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THE EFFECTS OF TETRA-ETHYLAMMONIUM IODIDE ON THE ELECTRICAL RESPONSE AND THE ACCOMMODATION OF NERVE[1]

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Tetra-ethylammonium salts have long been known to differ from most other quaternary ammonium salts in many respects. Two of these are that tetra-ethylammonium salts have only a feeble curariform action and that they produce fibrillary twitching in voluntary muscle [Brunton & Cash, 1884; Boehm, 1910]. Many investigators, some of whom are mentioned below, have established that the muscular twitchings are peripheral in origin. On the one hand Tillie [1890], Jacobj & Hagenberg [1902], Rothberger [1902] and Marshall [1914] have all brought forward evidence that the tetra-ethylammonium ion produces fibrillation by acting upon motor nerve-endings. On the other hand there is evidence, mainly from more recent investigations, of a direct action of the tetra-ethylammonium ion on nerve. Marshall himself stated that the threshold electrical stimulus necessary to excite the motor fibres of the frog's sciatic nerve diminished after the injection of small amounts of tetra-ethylammonium chloride. He also observed that whilst calcium salts would inhibit the twitchings produced in nerve-muscle preparations by tetra-ethylammonium chloride, they had little or no influence upon its curariform action. The experiments were performed both with preparations from frogs which had been injected with calcium chloride previously, and with preparations which were treated with calcium-enriched Ringer's solution containing tetra-ethylammonium

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chloride. Marshall established that tetra-ethylammonium ions do not remove calcium ions from solution through the formation of complexes. Loeb & Ewald [1916] found that immersion of the nerve only of a frog's sciatic-gastrocnemius preparation in Ringer's solution containing 6–42 millimols per litre of tetra-ethylammonium chloride caused twitching of the muscle, and that the onset of the twitching could be delayed or even prevented by the addition of calcium salts to the solution with which the nerve was treated. Unlike Marshall, they found no lowering of the threshold to induction shocks after treatment with tetra-ethylammonium chloride; in fact, they also found no change in the threshold of nerves that had been treated with tetra-ethylammonium chloride together with sufficient calcium chloride to prevent the development of twitchings of the muscle. They found that in nerves that had been treated with tetra-ethylammonium chloride-Ringer's solution in which part of the sodium chloride had been replaced by glucose the threshold was raised considerably; this procedure also inhibited the development of twitching in the muscle.

Cowan & Ing [1933, 1935] observed that in frog's isolated sciatic nerve, stimulated repetitively for short periods by maximal condenser shocks, the action current, measured ballistically as the area under a galvanometer deflexion and time curve, was increased from 50 to 100 p.c. after soaking in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide.

In the experiments described in this communication we examined, with an amplifier and oscillograph, the electrical response of nerve which had been treated with tetra-ethylammonium iodide. As we had anticipated, the response proved to be repetitive. This result led us to examine the nature of the changes of excitability responsible for the repetitive response. In this communication we have not attempted to deal with the action of tetra-ethylammonium iodide on the nerve-muscle preparation. This relatively more complex system than nerve will be discussed by one of us (S. L. C.) in a subsequent paper.

Materials

The frogs were English *Rana temporaria*, and except where otherwise stated, they were caught between mid-March and the end of May, and used within a fortnight of capture. Ringer's solution of the following composition was made up with "AnalaR" chemicals and water distilled in a porcelain still: NaCl 0·65, KCl 0·10, $CaCl_2$ 0·20, water to 100 c.c., and

contained 10 mg. per 100 c.c. of phosphorus in the form of phosphate buffer to maintain the *p*H at 7·2. The tetra-ethylammonium and tetramethylammonium iodides used were purified by recrystallization from absolute alcohol. The Ringer's solutions to which either of these substances had been added were diluted with distilled water to restore their original tonicity.

The electrical responses of nerve

Experimental

The sciatic nerve trunk was mounted in moist air (17–20° C.) in a closed ebonite chamber, and lay on four electrodes consisting of silver wires coated with silver chloride over which had been wound, just before the beginning of the experiment, thick thread soaked in Ringer's solution.

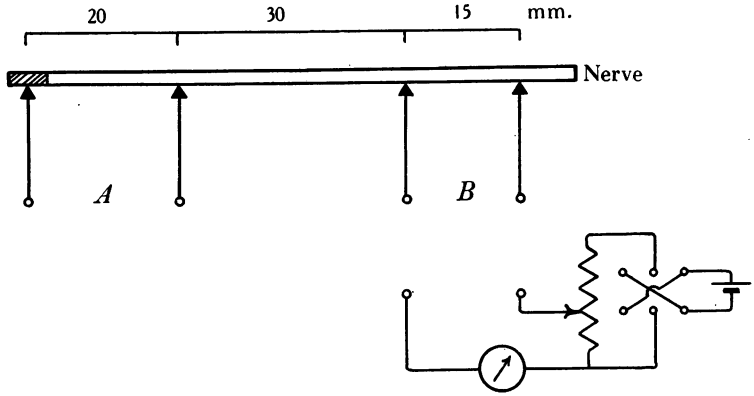

Fig. 1. The arrangement of the electrodes on the nerve trunk. *A*, leading-off electrodes to amplifier. *B*, electrodes for stimulus or polarizing current.

The purpose of the thread was to prevent the nerve making contact with the silver chloride, and the consequent disturbing action described by Cowan & Ing [1935]. One pair of electrodes was used for leading off to the input of an amplifier, a monophasic response being secured by first crushing the peripheral end of the nerve and then applying a drop of isotonic potassium chloride solution; the other pair of electrodes was used to apply stimuli or small polarizing currents to the nerve (Fig. 1).

The amplifier was resistance capacity coupled and had a time-constant of approximately 1 sec. The output was fed to a Matthews's moving-iron oscillograph, through the special output stage devised by Matthews [1928]. The action potentials were examined on the screen of a "standing

wave" camera similar to that described by Matthews [1929], but modified in one respect. Instead of the single contact breaker and cam on the driving shaft to give stimuli synchronized with the mirror, there were two cams operating two contact makers—one fixed and one adjustable. These enabled condenser charge and discharge shocks to be applied, and by altering the angular distance between them and watching the action potentials on the viewing screen it was easy to determine the refractory period of the nerve. The action potentials were recorded, when desired, on ciné bromide paper.

The stimuli usually applied to the nerve were condenser shocks ($RC = 250$ μsec.) and the voltage to which the condenser was charged was adjusted with a tapped battery in series with a potentiometer connected across a single cell. The shocks were just maximal except in the determinations of the absolute refractory period. The method of obtaining the polarizing currents is evident from Fig. 1.

Results

Treatment of nerves with dilute solutions of tetra-ethylammonium iodide produced a twofold change in the action potential response to single condenser shocks: a prolongation of the negative after potential; a repetitive discharge. The development of these effects is shown in Fig. 2. *A* is the monophasic response of a nerve trunk which had been soaked in aerated and phosphate-buffered Ringer's solution for $\frac{1}{2}$ hour after dissection; *B*, the response of the nerve trunk from the opposite side of the same frog after soaking in Ringer's solution for 2 hours; *C*, the response of the first nerve after it had been soaked for a further $\frac{3}{4}$ hour in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide; *D*, a response recorded under the same conditions as *C*, but with greater amplification; *E*, the response in the same nerve after it had been soaked for another hour in the 10 millimolar tetra-ethylammonium iodide solution, then re-mounted and a fresh injury made; *F*, taken at a slower speed, the response about 5 min. after painting the nerve with a 42 millimolar solution of tetra-ethylammonium iodide in Ringer's solution. From the records it can also be seen that the repetitive discharge began synchronously and became progressively more asynchronous.

In nerves which were examined after immersion for $\frac{3}{4}$ hour in 10 millimolar tetra-ethylammonium iodide solution no change was detected in the absolute or relative refractory periods, or in the threshold to the short stimuli employed. Further, in the records, the distance between the stimulus escape and the beginning of the action potential, that is the

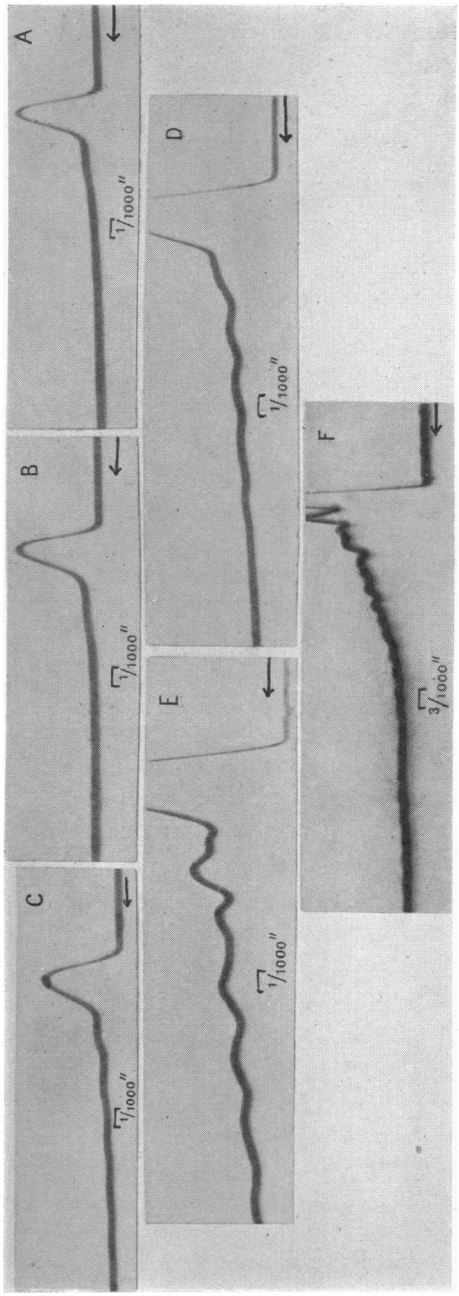

Fig. 2. Monophasic records of action potentials in frog nerve before and after treatment with tetra-ethylammonium iodide Ringer solution. Explanation in the text.

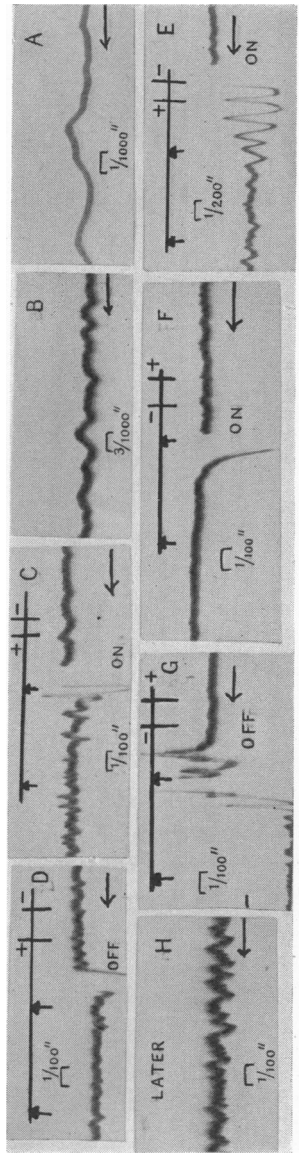

Fig. 3. Monophasic records of "spontaneous" activity in nerve after treatment with tetra-ethylammonium iodide solutions, and the influence of polarizing currents on the activity. Details in the text.

latent period of the nerve plus the conduction time, remained unchanged after tetra-ethylammonium iodide. From the constancy of the threshold to short shocks we infer that the "utilization time" was also constant and that it is probably justifiable to conclude that the conduction velocity was not affected by the dilute tetra-ethylammonium iodide solution.

Immersion of a nerve for 5–10 min. in a 42 millimolar tetra-ethyl-ammonium iodide solution produced "spontaneous" asynchronous activity; nerves immersed in more dilute solutions (15–30 millimolar) developed such activity after longer times (40–20 min.). Later in the year, June–July, nerves which had been immersed in 10 millimolar solution often developed "spontaneous" activity provided that they had not been subjected to preliminary soaking in Ringer's solution (0·02 p.c. $CaCl_2$) for any considerable time. Fig. 3*A*, taken at the same speed as Fig. 2*A–E* and with amplification three times greater than in *E*, is a record of the activity in a nerve that had been treated with the 42 millimolar solution of tetra-ethylammonium iodide. Fig. 3*B*, taken at the same speed as Fig. 2*F*, shows the form of the discharge more clearly. Records taken with a control nerve which had been immersed in Ringer's solution gave a perfectly steady base line.

Examination of the effects of constant currents on the discharge in nerves which were in a state of moderate "spontaneous" activity showed that the normal electrotonic changes of excitability at the anode and at the cathode, i.e. Pflüger's changes of excitability, had become reversed. For the records in Fig. 3*C–G* the current was one-quarter of the rheobase which was found for the normal nerve before treatment with tetra-ethylammonium iodide solution. Even this small current, approximately 0·25 μA., seems to have been a little above the threshold in these experiments. [Measurements, described later, with calomel half-cells as non-polarizable electrodes confirmed the lowering of the rheobase.] With the anode nearer to the leading-off electrodes the making of the current excited a small group of fibres. Fig. 3*C*, and Fig. 3*E* taken at a higher speed than 3*C*, show that the discharge began synchronously and became progressively more asynchronous in the 20–30 msec. immediately after the closure; from then onwards for about 30 sec. the "spontaneous" activity was considerably augmented. Breaking the current during the period of augmented "spontaneous" activity excited a few fibres and then the discharge rapidly sank to its previous resting value (Fig. 3*D*). With the polarity reversed a few fibres were excited on making the current, then the "spontaneous" activity was rapidly inhibited and remained so during further passage of the current (Fig. 3*F*). At the break again,

a few fibres were excited and there was an outburst of "spontaneous" activity which declined slowly (20 sec.) to its resting level (Fig. 3*G–H*).

The effects of polarizing currents could be observed equally well whether or not the part of the nerve between the leading-off electrodes had undergone treatment. This suggests that the reason why augmentation or inhibition effects were observable was that the impulses affecting the amplifier and oscillograph were chiefly those coming from the part of the nerve outside the leading-off electrodes and that impulses arising in that part of the nerve between the leading-off electrodes were distributed symmetrically giving only a very small net action potential.

Similar experiments to those already described, but with nerves that had been immersed for up to 2 hours in 10 millimolar tetramethylammonium iodide solution, showed no change in the action potential and no sign of "spontaneous" activity.

Measurements of accommodation in nerve

Loeb [1901] found that treatment of the nerves only of frog's nerve-muscle preparations with solutions of sodium salts whose anions remove calcium ions from solution, e.g. fluoride, carbonate, phosphate (Na_2HPO_4), oxalate, citrate or tartrate, set up twitchings in the muscles. He found too that the twitchings could be stopped by the addition of a sufficient quantity of calcium chloride. Further experiments led Loeb to the conclusion that the salts in question did not stimulate the nerve, but rather rendered it sensitive either to a contact or a mechanical stimulus—both, as Loeb employed them, stimuli of long duration. Mines [1908], who examined the "spontaneous" twitchings and changes of excitability of the frog's sartorius muscle when placed in calcium-free Ringer's solution, was able to put Loeb's conclusions into more quantitative terms. Like Loeb, Mines found that the threshold to break induction shocks was unchanged, but Mines also found that the threshold to constant currents of long ($\frac{1}{2}$ sec.) duration, in fact what we now call the "rheobase", was reduced to about one-third of its normal value.

Lucas [1907] established that each of the tissues, muscle and nerve, requires a characteristic "minimal current gradient" for its excitation, and later showed that in his own experiments and in those of Mines [1908, and reported in Lucas, 1908], the increase of rheobase produced by treatment with calcium-rich solutions was accompanied by a corresponding increase of the "minimal current gradient". Subsequently, Lucas [1910] drew attention to the association between "spontaneous" activity and the diminutions of "minimal current gradient" and of

rheobase. Kahn [1911], in experiments on the excitation of the intramuscular nerve fibres of the frog's isolated sartorius by currents increasing exponentially to steady values, also found that rates of rise, slower than those effective with preparations which had been soaked in normal or calcium-rich Ringer's solution, sufficed for preparations which had been soaked in calcium-deficient solutions.

Hill [1936] has pointed out that excitation by electric currents involves two independent time factors. For theoretical treatment he has assumed that the time factors are the time-constants of two exponential processes—a fast one and a slower one—whereby an excitable tissue reverts to its resting state upon the instantaneous removal of a subthreshold current. According to his theory, excitation by current of short duration involves only the shorter factor k, the time-constant of excitation, whereas excitation by slowly rising currents or currents near to the rheobase involves in addition λ, the time-constant of "accommodation". Also according to the theory, repetitive response is a consequence of slow "accommodation", i.e. long λ [see Katz, 1936].

In the part of Hill's theory which deals with excitation by currents increasing exponentially to constant values it is predicted that if the exciting current, instead of reaching the threshold value instantly, increases exponentially, then the threshold exponential current (I) divided by the rheobase (I_0) should bear a nearly linear relation to the time-constant of current rise (α), so long as α and λ are both large compared with K. The slope of the line should be $1/\lambda$ where λ is the time-constant of "accommodation" defined above. Hill has pointed out also that $1/\lambda$ should be equal to Lucas's "minimal current gradient". On testing experimentally the relation between I/I_0 and α Solandt [1936] found that it was linear, for values of α that were sufficiently large not to infringe the condition just stated. Changing of the calcium-ion concentration of the fluid bathing frog nerve caused the slope of the line to change through a wide range of values, but produced no departure from linearity. He concluded that the action of calcium ions in diminishing λ is almost a specific one and that k is changed comparatively little. He demonstrated also that removal of calcium ions from nerve by immersion in sodium citrate or oxalate solution caused λ to become very large and finally infinite when "spontaneous" activity began.

We anticipated that, like the agencies mentioned above, tetra-ethyl-ammonium ions would reduce the rheobase and would cause nerve to become more easily excited by slowly rising currents. It remained to determine whether the relation between I/I_0 and α was a linear one, and

if it was linear, whether the changes of λ so measured, together with changes of rheobase, would account for the repetitive responses and "spontaneous" activity produced by tetra-ethylammonium iodide.

Experimental

Our method of obtaining currents which rose exponentially, to constant values, with known time-constants of rise (α) was essentially the same as that employed by Solandt [1936] in his experiments in frog nerve. Since measurement of the small currents necessary for excitation would have prevented readings from being made in rapid succession we also adopted Solandt's plan of measuring the thresholds in volts at the potential source. The values of E/E_0 actually obtained from our experiments were the same as those that would have been measured by I/I_0, because the resistance of the circuit used was constant during any one set of observations and the current was proportional to the applied voltage.

The experiments were performed at room temperature (17–19° C.) with gastrocnemius-sciatic preparations which had been soaked for 1½–2 hours in Ringer's solution. A paraffin wax chamber with a closely fitting glass cover was used; moist air or oxygen could be passed through the chamber. The preparation lay horizontally, the muscle on the floor of the chamber and the nerve on two waxen shelves on which had been placed two filter paper strips (each 4 mm. wide and with 14½ mm. between their nearer edges) connected to large calomel electrodes used to apply the exponentially increasing currents. The presence of the minimal twitch, which was used as an indication of excitation in the motor nerve fibres, was detected by the movement of a fragment of silvered microscope cover-slip resting on the belly of the muscle. A beam of light was arranged so that it fell on the mirror and gave a spot on a ground glass scale.

Measurements were begun by determining the threshold voltage for a current of instantaneous rise (the rheobase), then the thresholds for currents of longer and longer time-constant of rise were determined. A return series, ending with the rheobase, was also performed, thus giving two thresholds which could be averaged for each time-constant of current rise. Throughout, the trials were made with inadequate stimuli which were increased until the threshold was reached. Finally, the effective resistance shunting the condenser was measured by substituting a low-resistance galvanometer in series with a small known E.M.F. in place of the condenser. When the first part of the experiment had been completed

the preparation was taken out, a small weight attached to the central end of the nerve, which was then put into the tetra-ethylammonium-iodide-containing solution, the effect of which it was intended to examine. A closed vessel was used so that the muscle was suspended in moist air and care was taken to prevent contact with the walls since any partial paralysis of the preparation by the quaternary salt would have vitiated the experiment. After a definite time had elapsed the muscle was rinsed with Ringer's fluid and the surplus drops wiped off. The preparation was re-mounted and a second series of measurements made. In some cases after the nerve had been soaked in a second solution a third series of measurements was made. The experiment was completed by the testing of a control preparation which had been taken from the opposite side of the same frog and which had been soaking in Ringer's solution.

Results

The relation between E/E_0 and α was substantially a linear one, even for nerves which had been treated with concentrations of tetra-ethyl-ammonium iodide sufficient to cause "spontaneous" activity to begin soon after the measurements had been made (e.g. Fig. 4 *B* and *C*, Fig. 5 *B* and *C*). Also, for all but large values of the slope of the line in question, i.e. with nerve treated with tetra-ethylammonium iodide, when short durations of α were employed there was curvature corresponding to a more rapid diminution of E/E_0 than of α; for large values of the slope, i.e. for Ringer-soaked but otherwise untreated nerve, the relation remained linear or nearly linear when short durations of α were employed. The first two results are of types which accord with Hill's theory and we have therefore concluded that, for nerve treated with tetra-ethyl-ammonium iodide, it is permissible to refer to the slope of the linear part of the curve relating E/E_0 and α as λ, the "time-constant of accommodation" of the theory. The third result no more than confirms Solandt's finding that when λ is small, then with small values of α, the curvature predicted by the theory is absent. Hill has pointed out (pp. 322 and 337) that the values of λ determined by this method are rather too large, and require correction, because the "observed" rheobase is slightly higher than the "true" rheobase. However, we have omitted the corrections throughout this paper, because they would have been small in comparison with the changes of λ studied [for calculation see Solandt, 1936], and, in the case of Ringer-soaked nerve, not of a kind to remedy the deviation from the predicted relation.

In experiments performed mainly during the first 2 weeks in May, values of λ between 10 and 18 msec. were found for nerves that had been soaked in Ringer's fluid for $1\frac{1}{2}$–2 hours (e.g. Fig. 4*A*, Fig. 5*A*), and between 13 and 20 msec. for nerves that had been soaked for 3–5 hours (e.g. Fig. 4*D*, Fig. 5*D*). Before use the frogs were kept at the laboratory

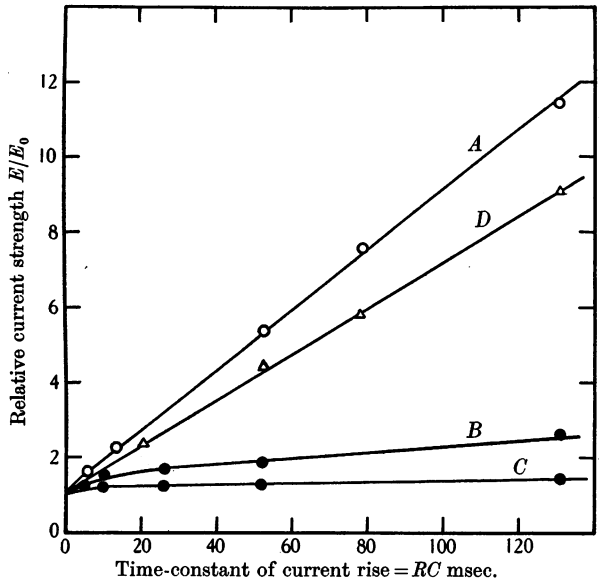

Fig. 4. "Accommodation" curves for frog nerve. *A*, nerve had been soaked in Ringer's fluid for $1\frac{1}{2}$ hours. $\lambda = 12{\cdot}7$ msec. *B*, after the same nerve had been immersed for $\frac{1}{2}$ hour in Ringer's fluid containing 10 millimols per litre of tetra-ethylammonium iodide, and then remounted. $\lambda = 122$ msec. The nerve was then immersed for 15 min. in 30 millimolar tetra-ethylammonium iodide Ringer's solution, re-mounted, and left undisturbed for 10 min. before the determinations. *C*, these were made as quickly as possible as the nerve was not in a steady state. $\lambda = 588$ msec. Less than $\frac{1}{2}$ hour later λ had become immeasurably long and there were occasional "spontaneous" discharges. *D*, control nerve that had been soaking in aerated Ringer's fluid for $3\frac{3}{4}$ hours. $\lambda = 15{\cdot}5$ msec.

temperature (16–20·5° C.). No measurements were made on nerves immediately after dissection. Our values of λ are slightly smaller than Solandt's for Ringer-soaked nerve—probably because our experiments were made not long after the breeding season, whilst his experiments extended over a longer period.

If present in sufficient concentration, tetra-ethylammonium iodide reduced the power of "accommodation" of nerve to vanishing point. Fig. 4 gives the results of an experiment.

For nerves that had been soaked in Ringer's solution for 1½–2 hours and then for ½–1 hour in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide, λ lay between 100 and about 600 msec. Immersion of Ringer-soaked nerves in 15 millimolar tetra-ethylammonium iodide Ringer's solution for about ½ hour made λ immeasurably great, and started "spontaneous" discharges, except in a few which had been soaked in normal Ringer's fluid for more than 3 hours, or previously in a weaker tetra-ethylammonium iodide solution for about an hour.

In a few experiments the painting on of tetra-ethylammonium iodide Ringer solution to the nerve as it lay on the electrodes was tried. However, the results were not very consistent, doubtless because the volume of fluid applied was not large in comparison with that of the nerve. To be reasonably certain of producing "spontaneous" activity by this method a concentration of 30 millimols per litre was required.

Calcium ions were found to reverse increases of λ which had been brought about by tetra-ethylammonium ions. The method was first to measure λ for a Ringer-soaked nerve; then to treat the nerve with tetra-ethylammonium iodide-containing Ringer's solution and to measure λ again; after that, to treat the nerve with Ringer's solution containing the same concentration of tetra-ethylammonium iodide as previously together with added calcium chloride, and to measure λ for a third time. Finally, λ was determined for a Ringer-soaked control nerve. The results of one such experiment are summarized in Fig. 5. Concentrations of calcium chloride 2–3 times that of normal Ringer's fluid (0·02 p.c.) were sufficient to reverse the changes produced by 10 millimolar tetra-ethylammonium iodide solution.

An adequate concentration of calcium chloride applied simultaneously with tetra-ethylammonium iodide could prevent the changes of λ that would have been produced by the quaternary salt alone. In two experiments Ringer-soaked nerves were immersed for 2½ hours in a solution containing 10 millimols per litre of tetra-ethylammonium iodide and 0·08 p.c. of calcium chloride (7·2 millimols per litre): in both of them λ, initially about 14 msec., was changed by less than 15 p.c.

In the various kinds of experiment described above a diminution of "accommodation" was accompanied by a diminution of rheobase. When λ was increased to 300–500 msec. the rheobase was lowered to about one-half of the value found before treatment with tetra-ethylammonium iodide solution; when λ had become immeasurably long, in the intervals between "spontaneous" discharges, the rheobase had a value 1/4 to 1/8

of the normal one (1·0–1·8 μA.). The lowerings of rheobase were reversed or prevented by calcium chloride in concentrations sufficient to reverse or prevent the changes of λ produced by tetra-ethylammonium iodide.

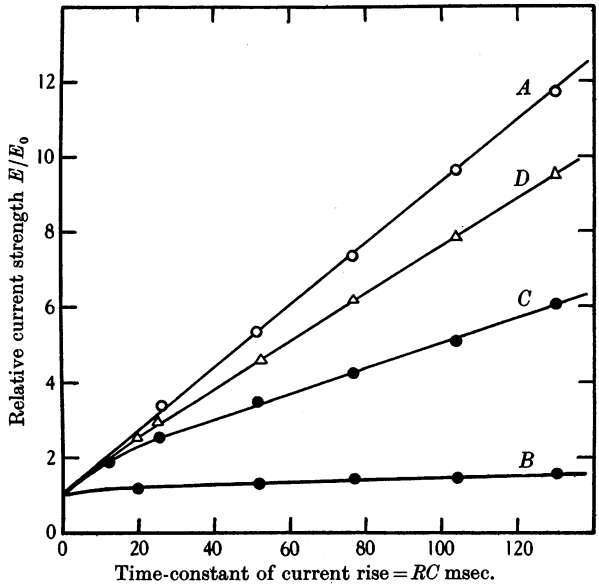

Fig. 5. The reversal of "accommodation" changes by calcium chloride. *A*, nerve which had been soaked in Ringer's fluid for 2 hours. $\lambda = 12{\cdot}0$ msec. *B*, after the same nerve had been treated for an hour with Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide. $\lambda = 323$ msec. The nerve was then immersed for an hour in Ringer's solution containing 10 millimols per litre of tetra-ethylammonium iodide and 0·04 p.c. calcium chloride. *C*, λ determined subsequently $= 30{\cdot}7$ msec. *D*, control nerve soaked in Ringer's solution for 5 hours. $\lambda = 15{\cdot}9$ msec.

Apparently with tetra-ethylammonium-iodide-treated nerve, as Katz [1936] found with sodium-citrate-treated nerve, the onset of "spontaneous" activity is the limiting result of reduction of the rheobase and of extremely slow "accommodation".

The effects of constant currents

Ordinarily in nerve when a constant electric current just sufficiently strong to excite at make is broken, no further excitation results. If, however, the strength of the current is increased to several times that required for excitation at make, then excitations are obtained both at make and at break. Apparently the threshold for break excitation is

higher than that for make excitation. The phenomena have been explained as a consequence of the rise of excitability which occurs at the cathode and of the fall of excitability which occurs at the anode of a polarizing current when applied before a test stimulus. According to this view, reversal of the changes of excitability should result in a fall of the threshold for break excitation below that for make excitation; abolition of them should result in break excitation and make excitation being obtained equally well.

Hill, to simplify the mathematical development of his theory, has treated the case of nerve from which the electrotonic changes of excitability are absent. He has pointed out that these changes are probably of secondary importance, since excitation and accommodation can take place independently of them. If a steady current of intensity I is applied to a nerve from which the electrotonic changes of excitability are absent, the duration of the repetitive response set up from the cathode should be

$$T = \lambda \log_e I/I_0,$$

where λ is the time-constant of accommodation, and I_0 is the rheobase. The same response should occur at the anode when the current is broken. If the electrotonic changes of excitability are taken into account, the normal changes might be expected to prolong the repetitive response at make, and to shorten or abolish the repetitive response at break; the reversed changes might be expected to have the opposite effects. On comparing the "observed" and "calculated" durations of repetitive response to constant currents Katz [1936] found an approximate agreement between them, but that the "observed" durations might be modified by the electrotonic condition of the nerve, or by changes of refractory period; also, that reversal of the normal electrotonic changes of excitability by cold resulted in the threshold for break excitation falling below that for make excitation.

Since our experiments on the reversal of the normal electrotonic changes of excitability at the anode and at the cathode referred to sciatic nerve trunks in a state of "spontaneous" activity, and our experiments on "accommodation" and rheobase to motor fibres only, we have made further experiments on the thresholds of motor fibres for make excitation and for break excitation, and on the influence of current intensity and direction on the duration of repetitive response to constant current. Nerve-muscle preparations were used, and, as at the time of these experiments the amplifier and oscillograph were not available, the mechanical response of the muscle was taken as a measure of the

response of the motor nerve fibres. Our observations, therefore, on the duration of repetitive response elicited from nerve are only semi-quantitative. The import of this limitation will be discussed later.

Experimental

Sciatic-gastrocnemius preparations, from winter frogs kept at 14–18° C., were soaked for 2 hours in Ringer's solution and then mounted in moist oxygen in a modified Lucas's trough closed by a cover through which the lever crank passed. The femur was held in a clamp, and the tendon was tied to the crank which was also connected to an isometric lever which, when writing on smoked paper, had a period of 1/30 sec. The central part of the nerve was supported on wicks leading to two calomel half-cells: the diameter of each wick was about 1·2 mm., and the distance between them was 20 mm.; the distance between the wick more distal from the muscle and the cut (central) end of the nerve was 6–10 mm. Constant currents in either direction were obtained as follows. One of the electrodes was connected to the moving contact of a graduated potential divider through a 200,000 ohm "metallized" resistance, the other electrode was connected directly to the fixed contact of the potential divider, thus completing the circuit. The ends of the potential divider were connected to a 4 V. battery through a reversing key. The resistance of the nerve and electrodes was measured and found to be 90–100,000 ohms, from this and the other constant of the circuit the current through the nerve was calculated. After the preparation had been mounted the experimental procedure was to determine the rheobase of the nerve and then to try currents of several times the rheobase until break excitation was observed. To avoid changes in the condition of the nerve due to the constant currents they were passed in descending and ascending directions alternately. When desired, records of the muscle response were made. After the preliminary determinations were finished the nerve was lifted off from the electrodes, without disturbing the muscle, and the central half immersed in tetra-ethylammonium iodide Ringer solution. The wicks leading to the electrodes were also immersed in the solution with which the nerve was treated. At the end of a definite time the nerve was re-mounted and fresh tests made.

Results

In the following paragraphs the expression "threshold for make excitation" or "make threshold" means the intensity of descending current necessary just to excite the motor nerve fibres at make, i.e. with

the cathode as the electrode nearer to the muscle; "break threshold" refers to current ascending nerve.

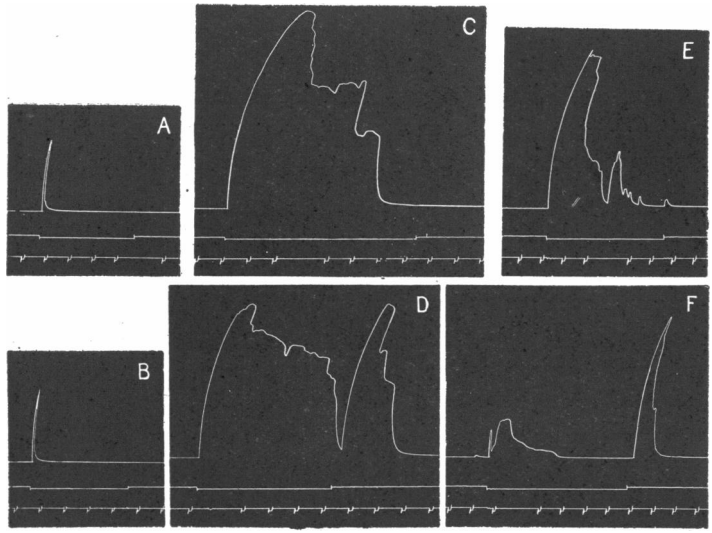

Fig. 6. The responses of a nerve-muscle preparation to constant currents applied to the nerve, before and after it had been treated with tetra-ethylammonium iodide. The time markings are in seconds; depression of the signal line indicates make of the current and rise indicates break of the current. The preparation was mounted after 2 hours soaking in Ringer's solution, and the threshold current for excitation of the motor nerve fibres at make was determined periodically. In $\frac{1}{2}$ hour it fell from 1·3 to 1·1 μA. and remained constant for the following $\frac{1}{2}$ hour. The break threshold was about (7 × 1·1) μA. *A*, response to descending current five times make threshold (1·1 μA.), recorded at 65 min. (this and the other times below are reckoned from the mounting of the preparation). *B*, response to ascending current five times make threshold, recorded at 66 min. The nerve was immersed in 30 millimolar solution from 70 until 93 min. and then re-mounted. Irregular outbursts of "spontaneous" activity were beginning to appear, and determinations of threshold, which could only be made in the quiescent intervals, became inexact. The average make threshold was about 0·3 μA.—a quarter of its former value; the average break threshold was about 0·25 μA.—one-thirtieth of its former value. *C*, response to descending current three times make threshold, recorded at 100 min. *D*, response to ascending current three times make threshold. At 130 min. the make and break thresholds were approximately equal (0·3 μA.). *E*, response to descending current five times make threshold (0·3 μA.), recorded at 133 min. *F*, response to the same current ascending.

Ringer-soaked nerves.

The condition of the nerves used in our experiments was such that a current intensity of 5–9 times the threshold for make excitation was required to produce excitation at break. The muscle responses elicited

at the make of currents of intensity up to seven times the (make) threshold were ones corresponding in size to a few (usually 1–3) maximal volleys in the motor fibres of the nerve. The size was independent of the direction of current flow (cf. Fig. 6*A* and *B*, Fig. 7*A* and *B*).

The influence of tetra-ethylammonium iodide on threshold.

The threshold for make excitation was reduced considerably, and the threshold for break excitation to a greater extent. In the case of nerve that had been treated with 30 millimolar solution determinations of threshold were made in the intervals between "spontaneous" discharges. Soon after treatment the break threshold fell below the make threshold; later, the break threshold rose again slightly. In nerves treated with smaller concentrations of tetra-ethylammonium iodide (10–15 millimols per litre) the break threshold never fell below the make threshold, nor was there a later rise of the break threshold relative to the make threshold.

The effects of tetra-ethylammonium iodide upon responses to descending currents 2–7 times threshold.

Following the make of a current applied to a nerve soon after it had been brought to a state just short of "spontaneous" activity by treatment with 15 millimolar solution the response elicited from the muscle was a tetanic one which continued until the break and then stopped (Fig. 7*C*). With nerves which had been treated with 30 millimolar solution, in the intervals between "spontaneous" discharges, the response was also a tetanic one, but there were superimposed small irregularities and it stopped rather suddenly after several seconds although the current was continued for longer (e.g. Fig. 6*C*). In a few instances further activity occurred in the interval between the end of the main response and the break of the current. Presumably this activity was "spontaneous" as it did not appear on applying the test current again some minutes later. The duration of response at make was greater with greater current intensity. After a time the response of preparations whose nerves had been treated with either of the concentrations of tetra-ethylammonium iodide grew progressively more irregular (e.g. Fig. 6*F*, Fig. 7*F*) and, to currents of constant strength relative to the (make) threshold, shorter. Eventually, the response began like a tetanus and quickly resolved into small twitchings which became infrequent and stopped after one or a few seconds although the current was continued for longer (e.g. Fig. 7*G*). The transition from the earlier types of response to the later type was

generally more rapid in "spontaneously" active nerves or nerves which had been subjected to a number of test stimulations (1–2 hours) than in nerves which suffered little activity (2–3 hours). The duration of the later type of response was also dependent on the intensity of the applied

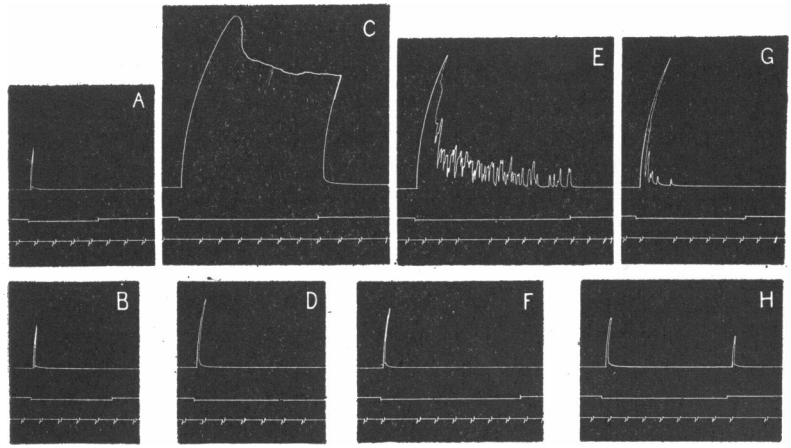

Fig. 7. The responses of a nerve-muscle preparation to constant currents applied to the nerve before and after it had been treated with tetra-ethylammonium iodide. The time markings are in seconds; depression of the signal line indicates closure of the current, rise indicates break of the current. The preparation was mounted after 1 hour 40 min. soaking in Ringer's solution. The make threshold remained steady at 1·4 μA. for an hour. The break threshold was about (9 × 1·4) μA. *A*, response to descending current three times make threshold, recorded 70 min. after mounting. *B*, response to ascending current, three times make threshold. *C*, response to descending current, five times make threshold (5 × 1·0) μA., recorded after the nerve had been immersed for 20 min. in 15 millimolar tetra-ethylammonium iodide Ringer's solution, and left mounted for 7 min. *D*, response to ascending current, five times make threshold. *E*, response to descending current, five times make threshold (5 × 0·8) μA., 20 min. after *C*. *F*, response to ascending current five times make threshold. During the next 90 min. the nerve was painted three times with fresh tetra-ethylammonium iodide solution. *G*, response to descending current, six times make threshold (6 × 0·6) μA., recorded 104 min. after *E*. *H*, response to ascending current, six times make threshold. At this time the break threshold was about (5·5 × 0·6) μA.

current. The greater the intensity: the longer did the initial tetanic part last before breaking up; the longer was the duration of the intermittent twitching; the shorter were the intervals between the earlier of the small twitches.

Excitation following break appeared only with intensities 5–7 times (make) threshold, and was very small (e.g. Fig. 6*E*). This difficulty of obtaining a response at break of descending current occurred with nerves

in which the break threshold had been reduced below, or made equal to, the make threshold, after treatment with 30 millimolar tetra-ethyl-ammonium iodide solution; with nerves in which the break threshold remained above the make threshold, after treatment with 15 millimolar solution (Fig. 7 *C* and *G*).

The effects of tetra-ethylammonium iodide on responses to ascending currents 2–7 times threshold.

Upon testing nerves at various times after treatment with 30 millimolar solution the muscle response following the make of an ascending current resembled qualitatively that following the make of a descending current in that it was a tetanic one which went on for a few seconds and then stopped quite suddenly although the current was maintained for longer, but differed quantitatively in that the duration and tension were both smaller than when the current was descending (cf. Fig. 6 *C* and *D*, Fig. 6 *E* and *F*). The response to ascending current lasted longer the greater the intensity of the current. The difference between the responses at make of ascending and of descending current was more marked with preparations whose nerves had been treated with 15 millimolar solution: the response with ascending current was of a size that would have been produced by a few maximal volleys in the untreated nerve (cf. Fig. 7 *C* and *D*, *E* and *F*, *G* and *H*).

Following the break of ascending current, preparations whose nerves had been treated with 30 millimolar solution gave a tetanic response which came to a well-marked stop, and it was seldom that any further excitation occurred for several seconds afterwards (e.g. Fig. 6 *F*). The duration of the break response was greater the greater had been the intensity of the applied current, but was always shorter than that of the make response preceding. For nerves which had been treated with 15 millimolar solution the ratio of break threshold to make threshold, although diminished, had values from 3 to 6, consequently break excitation was absent with the lower current intensities, and small with the higher intensities which we used (Fig. 7 *D*, *F* and *H*).

In the course of each experiment, as happened with the responses at make of descending current of constant intensity relative to the (make) threshold, the responses at make and at break of ascending current grew shorter and more irregular.

(i)

The possibility that failure of the transmission of excitation from nerve to muscle might have modified the tetanic responses described under the two preceding subheadings must be considered with respect to three phenomena.

(1) The relatively sudden ending of certain responses (e.g. Fig. 6*C* and *D*). The observed increase of duration of these responses with increase of applied current (which would have meant a greater initial frequency of nerve volleys owing to excitation earlier in the relative refractory period) is incompatible with junctional failure.

(2) The initial decline of tension in responses elicited by currents of high intensity (e.g. Fig. 6*C* and *D*, Fig. 7*C*). Probably this decline was due to partial failure of junctional transmission because with increased current it occurred earlier and was more marked.

[The simultaneous influence of the phenomena of 1 and 2 upon responses can perhaps be understood better by taking an example. In Fig. 6*D*, in the response following make of an ascending current three times threshold, there is a sudden fall of tension in the first second. A record of a response to a current five times threshold was made a few minutes after that shown in Fig. 6*D*. With the greater current the response lasted (the method of measurement is given below) 8·1 sec. against the 5·3 sec. of that in Fig. 6*D*, but the initial fall began earlier and amounted to about 30 p.c. of the maximum tension developed.]

(3) The intermittency and eventual failure in the later type of response. The effects of increase of current intensity on these indicate that they originated in the nerve and not in the neuromuscular junction. In the later responses apparently the initial tetanic part corresponds to synchronous excitation of the motor units of the preparation, and the smaller twitchings to asynchronous and infrequent excitation of single motor units or groups of motor units. Katz found that the response of sodium-citrate-treated nerve, which had been "spontaneously" active for some time, to constant current, became intermittent and failed rapidly. He has suggested that these events are due to lengthening of the refractory period, mainly at the point of stimulation [Bugnard & Hill, 1935].

(ii)

In two of our experiments with preparations whose nerves had been treated with 30 millimolar tetra-ethylammonium iodide solution attention was given to the effect of current intensity on the duration of repetitive response. Tests were begun soon after "spontaneous" activity appeared and continued as nearly in accordance with the following scheme as was consistent with avoiding the "spontaneous" outbursts. The response at make of a descending current was first recorded and then a few seconds later the response (if any) at break. About 3 min. later the responses at make and at break were recorded with the same current ascending. After another 5 min. the responses to a current of higher intensity were recorded.

Before stating our results it must be pointed out that the duration of excitation of the motor nerve fibres could most nearly have been measured as the interval between the beginning of the first and of the last action potential of the muscle; that we were concerned more with comparing the durations of excitation with currents of different strengths than with the absolute durations; that granting the interest of our experiments to lie mainly in the comparison, our use of the mechanical response of the muscle involves approximations which have to be described and examined. Although the ending of responses such as those of Fig. 6*C* and *D* was well marked, the beginning of the final fall of tension, which might have served as a rough indication of the end of excitation, was not sufficiently marked to be used for our measurements. Therefore, to obtain a well-defined end-point, even at the cost of including part of the relaxation, we have adopted the somewhat arbitary procedure of producing the line representing the final fall of tension until it cut the horizontal line of zero tension.

The two experiments yielded a practically linear relation between the logarithm of the current intensity relative to the threshold (I/I_0) and the duration of response (measured to our arbitrary end-point): at make of descending current; at make of ascending current; at break of ascending current. The results of one of the experiments (the one from which the records shown in Fig. 6 have been taken) are summarized in Fig. 8. It is to be noted that we have drawn the three straight lines in the figure as passing through the origin, although other straight lines lying equally well among the small number of experimental points and cutting off small positive intercepts on the ordinate axis might perhaps have

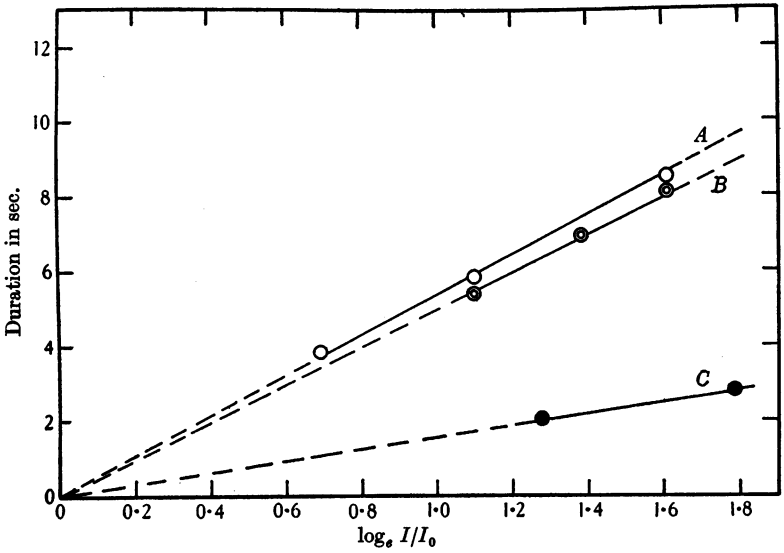

Fig. 8. The relation between the duration of repetitive response given by a nerve-muscle preparation, and the strength of current applied to the nerve, which had been treated with 30 millimolar tetra-ethylammonium iodide solution a short time before the tests. *A*, at make of descending current (strength relative to the make threshold). From the slope of the line, the time-constant of cessation of response at the cathode is 5·3 sec. *B*, at make of ascending current (strength relative to make threshold). *C*, at break of ascending current (strength relative to break threshold), whence the time-constant of cessation of response at the anode is 1·6 sec.

been drawn. It is impossible to say whether we have done rightly. An intercept would have been expected, on account of the part of the muscle relaxation process included in each of our measurements of duration, had not two other factors been operating: the well-known slowing of relaxation after activity [see Hill, 1931; Feng, 1931; Parkinson, 1933], which would have increased the slope of the line; the progressive shortening of the responses in the course of each experiment (see above and discussion later), which would have affected the later responses, to higher current intensities, more than the earlier ones, to lower current intensities, thus diminishing the slope of the line.

Discussion

The effects of tetra-ethylammonium iodide on "accommodation" and on threshold

Tetra-ethylammonium ions produce the same changes in frog nerve that removal of calcium ions produces. The experiments of Netter [1928], upon the action of various ions on the injury potential, indicate that the nerve membrane acts as an ionic sieve permeable only to cations below a certain size. He has concluded that the effective "mesh" of the sieve is affected little by changing the cations in the solution bathing the nerve. Netter found that neither tetra-ethylammonium ions nor calcium ions influenced the injury potential of frog nerve which had been previously soaked in glucose solution, and he has inferred that the nerve membrane is impermeable to these cations. Chemical studies also indicate that the nerve membrane is impermeable to calcium. Tipton [1934] has found that the calcium in nerve is present in a diffusible fraction, which he regards as present in the extracellular spaces, and an indiffusible fraction, which he regards as intracellular. We conclude, therefore, that the principal factor involved in the effects which we have studied is probably displacement by tetra-ethylammonium ions of calcium ions from the position that they normally occupy at the interface between the exterior of the nerve membrane and the surrounding solution.

Any more detailed explanation of the antagonism between calcium ions and tetra-ethylammonium ions necessitates answering two questions:

Why are the other cations of Ringer's solution, sodium and potassium, not involved in the same way that calcium is?

Why is tetra-ethylammonium the only symmetrical tetra-alkyl-ammonium cation that produces "spontaneous" activity?

An answer to the first of these is suggested by the work of Höber [1936] and of Wilbrandt [1937]. Since calcium ions and tetra-ethyl-ammonium ions are too large to penetrate the nerve membrane, and they are considerably more hydrophobic than sodium and potassium, their concentrations at the interface may well be greater than in the bulk of the surrounding solution. Increasing hydrophobic character in passing from lower to higher members of the series of tetra-alkylammonium ions [for estimates see Ing & Wright, 1931] must enter into the answer to the second question, but this factor alone cannot explain the uniqueness of the action of the tetra-ethylammonium ion. The other factor concerned is unknown. Extrapolation of conductivity data and calculation of the ionic radius in free solution by the Stokes-Einstein equation give

the values 2·78 Å. for the tetra-ethylammonium ion [Ing & Wright, 1933; Ing, 1936], and 3·0 Å. for the calcium ion [Landolt-Börnstein, 1927]. Whilst the approximate equality of these radii may be only a coincidence, it is possible that the other factor is size.

The effects of tetra-ethylammonium iodide on thresholds and on responses to constant current

Clearly, our findings involve more than a "reversal" of the normal electrotonic changes of excitability.

The dependence of the duration of repetitive response of nerve which had recently been treated with 30 millimolar solution upon current intensity, over a range of 2–7 times threshold, is a point of interest. Residual "accommodation" may have been one of the factors concerned in the ending of the response (and in the rarity with which "spontaneous" discharges occurred within a few seconds after the end of the response, if it be allowed that a part of a nerve remote from the point of stimulation can "accommodate" to its own action potential), although if any such "accommodation" were present in the quiescent intervals it was too small for us to measure. (Residual "accommodation" would hardly have been expected in view of our finding that the response of nerve which had been treated with 15 millimolar solution, to descending current, went on until break.) Probably a second factor contributing to the ending of the response and to the subsequent rarity of "spontaneous" discharges was a generalized failure of excitation ("refractoriness" or "fatiguability"). Such a failure, however, would not explain why, when the break threshold was below the make threshold, the duration of response at break of ascending current was shorter than that of the response at make with the same current descending. Local failure of excitation, which developed more rapidly at the anode following the break of current than at the cathode following the make of current, appears to have been the principal factor operating.

If it be assumed that for the response at make to be smaller when a current of a certain intensity is ascending nerve than when the current is descending indicates that impulses starting from the cathode, when more distal, are partly blocked in the anodic region, then in our experiments with Ringer-soaked preparations the independence of the size of muscle response of current direction indicates that the condition of the nerves was one in which currents of intensity up to seven times (make) threshold did not cause any block at the anode. Comparison of the responses given by the same preparations at make of descending and of

ascending current of 2–7 times threshold intensity after their nerves had been treated with 15 or with 30 millimolar solution indicates that then, however, the anode exerted a weak blocking action.

If an explanation similar to that above be assumed for the failure of preparations whose nerves had recently been treated with 30 millimolar solution to give responses with any but the highest intensities of descending current, it is to be concluded that an almost complete block was built up at the cathode in the few seconds which elapsed between make and break of current.

When the small irregularities which were superimposed on the earlier responses are taken into account it becomes evident that the local failures of excitation postulated in the three preceding paragraphs would have to be made up of two components: a depression (at the anode or at the cathode) developing smoothly after the application of current, and dependent upon the intensity of the current and upon the concentration of tetra-ethylammonium ions with which the nerve had been treated; a local prolongation of the relative refractory period at the point of stimulation. The transition to the intermittency and rapid failure exhibited in the later type of response would be accounted for by a progressive increase in the importance of the second factor.

The following observations by other workers suggest that certain of the effects of potassium ions are qualitatively similar to those of tetra-ethylammonium ions: the threshold for excitation at break of constant current was lowered relative to the threshold for excitation at make [Biedermann, 1898]; during the passage of small polarizing currents the threshold for excitation by superimposed long-lasting current was increased at the cathode and diminished in some, but not in all, instances at the anode [Chweitzer, 1935, 1937; Bouman, 1937]; during the passage of larger polarizing currents the threshold for superimposed long-lasting currents was increased at the anode and at the cathode [Chweitzer, 1935, 1937]. Should these apparent similarities in the effects of the two cations prove to be real they may be of interest on account of certain similarities in their chemical and physical properties.

Katz has recorded that treatment of nerve with sufficient sodium citrate to produce "spontaneous" activity reduced the rheobase to 1/8 to 1/10 of its initial value. In our experiments the action of tetra-ethylammonium ions on the electrotonic changes of excitability probably accounts for the smaller fall observed—1/4 to 1/8 of the initial value. So far as we know sodium citrate is without influence on the electrotonic changes of excitability of nerve.

SUMMARY

1. Treatment of frog nerve with Ringer's solution containing tetra-ethylammonium iodide (10 millimols per litre) produced a twofold change in the action potential: a prolongation of the negative after potential; a repetitive discharge in response to a single shock. Greater concentrations of tetra-ethylammonium iodide (15 millimols per litre and more) caused "spontaneous" asynchronous activity in nerve.

2. According to Hill's [1936] theory of excitation repetitive response in nerve is due to an increase in the time-constant of "accommodation" (λ) of the tissue. In these experiments this time-constant was measured and found to increase with increase in the concentration of tetra-ethylammonium iodide applied to the nerve.

3. Treatment of nerve with tetra-ethylammonium iodide-Ringer solution reduced the rheobase considerably below its normal value.

4. The "spontaneous" activity produced in nerve by tetra-ethyl-ammonium ions is the limiting result of lowering of the rheobase and an increase of the time-constant of "accommodation".

5. The changes in the time-constant of "accommodation" and rheobase could be prevented or reversed by an adequate concentration of calcium ions.

6. Tetra-ethylammonium ions reduced the threshold for excitation at break of constant current to a greater extent than the threshold for excitation at make. From examination of the responses of tetra-ethyl-ammonium iodide-treated nerve to constant currents 2–7 times make threshold it is inferred that local depressions of excitability were produced at the anode and at the cathode.

Our sincere thanks are due to Prof. Sir Joseph Barcroft and to Mr E. B. Verney for the facilities placed at our disposal in their laboratories at Cambridge, where the greater part of this work was done. We are similarly indebted to Prof. J. H. Gaddum for facilities which enabled one of us (S.L.C.) to finish the work in London.

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