG_{Cl}, $0{\cdot}25\times10^{-6}$ mho, represents the chloride conductance for the whole neuronal surface, and hence is to be compared with the approximate measured value, 10^{-6} mho, for the total conductance (Coombs *et al.* 1955*a*). This proportion of 20–30 % of the total membrane conductance is in good agreement with the proportions calculated for the chloride conductance of giant axons and striated muscle fibres (Hodgkin & Katz, 1949; Hodgkin, 1951; Hodgkin & Huxley, 1952*b*; Shanes, Grundfest & Freygang, 1953). The most unreliable estimate used in the above calculation was the value for the volume of the motoneurone. It has been suggested that this estimate could be too low by a factor of two (Coombs *et al.* 1955*a*). By assuming double the value for v, G_{Cl} is doubled in size and so would account for about half of the total conductance. If an area of 5×10^{-4} cm^2 is assumed for A, i.e. for the effective surface area of the motoneurone (Coombs *et al.* 1955*a*), a value of 0·07 pmole/sec for the whole neuronal surface corresponds to a value for M_{Cl} of 140 pmole cm^{-2} sec^{-1}, which is several times greater than the largest ionic fluxes observed for giant axons or muscle fibres (cf. Hodgkin & Keynes, 1954).

C. *The equilibrium potential for the ionic fluxes during the i.p.s.p.*

The inhibitory response of the motoneurone may be considered to be due to an increased ionic conductance in those parts of the motoneuronal membrane under the inhibitory synapses. The consequent ionic fluxes would tend to change the potential of these inhibitory patches to a new equilibrium value, but local currents through the capacity and resistance of the remainder of the membrane would limit the recorded change. The actual potential attained by the i.p.s.p. results from this in-parallel relationship of the inhibitory patches and the rest of the membrane. These local currents are eliminated when, by a background of applied current, the membrane potential is adjusted so that it is not changed by the i.p.s.p., which is precisely the reversal-potential for the i.p.s.p. as reported in §§ A, C and D of the Results (cf. Table 1). If it be assumed that the applied current has not modified the action of the inhibitory transmitter on the post-synaptic membrane, the reversal-potential for the i.p.s.p. would give the value for the inhibitory equilibrium potential under any given condition of ionic concentrations.

If the assumption be made that there is a uniform electric field through the

membrane, the net flux outwards across unit area of the membrane will be expressed for any cation species by

$$M_c = P_c \frac{EF}{RT}\left(\frac{C_{co} - C_{ci}\, e^{EF/RT}}{1 - e^{EF/RT}}\right), \tag{4}$$

and for any anions species by

$$M_a = -P_a \frac{EF}{RT}\left(\frac{C_{ai} - C_{ao}\, e^{EF/RT}}{1 - e^{EF/RT}}\right), \tag{5}$$

where E is the membrane potential, inside with respect to outside, R, T, and F have the usual connotations, P_c and P_a are the permeabilities for the cation and anion species considered, while C_{co} and C_{ci} are the respective external and internal concentrations for the cations, and C_{ao} and C_{ai} for the anions (Goldman, 1943; Hodgkin & Katz, 1949). The i.p.s.p. will be generated by the current due to the fluxes of all the ion species involved. The current density,

$$I_{\text{i.p.s.p.}} = F\Sigma(M_c - M_a). \tag{6}$$

At the reversal or equilibrium potential for the i.p.s.p., $F\Sigma(M_c - M_a) = 0$.

It must be assumed that the permeability to Na^+ ions is not significantly increased by the inhibitory transmitter, otherwise it would not be possible to account for the experimental observations on the i.p.s.p., for example, its hyperpolarizing character at the normal resting potential. Hence we may regard the fluxes of K^+ and Cl^- ions as alone producing the i.p.s.p. Since the inhibitory transmitter appears to act by converting the inhibitory areas into a sieve selective towards the size of ions, but indifferent to their charge (§ A) it may be postulated, as a first approximation, that during the inhibitory process the inhibitory areas are equally permeable to ions as similar in size as K^+ and Cl^- ions (cf. Table 2); hence it will be assumed that $P_K = P_{Cl}$ and at the inhibitory equilibrium potential that $M_K - M_{Cl} = 0$. Substituting Eqns. (4) and (5) for M_K and M_{Cl} respectively and cancelling common factors,

$$[K]_o - [K]_i \exp(E_{\text{i.p.s.p.}}F/RT) + [Cl]_i - [Cl]_o \exp(E_{\text{i.p.s.p.}}F/RT) = 0, \tag{7}$$

where $[K]_i$, $[K]_o$, $[Cl]_i$ and $[Cl]_o$ are the intracellular and extracellular concentrations of K^+ and Cl^- ions and $E_{\text{i.p.s.p.}}$ is the equilibrium potential for the i.p.s.p. This may be expressed by

$$E_{\text{i.p.s.p.}} = -\frac{RT}{F}\ln\left(\frac{[K]_i + [Cl]_o}{[K]_o + [Cl]_i}\right). \tag{8}$$

The external ionic concentrations may be assumed to be those of an ultrafiltrate of cat's blood plasma, i.e. $[K]_o = 5{\cdot}5$ mM and $[Cl]_o = 125$ mM (mean values derived from Davson, Duke-Elder & Benham (1936), D'Silva (1936) and Krogh (1946) for cat's plasma potassium and from Davson, Duke-Elder & Maurice (1949), Eggleton (1937), Davson *et al* (1936) for cat's plasma chloride,

the ratios of ultrafiltrate to plasma concentration being taken as 0·93 and 1·015 for potassium and chloride respectively (Manery, 1954)). There is good reason for regarding the equilibrium potential for the positive after-potential (mean value −88·5 mV in Table 1 column 6) as the equilibrium potential for K^+ ions, i.e. as E_K, (Coombs *et al.* 1955*a*); hence it is possible to calculate $[K]_i$ according to the formula

$$[K]_i = [K]_o \exp(-E_K F/RT) = 5{\cdot}5 \exp(88{\cdot}5/26{\cdot}8) = 151 \text{ mM}.$$

If the mean equilibrium potential for the i.p.s.p. ($E_{i.p.s.p.}$) is taken as −79 mV (cf. columns 4 and 5, Table 1), $[Cl]_i$ can be directly calculated from eqn. 8 as having the value of 8·9 mM.

Given $[Cl]_o$ as 125 mM, the equilibrium potential for Cl^- ions may be determined from the Nernst equation as being −71 mV, which is in close agreement with the normal resting potential across the motoneuronal membrane (cf. Brock *et al.* 1952; Coombs *et al.* 1955*a*; Table 1, column 3). Thus it appears that normally Cl^- ions are in electro-chemical equilibrium across the membrane. This conclusion agrees with that derived from investigations on other membranes, e.g. of muscle fibres and giant axons (Hodgkin, 1951) and of erythrocytes (Krogh, 1946). However, the significance of our calculation must be evaluated against the assumption on which it was based, viz. that the inhibitory membrane is equally permeable to K^+ and Cl^- ions.

D. *The ionic fluxes generating the i.p.s.p.*

It is now possible to construct a diagrammatic representation of the explanation that has been proposed for the generation of the i.p.s.p. On the left side of Fig. 18A there is shown an element of an inhibitory patch of the motoneuronal membrane with K^+ and Cl^- resistance-voltage components in parallel and on the right side there is an ordinary element of the membrane. The battery on the right side represents the resting membrane potential with a voltage of −70 mV. For present purposes it is sufficient to operate the inhibitory components in an all-or-nothing manner by a ganged switch as shown. The actual time course of operation will be considered in a later paper. In accord with the above considerations the K^+ and Cl^- components are shown with identical resistances and with batteries of −90 and −70 mV respectively. It is to be understood that the separation between K^+ and Cl^- elements is for diagrammatic purposes only. Presumably the respective ionic fluxes occur through the same channels in the sieve-like membrane. With the switch open at the resting membrane potential, there will be no current through the ordinary membrane element. Closure of the inhibitory switch will cause an inward current to flow through the K^+ component, so hyperpolarizing the membrane condenser. The Cl^- component and the ordinary membrane elements will in part shunt this effect, i.e. it is postulated that, under the conditions of

normal resting potential and ionic composition, the hyperpolarizing i.p.s.p. is generated solely by the flux of K^+ ions. By applying an extrinsic current the membrane potential can be preset at any desired level (Results, § A). If it is preset at -80 mV, on closing the inhibitory switch in Fig. 18A, the inward current through the K^+ component just equals the outward current through the Cl^- component, i.e. the inhibitory element provides no current for the remainder of the membrane. This is precisely the condition obtaining at the equilibrium potential for the i.p.s.p.

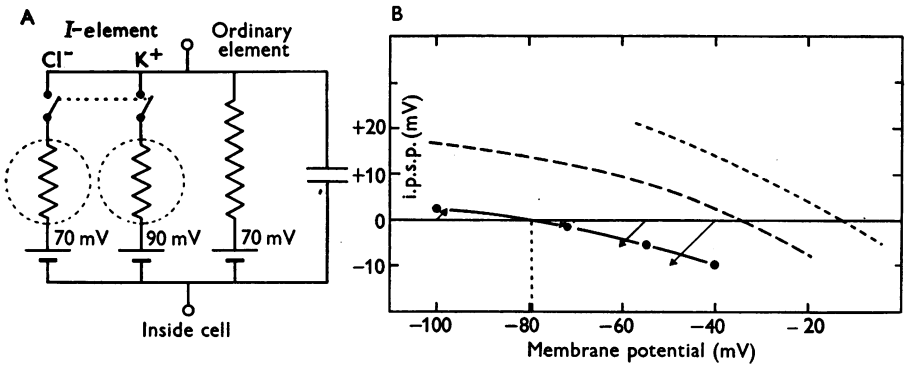

Fig. 18. A. Diagrammatic representation of the electrical properties of an ordinary element of the neuronal membrane and of an inhibitory element with K^+ and Cl^- ion components in parallel. Further description in text. B. I.p.s.p./membrane potential curves drawn on co-ordinates having equal values as described in the text. The arrows give the actual potential loci for i.p.s.p. responses corresponding to the four points designated ● on the continuous line. Further description in text.

When the membrane potential is displaced from this equilibrium potential, Fig. 2 shows that the i.p.s.p. is changed in the direction that counteracts this initial displacement, i.e. it can be considered as effecting a partial restoration. The mechanism of this restoration can be appreciated by reference to Fig. 18A. Closure of the *I*-switch will cause current to flow through the *I*-element in the direction which will displace the membrane potential back towards the equilibrium potential of -80 mV. The effectiveness of this restorative action is illustrated in Fig. 18B, where the curves of Figs. 2A, 14A (+) and 14C (▲) are drawn on equi-valued potential co-ordinates. For the curve of Fig. 2A the potential loci during the i.p.s.p.'s are represented by arrows for a series of initial membrane potentials. A background of extrinsic current sets the level of each membrane potential and continues throughout the response. The loci are drawn at 45° because, when the i.p.s.p. attains any potential, there is necessarily an equivalent alteration of membrane potential. If there was 100% restoration at the summit of the i.p.s.p., the arrows would end on the vertical line through the equilibrium potential of -80 mV. It will be seen that

the restoration was as large as 25% for the depolarized membrane, whereas it was only 16% at the normal resting potential. This variation is of course also indicated by the increasing steepness of the i.p.s.p./membrane potential curve as the membrane becomes more depolarized (cf. Figs. 2, 4, 6, 8E, 14 and 16B). Fig. 18A is defective in that it does not account for this non-linear behaviour. In order to do so, account must be taken of the Cl^- and K^+ ion concentrations on the two sides of the membrane. The more depolarized the membrane the less effectively will it impede the movement of ions from the side on which they are in greater concentration—if cations, outward, or if anions, inward. The reverse situation occurs when the membrane is hyperpolarized. Fig. 18B also indicates the importance of these relative ionic concentrations, for the restoration for the curves derived from Fig. 14A and C was increased to as much as 51 and 60% respectively. Thus, for any given ionic composition, the resistances in the K^+ and Cl^- components behave in a non-linear fashion, a property which is symbolized by the encircling dots in Fig. 18A.

When it is assumed, as in the preceding section, that K^+ and Cl^- are the only ion species normally contributing to the generation of the i.p.s.p., and that during the inhibitory response the inhibitory areas of the membrane have the same permeability coefficient, P_I, to these two species, the equations 4, 5 and 6 may be arranged in the form,

$$\frac{I_{\text{i.p.s.p.}}}{FP_I} = \frac{EF}{RT}\left(\frac{[\text{K}]_o + [\text{Cl}]_i - ([\text{K}]_i + [\text{Cl}]_o)\, e^{EF/RT}}{1 - e^{EF/RT}}\right) \tag{9}$$

In the application of this equation a fundamental assumption is that the permeability factor P_I is not changed in value by changes in the membrane potential. This is reasonable in view of the chemical transmission hypothesis, according to which the peculiar changes in the post-junctional membrane which are elicited by the junctional transmitter cannot be elicited by electrical means. In plotting eqn. 9 (Fig. 19A) the values for $I_{\text{i.p.s.p.}}/FP_I$ have been calculated for various values of the membrane potential (E) from -140 to 0 mV when the assumed values for $[\text{K}]_o$, $[\text{K}]_i$, $[\text{Cl}]_o$, $[\text{Cl}]_i$ are 5·5, 150, 125 and 9 mM respectively, i.e. approximately at normal levels (cf. § C). No further assumption is made in plotting the curve. The two other curves drawn as broken lines show the curves for the two components,

$$\frac{EF}{RT}\left[\frac{[\text{K}]_o + [\text{Cl}]_i}{1 - e^{EF/RT}}\right] \quad \text{and} \quad \frac{EF}{RT}\left[\frac{([\text{K}]_i + [\text{Cl}]_o)\, e^{EF/RT}}{1 - e^{EF/RT}}\right]$$

of the right side of eqn. 9, which plot respectively ionic fluxes giving internal positivity (the upper broken line) and internal negativity (the lower broken line). The continuous line represents the sum of these two curves.

Since P_I has been assumed to be constant for any particular inhibitory response, the values for the ordinates of Fig. 19 A, i.e. for $I_{\text{i.p.s.p.}}/FP_I$, would be proportional to the ionic current densities ($I_{\text{i.p.s.p.}}$) at the respective membrane potentials. Furthermore, since the membrane capacity may be assumed to be unaltered, these values would be approximately proportional to the maximum rates of rise of the recorded i.p.s.p.'s, the additional assumption being made that the intracellular microelectrode picks up a constant fraction of the i.p.s.p. that is generated by the currents through the inhibitory patches. This assumption appears to be justified because the membrane conductance is not greatly altered over the range of membrane potentials employed in our experiments (Coombs *et al.* 1955 *a*). Hence, with suitable scaling of the ordinates,

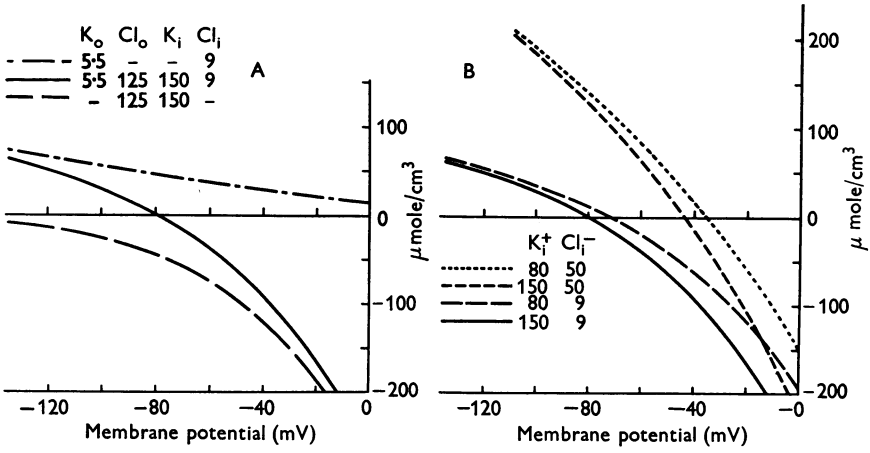

Fig. 19. A. The continuous line gives the curve when computed values for $I_{\text{i.p.s.p.}}/FP_I$ at the assumed normal intracellular ionic concentrations are plotted against membrane potential as described in the text (cf. eqn. 9). Since the ordinate scaling is for the calculated net ionic flux rates divided by the permeability constant of the membrane it has the dimensions of a concentration as shown. The derivation of the two broken lines is described in the text. B. The continuous line is plotted as in Fig. 18 A, while the other lines represent the computed curves when the intracellular ionic concentrations are altered as described in the text, and indicated in the figure.

the curve shown by the continuous line in Fig. 19 A can be regarded as plotting the theoretical curve for the effect of variations in the membrane potential on the maximum slope of an i.p.s.p. However, our experimental curves have been drawn, not for the maximum slopes of the i.p.s.p.'s, but for their peak amplitudes, which on two counts are less satisfactory for comparison with Fig. 19 A: first, the potentials attained by the peak amplitudes are much more modified by decay due to current flow through the general neuronal membrane than are the maximum slopes; secondly, there must necessarily be a considerable change in membrane potential, i.e. in abscissal scaling, at the peak amplitude

(cf. arrows in Fig. 18B), whereas the maximum slope of the i.p.s.p. occurs when there is a relatively small change in membrane potential. However, the time courses of the i.p.s.p.'s are approximately constant over our experimental ranges of membrane potential (cf. Fig. 1), so the forms of the experimental curves for i.p.s.p. summits should not deviate widely from those for slopes. As expected, good agreement has been observed when both curves were plotted for i.p.s.p.'s that were recorded with a much faster time-base than those of Figs. 1, 3 and 13. It is therefore significant that the experimental curves plotting the peak potentials of the i.p.s.p. against membrane potentials (Figs. 2, 4, 6, 8E, 14 and 16B) agree closely with the theoretical curve of Fig. 19A, having approximately the same reversal potential and upward convexity.

Small deviations between the slopes and peak potentials of the i.p.s.p. occur close to the reversal point for the i.p.s.p., where the time course shows a diphasic form (cf. Fig. 9B, C). Also a significant deviation sometimes occurs with large i.p.s.p.'s in the depolarizing direction (cf. Coombs *et al.* 1953, fig. 2B), where it appears that a spike-like process is superimposed on the i.p.s.p. This effect is presumably caused by a partial activation of the sodium-carrier mechanism by the large depolarization, and it causes a large increase in the values for the two upper plotted points of the derived curve (Coombs *et al.* 1953, fig. 3, ○ points), and hence an apparent linear relationship of i.p.s.p. to membrane potential instead of the curve with upward convexity as in the experimental curves of Figs. 2, 4, 6, 8E, 14, 16B and the theoretical curves of Fig. 19.

In Fig. 19B the normal curve is combined with a family of curves calculated for various alterations of $[Cl]_i$ and $[K]_i$, such as are assumed to occur experimentally. The calculated curve for an increase in $[Cl]_i$ to 50 mM would be approximately the condition obtaining after passing a hyperpolarizing current through a chloride microelectrode as, for example, in Figs. 4, 6 and 17C. There is good agreement with the experimentally determined curves. Furthermore, if it be assumed that during the i.p.s.p. there is the same increase in permeability towards Br^-, NO_3^- and SCN^- ions, the internal concentrations of such ions would be merely added to the $[Cl]_i$ value and a similar curve would be obtained by calculation, which again agrees with experiment.

With the passage of a depolarizing current through a Na_2SO_4- or $((CH_3)_4N)_2SO_4$-filled microelectrode it has been postulated that both the decreased $[K]_i$ and increased $[Cl]_i$ would displace the i.p.s.p. in the depolarizing direction. The calculated curves of Fig. 19B reveal that an assumed diminution of $[K]_i$ from 150 to 80 mM has a much smaller effect than an assumed increase of $[Cl]_i$ from 9 to 50 mM for all membrane potentials beyond the very low value of -14 mV, though the actual increase in Cl^- ions was less than 60% of the decrease in K^+ ions. The combined effect of $[Cl^-]_i$ increase and $[K^+]_i$ decrease, however, gives a curve which is in reasonable agreement with the curve plotted in Fig. 14A (+ points) after a depolarizing current that would cause approximately this depletion of $[K^+]_i$ ions, but probably a smaller increase in $[Cl^-]_i$ ions.

CONCLUSIONS

A simple hypothesis has been developed which provides satisfactory explanations of the effects produced on the inhibitory post-synaptic potential of motoneurones by variations of their membrane potential and of their ionic composition. According to this hypothesis the inhibitory transmitter substance that is liberated from the inhibitory presynaptic terminals acts on the inhibitory patches of the post-synaptic membrane and greatly increases their permeability to all ions below a critical size, i.e. to K^+, Cl^-, NO_3^-, Br^- and SCN^-. Under ordinary physiological conditions K^+ and Cl^- ions are the important permeable ions, the membrane being about equally permeable to them, while the relative impermeability of the membrane to Na^+ ions is unchanged. The equilibrium potential for the inhibitory post-synaptic potential is about -80 mV, i.e. about 10 mV more negative than the normal resting potential, while the equilibrium potential for the positive after-potential, i.e. for K^+ ions only, is about -90 mV (Coombs *et al.* 1955*a*). From these values and the external K^+ and Cl^- ion concentrations, the internal Cl^- concentration has been calculated to be about 9 mM which indicates that there is approximately chloride equilibrium at the normal resting potential. Thus, according to the hypothesis, at the resting membrane potential the hyperpolarizing inhibitory potential would be entirely due to the flux of K^+ ions, the flux of Cl^- ions actually limiting its size. However, when the membrane is depolarized, as would occur during an excitatory post-synaptic potential, the Cl^- ion flux will operate in the same way as the K^+ ion flux to counteract the depolarization. The effectiveness of the conjoint K^+ and Cl^- fluxes in preventing the generation of an impulse by the excitatory post-synaptic potential will be discussed in a later paper (Coombs, Eccles & Fatt, 1955*b*), where it will be shown that it is possible in this way to account satisfactorily for the observed inhibitory effects. That paper will also deal with the time course of the raised ionic permeability produced by a single inhibitory volley.

SUMMARY

1. Two new techniques have been applied to motoneurones in an investigation of the ionic mechanisms concerned in producing the inhibitory response known as the inhibitory post-synaptic potential, (i.p.s.p.), which normally is a hyperpolarization of the neuronal membrane.

(A) Double-barrelled microelectrodes have been inserted into motoneurones of anaesthetized cats so that the inhibitory potentials can be recorded through one barrel when the membrane potential is preset at any desired value above or below the resting potential by current through the other barrel.

(B) By passing through the intracellular microelectrode a measured current for a measured period, the ionic composition of the neurone has been altered

by injecting known amounts of ions. Currents that hyperpolarize the neuronal membrane are almost entirely carried out of the microelectrode by anions, which are thus injected from the microelectrode into the neurone. Similarly, cations are injected by the reverse current. Complications arise because there is a background diffusion of ions from the electrode into the neurone, and also on account of the ionic flux which carries the applied current across the neuronal membrane. It has been possible experimentally to make approximate estimates of the effects produced by these complications.

2. Using technique (A) it has been possible to construct curves expressing the relationship of the i.p.s.p. to the membrane potential. Normally the i.p.s.p. is reversed to a depolarizing response when the membrane is hyperpolarized beyond about 10 mV above the resting level.

3. Using technique (B) it has been shown that the i.p.s.p. is converted to a depolarizing response by increase in the intracellular concentration of some monovalent anions, Cl^-, Br^-, NO_3^- and SCN^-, which appear about equipotent in this respect. The larger monovalent anions, HCO_3^-, CH_3COO^- and glutamate have little or no effect and the large polyvalent anions SO_4^{2-} and HPO_4^{2-} are quite ineffective. During any injecting current the changes develop exponentially towards an equilibrium with a time constant of about 20 sec, and on cessation of the current there is usually a fairly complete recovery along an exponential time course, with a rather longer time constant, about 30 sec.

4. When a depolarizing current is passed through the microelectrode, it appears that the changes in the i.p.s.p. are largely attributable to the ionic flux that conveys the current across the neuronal membrane, the neurone suffering both a loss of potassium and a gain of chloride. Comparison of experiments with potassium, sodium and tetramethylammonium salts in the electrode has indicated that the displacement of the i.p.s.p. in the depolarizing direction is attributable not only to the gain in chloride, but also to the loss of potassium. As indicated by the changes in the i.p.s.p., when the potassium is replaced by tetramethylammonium, the lost potassium of the neurone is recovered very slowly if at all. Presumably the neurone has difficulty in eliminating the tetramethylammonium. When it is replaced by sodium, there is always complete recovery with a time constant of about 5 min, which may be regarded as the time-constant for elimination of the excess sodium by the sodium pump.

5. The depolarizing current also appears to act by depressing the diffusion of anions (Cl^-, Br^- or NO_3^-) from the microelectrode into the neurone. As would be expected, on cessation of the current the diffusion from the microelectrode again increases the anion content with the same time constant of about 30 sec that governs diffusional exchange between the cell and its environment.

6. There is a brief theoretical treatment of the diffusional relationships between the motoneurone and its environment. The calculated resting rate of chloride diffusion across the membrane is considerably higher than the values that have been given for corresponding areas of giant fibre membranes and it accounts for a considerable fraction (up to half) of the membrane conductance.

7. It is postulated that the inhibitory transmitter substance greatly increases the permeability of the inhibitory patches of the post-synaptic membrane to some ions (K^+, Cl^-, NO_3^-, Br^-, SCN^-) and not at all or very slightly to others (Na^+, $(CH_3)_4N^+$, SO_4^{2-}, HPO_4^{2-}, HCO_3^-, CH_3COO^-). On the basis of this hypothesis explanations have been offered for all the changes that are produced in the i.p.s.p. by varying the membrane potential and by the various changes in intracellular ionic composition. Since all permeable ions have diameters (calculated from limiting ion conductances) smaller than all the non-permeable, it seems that the inhibitory transmitter acts by converting the membrane into a sieve with pores that are small enough to block the passage of sodium and all larger ions.

APPENDIX

Changes in intracellular ionic concentrations produced by injection from a microelectrode

(A) *Ionic injection by diffusion*

Since the intracellular microelectrode has in our experiments been filled with a concentrated salt solution, at least 1·2 equiv/l., the ionic movements from the electrode into the cell would always have been much greater than from the relatively low concentration in the cell, about 0·15 equiv/l., into the electrode. When the tip of a microelectrode is immersed in a solution identical with that filling it, the ionic movements into and out of the orifice will be equal, and, if the ionic conductance for any one ion (G_n) is known, the corresponding flux for that ion (M_n) can be calculated from the relationship given by Hodgkin (1951),

$$M_n = \frac{RT}{z^2F^2} G_n,$$

where R, T, z and F have the usual connotation. For example, when an electrode filled with 3M-KCl has a resistance of $3{\cdot}5 \times 10^6\,\Omega$ when immersed in 3M-KCl, it may be assumed that, on account of the similar mobilities of the K^+ and Cl^- ions, the conductance for each will be $1{\cdot}4 \times 10^{-7}$ mho and hence the efflux of each ion species out of the electrode can be calculated from the above equation to be about 0·04 pmole/sec. When such an electrode is withdrawn from the 3M-KCl and similarly immersed in a 0·15M-KCl solution, i.e. in a solution approximately isotonic with the interior of a cell, the resistance rises within a second or so to a new steady level almost three times the value measured in a 3M-KCl solution; for example, for the electrode considered above, the resistance rises to about 10 MΩ. A ratio of this order has been calculated on theoretical grounds and is also implicit in the calculated values reported by Nastuk & Hodgkin (1950). This increase in resistance is attributed to the dilution of the KCl solution in the terminal segment of the microelectrode due to the outward diffusion of KCl at the tip, where there is initially a very high concentration gradient. It should be noted that, on immersion in the lower concentration of KCl, almost all of the raised resistance occurs on account of dilution within the electrode, for only about 200,000 Ω could occur on account of the increased specific resistance of the external medium.

When immersed in 0·15 M-KCl the efflux of ions from the electrode cannot be calculated from the above equation because there will no longer be equality of efflux and influx. The reduction in concentration of KCl within the microelectrode near the orifice will diminish the efflux below the level calculated for the immersion in 3 M-KCl. On the other hand, the efflux would be considerably higher than the value calculated according to the above equation from the conductance (10^{-7} mho) of the electrode in the 0·15 M solution. For present purposes it is sufficient to regard 0·04 pmole/sec as being an upper limit for the efflux of K^+ and Cl^- ions from an electrode filled with 3 M-KCl and having a resistance of 10 MΩ, either when it is immersed in a solution of 0·15 M-KCl, or when it is implanted in a cell where the solution is of about the same concentration. The ionic efflux varies inversely with the resistance of the microelectrode. For example, with a KCl-filled electrode of 5 MΩ resistance, which is the lowest resistance ordinarily used, an approximate estimate of the ionic efflux would be 0·06 pmole/sec, which is the value quoted in Results, § B, of the text.

If the potential of the intracellular microelectrode is altered with respect to the indifferent electrode, current will flow from it into the cell or vice versa, and as a consequence there will be modification of the ionic exchanges which are occurring by diffusion across the two interfaces between the cell and its environment, i.e. between the cell and the microelectrode and between the cell and the surrounding medium. The changes so produced in the ionic composition of a cell by current in either direction are complex, and not simply attributable to the injection by the current of anions or cations from the microelectrode. The ionic injections with the two types of current are considered in the next two sections. The effects on the intracellular ionic concentrations are dealt with in § B of the Discussion.

(B) *Ionic injection by hyperpolarizing currents*

When the microelectrode is made electrically negative to the indifferent electrode, current will flow from the indifferent electrode through the cell membrane into the neurone and then into the microelectrode, i.e. it will pass through the cell membrane in the direction that will hyperpolarize. The potential gradient giving such a hyperpolarizing current will modify the ionic diffusion obtaining between the microelectrode and the cell, increasing the anion movements into the cell and decreasing the cation movements. In fact the current is carried from the cell into the electrode by the sum of the two changes in these ionic currents, $-i = F(m_c - m_a)$, where i is the current in amperes, the negativity denoting hyperpolarizing current, m_c and m_a are the respective changes in the diffusional transport of cations and anions measured in equivalents per sec, and F is the Faraday. With the largest size of KCl-filled microelectrode that we have used (5 MΩ resistance) the rates of diffusion of K^+ and Cl^- ions into the cell have been calculated to be each about 0·06 p-equiv/sec (Appendix, § A), while the rates of diffusion in the reverse direction (cell to microelectrode) would be so much less that they may be neglected in the present approximate calculations. A very small hyperpolarizing current would be carried in about equal proportions by the increase of the anion movement into the cell and by the decrease of the cation movement, e.g. with $0{\cdot}5 \times 10^{-8}$ A, the Cl^- efflux from the microelectrode would be increased from 0·06 to about 0·085 p-equiv/sec, while the K^+ efflux would be decreased from 0·06 to about 0·035 p-equiv/sec. However, the current applied through the microelectrode has been much larger, often about ten times. Assuming complete suppression of the background K^+ efflux, which would carry a current of about $0{\cdot}6 \times 10^{-8}$ A, the Cl^- efflux would have to be increased from 0·06 to 0·5 p-equiv/sec in order to carry a hyperpolarizing current of 5×10^{-8} A. This increase in Cl^- efflux is overestimated by the small amount of K^+ influx from the cell into the microelectrode. Usually the microelectrode has had a resistance of 10–20 MΩ, so the effects of background ionic diffusion from the microelectrode were usually much smaller than in this numerical illustration. When the microelectrode is filled with other salts, there must be a similar rate of injection of other anions by a hyperpolarizing current (approximately 0·1 p-equiv/sec for each 10^{-8} A of current).

However, it cannot be assumed that the injected anions simply accumulate progressively in the cell. For example, chloride ions (and also bromide, nitrate and thiocyanate ions) diffuse freely across the cell membrane (Discussion, § B), and their outward flux across the membrane will be

increased as the internal concentration rises and also on account of the larger driving force exerted by the increased potential gradient which the current produces in the membrane. Since the internal mobile anions would be initially in low concentration, the inward membrane current (i.e. the hyperpolarizing current) would be carried largely by the influx of K^+ and Na^+ ions, a likely proportion being about 30% for the anionic share of the current (cf. Discussion, §B). With increasing concentration of internal anions, however, the outward anion flux would contribute progressively more to this current. A further mechanism tending to limit the progressive increase in the concentration of internal anions is provided by the osmotic influx of water across the cell membrane which would occur on account of the increase in salt content produced in the cell due to the Na^+ and K^+ ions coming from outside and anions from the electrode. For example, when passed through a chloride-filled electrode, a hyperpolarizing current of 5×10^{-8} A injects about 0·5 p-equiv/sec of Cl^- ions into the cell. If the exponential time constant for Cl^- equilibrium between the cell and its environment is 30 sec (Discussion, §B), a steady state is reached during the passage of the current when the Cl^- content of the cell is increased by 15 p-equiv. However, due to the increased membrane potential the time constant may be decreased even to one-half (cf. Fig. 5A), in which case the Cl^- content of the cell would be increased by only 7·5 p-equiv. Nevertheless, there would still be a considerable increase in the concentration of Cl^- ions, more than 30 mM, if the cell volume is assumed to be $2{\cdot}3 \times 10^{-7}$ cm³ (cf. Coombs *et al.* 1955*a*). This represents a large relative change, for the normal concentration has been calculated to be only about 9 mM (Discussion, §C). In making this calculation the osmotic influx of water across the membrane has been neglected, but it can be shown to have a relatively small effect for such injections of anions. For example, an increase in both the anion and cation concentrations by 30 mM increases the osmotic pressure of the cell by about 20%. Thus the consequent influx of water would cause a 20% increase in the water content of the cell, and hence the increase in anion concentration would be reduced from 30 to 24 mM. However, the osmotic influx of water would be much more important when relatively impermeable anions such as sulphate or phosphate were injected. Probably osmotic influx of water then provides the only significant check against a progressive increase in anion concentration during a hyperpolarizing current.

(C) *Ionic injection by depolarizing currents*

When the microelectrode is made electrically positive to the indifferent electrode, i.e. when a depolarizing current is passed, the ionic diffusion between the microelectrode and the cell will be affected in the direction opposite to that for a hyperpolarizing current, the anion movements into the cell being decreased or even suppressed, while the cation movements are increased. It can be assumed, likewise, that the cation flux out of the microelectrode will inject about 0·1 p-equiv/sec of cations into the cell for each 10^{-8}A of current. On analogy with the membranes of other excitable cells (Hodgkin & Huxley, 1952*a*, *b*), it may be assumed that normally more than half (perhaps about 0·7) of the depolarizing current is carried across the membrane by the net outward flux of K^+ ions, with the inward flux of Cl^- ions carrying most of the remainder (cf. Discussion, §B).

With a K_2SO_4-filled electrode there would be virtually no progressive change in the proportional carriage of current across the membrane. Since current would be carried out of the electrode almost exclusively by K^+ ions, and the transference number for K^+ ions is probably about 0·7 for the outward current across the membrane, there would tend to be an increase in intracellular K^+ ion concentration. Since the remaining 0·3 of the membrane current would be carried largely by the inward flux of Cl^- ions, for each 10^{-8}A of depolarizing current there would be an addition of about 0·03 p-equiv/sec of K^+ and Cl^- ions to the cell. This increase in salt concentration would cause an osmotic influx of water across the surface membrane which would tend to restore the osmotic pressure to normal, i.e. to bring the K^+ ion concentration down to 150 mM. An approximate value for this rate of osmotic change may be derived from measurements on the giant axons of cephalopods (Hill, 1950). Making due allowance for the different volume to surface ratios, the time constant for osmotic equilibration would lie between 4 and 8 sec for the motoneurone. Probably the value would be still shorter, for the motoneuronal membrane has been calculated to be several times more permeable to ions than the giant fibre membrane (Discussion, §B), and hence

presumably it is also more permeable to water. Thus the time constant for osmotic equilibration might be well less than 10% of that for ionic equilibration, which is about 30 sec for the small anions. Under such conditions, during the passage of a depolarizing current through a K_2SO_4 electrode, there would be a negligible increase in the intracellular K^+ concentration above its normal value of 150 mM, for the influx of water across the cell membrane would virtually parallel the net gain of K^+ ions produced by the current flow; for example, the usual intensity of current, 5×10^{-8} A, would cause an increase of only 4% in the intracellular K^+ ion concentration. As with a hyperpolarizing current, this osmotic influx of water would not seriously depress the relative increase in the internal Cl^- concentration by the influx across the membrane, for, in contrast to K^+, the initial Cl^- concentration is very low. However, the respective transference numbers that have assumed for the hyperpolarizing and depolarizing currents indicate that the rate of addition of Cl^- by a depolarizing current would be less than half of that produced by a similar hyperpolarizing current.

Very different conditions prevail when the depolarizing current is applied through a Na_2SO_4-filled electrode. Throughout there would be a sodium transference number of nearly unity for the current from electrode to cell. As above, the current would be carried initially across the membrane by the outward flux of K^+ ions and the inward flux of Cl^- ions with respective transference numbers probably of about 0·7 and 0·3. Thus there will be a progressive loss of K^+ ions and gain of Na^+ and Cl^- ions. The consequent rapid depletion of intracellular K^+ ions (initially at about 0·07 p-equiv/sec for every 10^{-8} A) will result in a progressive change in the transference numbers across the membrane: the potassium efflux will fall progressively, while the chloride influx will rise (perhaps even to double the initial value), and to a smaller extent there will be an increase in the net outward flux of Na^+ ions. Thus the principal effects of the current on the intracellular ionic composition will be a substitution of Na^+ ions for K^+ ions and an increase in Cl^- ions. This latter increase will be considerably larger than when the current is passed through a K_2SO_4-filled electrode. Similar changes in the K^+ and Cl^- ion concentrations would be expected when the depolarizing current is passed through a $((CH_3)_4N)_2SO_4$-filled electrode.

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