ON THE EFFECT OF AMMONIUM AND LITHIUM IONS UPON FROG NERVE DEPRIVED OF SODIUM

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Overton demonstrated in 1902 (13) that sodium ions are necessary to maintain the excitability of the nerve fibers, since (*a*) the nerve fibers become inexcitable if the concentration of sodium ions in their external medium is decreased below a certain value (about 0.012 N) and (*b*) the excitability of the nerve fibers can be restored by increasing the external concentration of sodium ions. In recent years the role of sodium in nerve function has been analyzed by Lorente de Nó (6–12) and by Gallego (3) with frog nerve, and by Hodgkin and Katz (5) with invertebrate nerve fibers.

Overton also demonstrated that lithium ions can substitute for sodium ions in so far as they can restore the excitability of nerve fibers that have become inexcitable in a sodium-free medium; the restoring action of lithium ions, however, is only temporary since the continued presence of lithium ions in the external medium again renders the nerve fibers inexcitable. The ability of lithium ions to substitute for sodium ions has been confirmed by Hodgkin and Katz who worked with the giant axon of the squid.

In this paper an analysis is made of the effect of lithium ions upon frog nerve deprived of sodium. It has been found that lithium ions can restore the excitability of fibers of fast conduction (A fibers in Erlanger and Gasser's classification, *cf.* Erlanger, 1) as well as the excitability of fibers of slow conduction (C fibers in Erlanger and Gasser's classification). The effect of lithium ions upon sodium-deficient B fibers has not been investigated. On the other hand, it has been found that lithium ions cannot substitute for sodium ions in other aspects of nerve function, as should be expected from the observation made by Overton that prolonged action of lithium ions renders the nerve fibers inexcitable, and from the observation made by Gallego and Lorente de Nó (4) that lithium ions when present in the external medium of the nerve fibers at a high concentration cause a depolarization of the nerve fibers.

According to Overton (13) ammonium ions are not able to restore the excitability of nerve deprived of sodium. Reexamination of this question seemed desirable, however, because Overton worked only with fibers of the A group (motor fibers) while Lorente de Nó (8) has shown that a number of quaternary ammonium ions, which are not able to restore the excitability of A fibers,

restore the excitability of fibers of the B and C groups. For the reason that among the simple ones only quaternary ammonium ions having two or more ethyl groups restore the excitability of sodium-deficient nerve fibers it was not expected that ammonium ions would be able to substitute for sodium ions and restore the excitability of Et fibers. In fact, experiments to be reported in this paper have shown that ammonium ions cannot substitute for sodium ions.

I

Technique

Since the technique has been described in detail elsewhere (8, 10) it will be sufficient to give here an outline of the procedures that have been used.

The nerves were allowed to become inexcitable in a large volume of a sodium-free medium (0.11 M diethanoldimethylammonium chloride) which was continuously renewed and stirred during the initial 4 hours, and several hours after total inexcitability had developed the nerves were mounted in humid chambers. The excitability of the central segments of the nerves was restored with Ringer's solution (practically 0.1 M sodium chloride); no less than 1 hour was allowed for the recovery to become complete. After tests had been made of the inability of the impulses initiated in the restored central segments to propagate themselves into the peripheral (sodium-free) segments a solution containing lithium or ammonium ions was placed in contact with the peripheral segment and the restoration of excitability was followed at short intervals of time by recording the action potential of those impulses which had become able to propagate themselves into the peripheral segment.

Recordings were also made of the electrotonic potentials produced in the peripheral segments by rectangular pulses of applied currents, before and after the application of the solutions containing lithium or ammonium ions.

Measurements of changes in the value of the membrane potential (Fig. 3) were made with the technique previously described (4, 7).

II

Ammonium Ions

For the reason that ammonium ions at the concentrations 0.05 and 0.1 N are powerful depolarizing agents (4) precautions had to be taken to prevent the depolarizing action of ammonium ions from masking the effect that these ions could have upon the excitability of the nerve fibers.

Observations were made with the use of ammonium ions at the concentration 0.025 N or 0.03 N with nerves that had been treated with sodium ions at the concentration 0.015 or 0.02 N until the C fibers and a small number of A fibers had regained the ability to conduct impulses. Under conditions such as these 0.025 N or 0.03 N sodium ions would have produced important increases in the

number of conducting fibers within 5 seconds (*cf.* reference 10, Fig. 3; reference 12, Fig. 6). However, solutions containing ammonium ions at the concentration 0.025 N or 0.03 N (25 or 30 parts 0.1 M ammonium chloride, 75 or 70 parts sodium-free solution) did not increase the number of conducting fibers, which shows that ammonium cannot substitute for sodium and restore the excitability of nerve fibers. In addition, those fibers which had already been restored by sodium ions became inexcitable, which shows that ammonium ions cannot maintain the excitability of the nerve fibers.

During the experiments, however, the definite impression was gained that in the presence of ammonium ions the rate of development of inexcitability was less than it would have been in the ordinary sodium-free medium (0.11 M diethanoldimethylammonium chloride). It is obvious that before this impression can become a certainty carefully controlled experiments must be carried out; if it could be shown, however, that in the absence of external sodium ions ammonium ions can delay the onset of inexcitability a fact of considerable importance would have been discovered.

Ammonium ions cannot substitute for sodium ions in the establishment of the nerve reaction. In the presence of ammonium ions the magnitude of the L fraction and the efficiency of the nerve reaction continue to decrease, as fast or somewhat faster than in the inert sodium-free medium. In this respect the effect of ammonium ions upon nerve deprived of sodium is similar to the effect of lithium ions (see below).

III

Lithium Ions

Excitability of the Nerve Fibers.—As was described by Overton (13) the effect of lithium ions upon the excitability of sodium-free nerve develops in two phases: during the first phase lithium ions restore to the nerve fibers the ability to conduct impulses; during the second phase lithium ions again render the nerve fibers inexcitable.

Fig. 1 illustrates the results of a typical experiment that was done with the use of 0.11 M lithium chloride. The observations were begun after the peripheral segment of the nerve had been kept in the sodium-free medium for over 20 hours; *i.e.*, for several hours after all the fibers of the nerve had become inexcitable. Records *1* and *2* show that the impulses initiated in the restored central segment were unable to propagate themselves into the peripheral segment. Record *1* was obtained with the use of a rectangular pulse of cathodal current (100 μa.) of duration sufficient to stimulate all the fibers of fast conduction (A fibers) and record *2* with a pulse of duration sufficient to stimulate all the fibers (A, B, C) of the nerve. In record *1* there is no sign of a conducted spike, since there appears after the shock deflection only a small deflection that

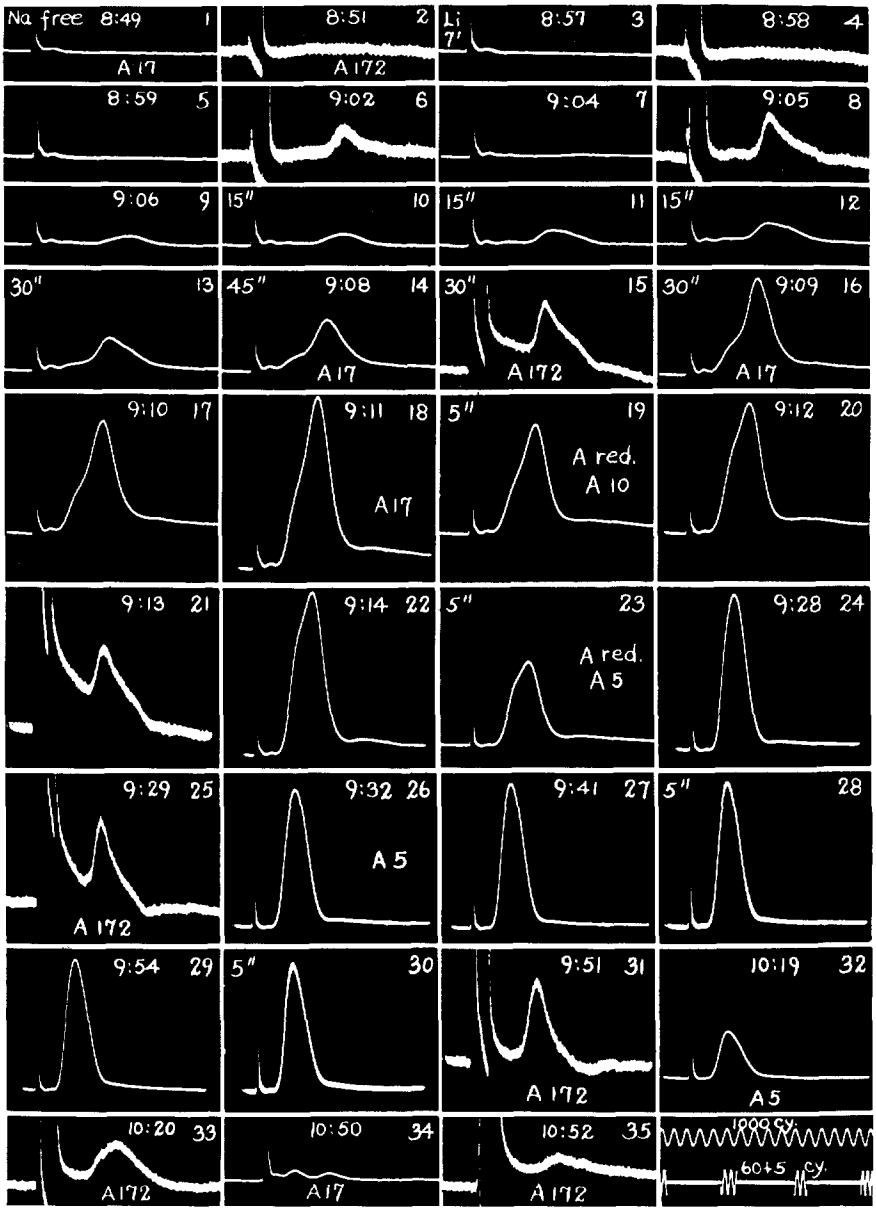

FIG. 1. Effect of 0.11 M lithium chloride upon a nerve that had been kept in a sodium-free medium for slightly over 20 hours. The restoring solution was applied to the nerve at 8:50 p.m. The times at which the individual records were obtained are given with the records either directly or by giving in seconds in the upper left corner of the records the intervals of time elapsed between successive records. Records *28*, *30* were obtained with the use of repetitive stimulation at the frequency of approximately 50 per second. In this and in the following figures the amplification (*A 17*, *A 172*, etc.) is given in millimeter deflection per millivolt input when the distance between consecutive vertical lines separating records measures 50 mm. The C spikes were recorded at constant amplification (172 mm. per mv.). The 1000 cycles time line applies to the A spikes; the 60 cycles time line, to the C spikes.

was referable to electrotonic spread of the action potential of impulses blocked at the margin of the peripheral segment of the nerve, nor can a sign of a conducted spike be detected in record *2*.

The peripheral segment of the nerve was placed in contact with 0.11 M lithium chloride at 8:50 p.m. No conducted response was observed during the first 9 minutes (records *3* to *5*) but after 12 minutes a considerable number of C fibers had become able to conduct impulses (record *6*); and 2 minutes later also a few A fibers had become able to conduct (record *7*). The recovery then proceeded with remarkable rapidity: records *6*, *8*, and *15* illustrate the rapid growth of the C spike and records *9* to *14* and *16* to *18* the rapid growth of the A spike. Further increases in the conducted A spike, *i.e.* in the number of conducting A fibers, were observed for several additional minutes until after 35 minutes of the action of lithium ions the A spike reached the relatively great height with which it appears in record *24*. Thereafter the A spike decreased progressively in size (records *26* to *30* and *32*) and finally, after 2 hours of the action of lithium ions, only an exceedingly small number of A fibers were still able to conduct impulses (record *34*). It may be estimated that when the A spike passed through its maximal height (record *24*) this height was only 60 per cent of the height that would have been observed if the excitability had been restored by sodium ions, which in all probability means that not all the A fibers had become excitable before the deleterious effect of lithium ions began to render some fibers inexcitable.

The C spike continued its increase after the A spike had begun to decrease (records *21*, *25*, and *31*); it reached practically the same height that it would have reached in Ringer's solution; ultimately, however, also the C spike underwent a progressive decrease (records *33* and *35*), which continued until all the C fibers became inexcitable.

An important detail to be observed in Fig. 1 is that during the initial stages of the recovery the nerve fibers conducted impulses at a very low speed, for which reason the shock spike times were relatively very long. With advancing recovery the speed of conduction of the restored fibers increased, but even at the time when the conducted spikes passed through their maximal heights (Fig. 1, *24*, *31*) the speed of conduction still was markedly subnormal. According to observations presented elsewhere (3, 10) if the restoration of excitability had been effected by means of sodium ions the normal speed of conduction would have been approached in about 60 minutes.

If lithium ions at the concentration 0.11 N are allowed to act upon freshly excised nerve for several hours the changes produced in the nerve fibers are not wholly reversible by transfer of the nerve to Ringer's solution (4). The deleterious effect of 0.11 M lithium chloride develops faster if the nerve has been previously deprived of sodium for 15 to 20 hours, and complete recovery in Ringer's solution is not obtained if the lithium ions are allowed to act upon the

nerve for longer than 2 to 3 hours. The series of records reproduced in Fig. 2 demonstrate that the inexcitability caused by 0.11 M lithium chloride acting for 90 minutes is fully reversible by 0.1 M sodium chloride (*cf.* also below, Figs. 4 to 6). Records *1* to *11* and *13* of Fig. 2 illustrate the restoration of excitability

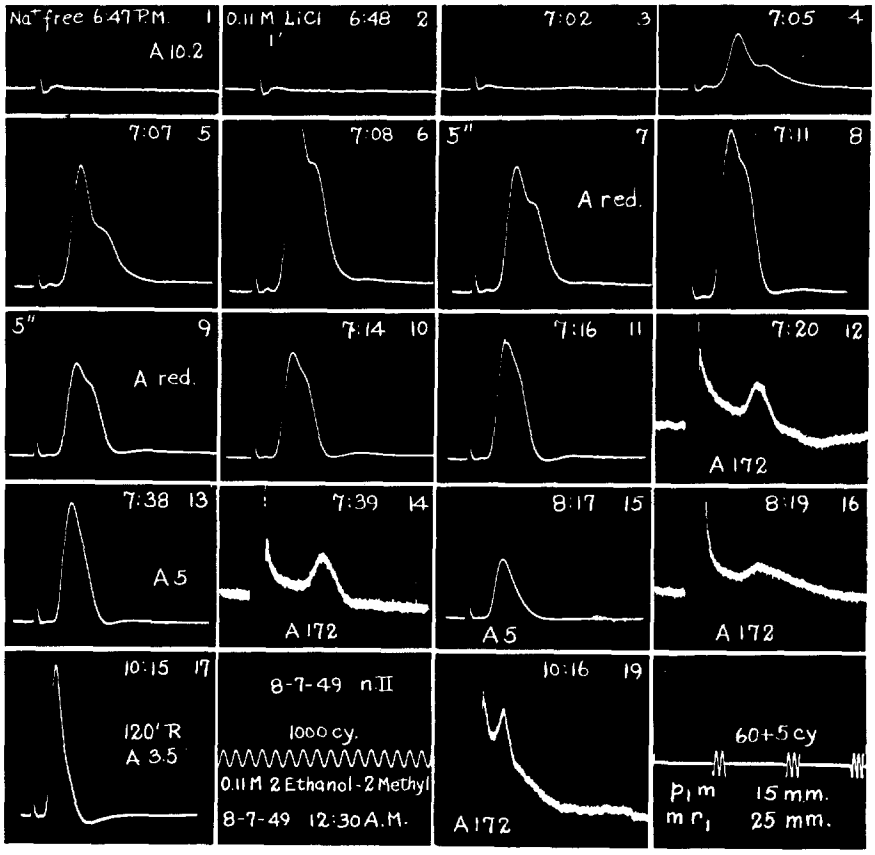

FIG. 2. Effect of 0.11 M lithium chloride upon a nerve that had been deprived of sodium for slightly over 18 hours (records *1* to *16*). Records *17* and *19* present the A and the C spikes after restoration by Ringer's solution. *R* denotes Ringer's solution.

of sodium-deficient A fibers by lithium ions and records *12* and *14* present the restored C spikes. After the continued action of lithium ions had decreased the heights of the spikes to the sizes with which they appear in records *15* (A spike) and *16* (C spike) the nerve was placed in contact with Ringer's solution, which resulted in a total recovery of the function of the nerve fibers. Records *17* and *19* that were obtained 2 hours after records *15* and *16* present the A and C spikes with full heights and with normal shock spike times.

Membrane Potential.—Since lithium ions ultimately cause a depolarization of the nerve fibers it would be reasonable to assume that the inexcitability that develops after the restoration of sodium-free nerve by lithium ions is referable to a depolarization of the nerve fibers. Such an assumption, however, encounters the difficulty that with freshly excised nerve the action of lithium ions develops in two phases (Fig. 3, *2*): during the first phase the membrane potential undergoes an increase and during the second phase, a decrease.[1] According to the demarcation potential curve (Fig. 3, *2*) it should be expected that A fibers restored by lithium ions would begin to become inexcitable not earlier than after about 3 hours, *i.e.* when the absolute value of the A-B demarcation potential measures 4–5 mv., unless the depolarizing action of lithium ions should begin earlier with sodium-free nerve than with freshly excised nerve.

Curves *1* and *2* of Fig. 3 establish a comparison between the action of 0.11 M lithium chloride upon nerve kept in the sodium-free medium for 19 hours (curve *1*) and upon freshly excised nerve (curve *2*). It should be mentioned that nerves kept in 0.11 diethanoldimethylammonium chloride maintain their membrane potential at the same level as nerves kept in Ringer's solution (8).

As can readily be noted the action of 0.11 M lithium chloride upon sodium-free nerve has a temporal course of its own. The initial phase of hyperpolarization is not produced; instead there appears a continuous phase of progressive depolarization beginning a few minutes after the nerve is placed in contact with the test solution. This change in the mode of action of lithium ions is wholly referable to the lack of sodium. Curves *3* and *4* of Fig. 3 were obtained with two nerves taken from the same bullfrog. One nerve (curve *3*) was kept for 15 hours in the sodium-free medium and the other nerve (curve *4*), in Ringer's solution for the same length of time. In curve *4* there appear the two phases (hyperpolarization, depolarization) that are customarily observed with freshly excised nerve. Curve *3* shows again that with sodium-free nerve lithium ions produce only a continuous phase of depolarization.

A plausible explanation of the change in the mode of action of lithium ions seems to be this. With freshly excised nerve the phase of hyperpolarization is produced during the time that lithium ions are penetrating into the nerve fibers and displacing sodium ions; the phase of depolarization begins when a significant number of internal sodium ions have been replaced by lithium ions. In the

[1] In comparing the curves reproduced in Fig. 3 with previously published curves (4, fig. 5) attention should be given to the fact that the curves reproduced in Fig. 3 were obtained with bullfrog (*R. catesbiana*) nerves, while the published curves (4, fig. 5) were obtained with *R. sphenocephala* nerves. The mode of action of substances upon the membrane potential has been found to be the same with *R. catesbiana* and with *R. sphenocephala* nerves; the absolute magnitude of the demarcation potentials, however, is usually greater with *R. sphenocephala* nerves. Knowledge of this detail may be useful in the planning of experiments.

absence of sodium, lithium ions are able to initiate the depolarization without delay.

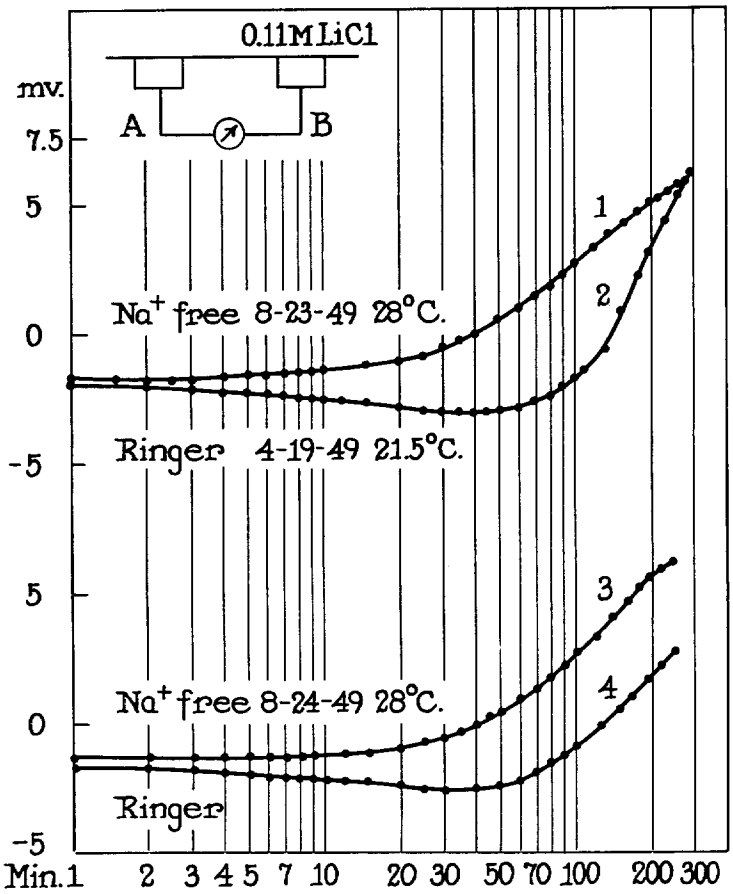

FIG. 3. Demarcation potentials resulting from the action of 0.11 M lithium chloride upon the *B* segment of the nerve. *1*, nerve kept in the sodium-free medium for 19 hours; *2*, freshly excised nerve, *3*, nerve kept in the sodium-free medium for 15 hours; *4*, nerve kept in Ringer's solution for 15 hours. The A segments were kept in the sodium-free medium (curves *1* and *3*) or in Ringer's solution (curves *2* and *4*).

Whether or not the decrease in the value of the membrane potential is the direct cause of the inexcitability is a question that cannot be answered solely by considering the magnitude of the demarcation potentials measured by curves *1* and *3* of Fig. 3. With recently excised nerve a depolarization by the amounts indicated by curves *1* and *3* at 120 minutes would not be sufficient to reach the critical excitability level of the membrane potential (*cf.* reference 7, pt. 1,

page 51) in all the A fibers but the situation in the case of sodium-free nerve may be different, in so far as a small depolarization may be sufficient to cause inexcitability. There is, however, a reason to believe that the inexcitability is not due solely to the depolarization. If, in addition to the depolarization, lithium ions had not produced an especial change in the nerve membrane, an increase in the value of the membrane potential created by an externally applied anodal current would have restored to the nerve fibers the ability to conduct impulses (*cf.* references 2, 7), and contrary to this expectation nerve fibers that have become inexcitable in the presence of lithium ions do not respond readily to the break of the anodal current. On the other hand, the inexcitability produced by lithium ions soon becomes irreversible. Therefore, it is necessary to conclude that, although the depolarization created by lithium ions may contribute to produce the inexcitability, the action of lithium ions results in especial changes in the properties of the nerve membrane. An important change can be detected by means of a study of the electrotonic potentials created by applied currents.

Electrotonic Potentials.—The effect of lithium ions upon the electrotonic potentials of nerve deprived of sodium is precisely the opposite of the effect which is produced by sodium ions and by those quaternary ammonium ions which are able to substitute for sodium.

According to available information (7, chapter VIII; 8, section 9), in nerves that have become inexcitable in a sodium-free medium the L fraction of the membrane potential has a subnormal value, the polarizability of the membrane by the applied anodal current is less than in normal nerve, and the nerve reaction is established in a defective manner, the inefficiency of the nerve reaction being the reason why (*a*) the slow electrotonus does not display overshootings after the end of the applied current and (*b*) the slow anelectrotonus does not pass through a maximum if the applied current is small; in the advanced stages the slow anelectrotonus does not display a maximum even when the applied current is large. Sodium ions and the quaternary ammonium ions of the restoring type increase the L fraction of the membrane potential and the polarizability of the membrane by applied currents; they also increase the efficiency of the nerve reaction. Lithium, on the contrary, decreases the L fraction and the polarizability of the membrane and reduces the effectiveness of the nerve reaction.

Figs. 4 to 6 illustrate the effect of lithium upon nerve deprived of sodium. Records *1* to *12* of Fig. 4 present the electrotonic potentials that were observed with the sodium-free nerve. They should be compared with records *9* to *20* of Fig. 6 that were obtained after restoration of the nerve by means of sodium ions. After the restoration the electrotonic potentials were approximately normal; *i.e.*, they were approximately such as would have been observed if the nerve had been kept in Ringer's solution all the time.

At the time when the observations were begun the L fraction of the membrane potential still had a considerable value, as is shown by the relatively large slow

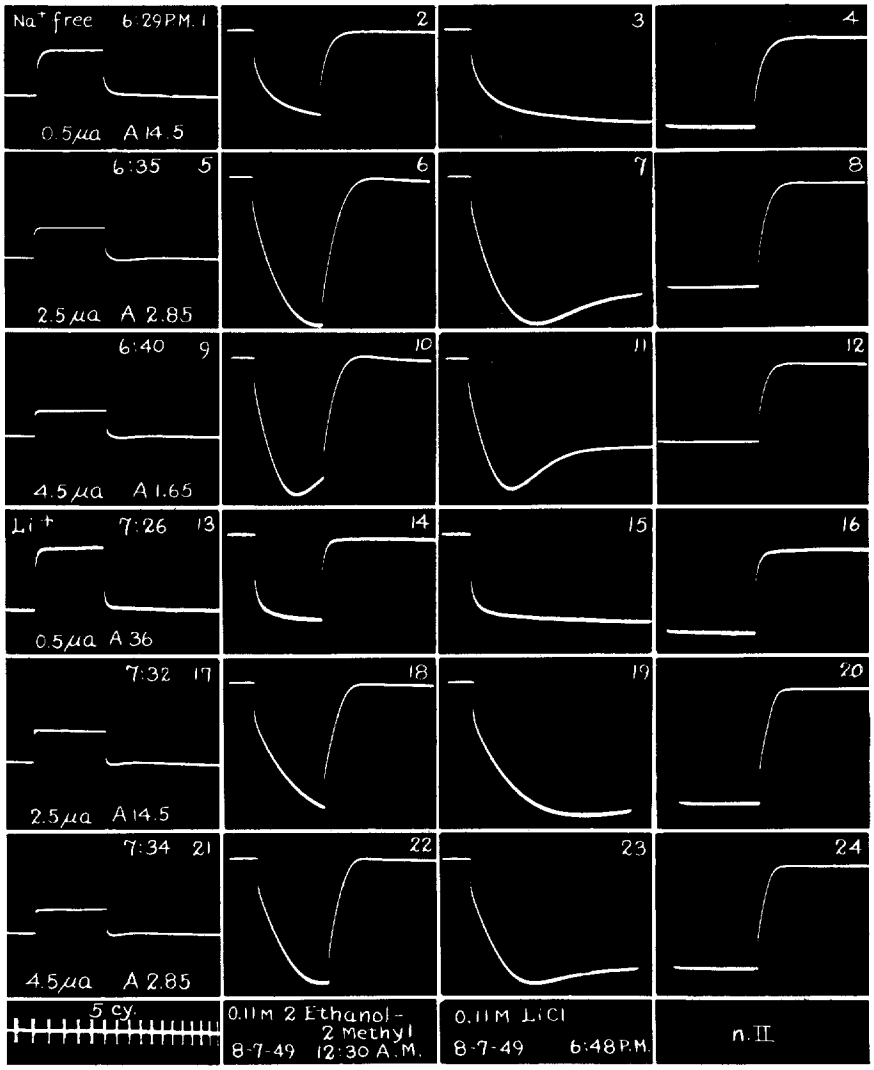

FIG. 4 (continuation of Fig. 2). Electrotonic potentials that were produced by rectangular pulses of current in the peripheral segment of the nerve at 5 mm. from the polarizing electrode (*cf.* fig. *1*, I in reference 10) with the nerve in the sodium-free medium (records *1* to *12*) and after 88 minutes of the action of lithium ions (records *13* to *24*).

component of the electrotonus in records *1*, *5*, and *9* of Fig. 4. The polarizability of the membrane of the anodal current also was relatively high, since the anelectrotonus had large slow components. That the nerve reaction, however,

was established in a deficient manner is proven by the fact that the overshootings of the slow anelectrotonus (Fig. 4, *2*, *4; 6*, *8; 10*, *12*) were considerably smaller than in nerve treated with sodium (Fig. 6, *10*, *12; 14*, *16; 18*, *20*), or absent; nevertheless, the nerve reaction still was sufficiently intense to produce small overshootings after the end of cathodal pulses (Fig. 4, *1*, *5*, *9*) and maxima in the anelectrotonus created by relatively large currents (2.5 μa., records *6*, *7;* 4.5 μa., records *10*, *11*).

After 40 minutes of the action of lithium ions and before the nerve fibers had begun to become inexcitable (*cf.* Fig. 2, *11* and *13*) the electrotonic potentials were found to have undergone important changes (Fig. 4, *13* to *24*). The L fraction of the membrane potential had decreased, since the slow catelectrotonus displayed only a very small height (Fig. 4, *13*, *17*, *21*); the polarizability of the membrane by the anodal current had decreased, and the efficiency of the nerve reaction had become less, since the maxima of the anelectrotonus (records *18*, *19; 22*, *23*) were less pronounced than they had been previously (records *6*, *7; 10*, *11*). In comparing the two sets of records of Fig. 4, *1* to *12* and *13* to *24*, attention should be given to the ratio of the heights of the fast and slow components of the electrotonus, rather than to the absolute magnitude of the deflections. When the nerve was placed in contact with the 0.11 M solution of lithium chloride the external longitudinal resistance of the nerve fibers decreased and therefore the total height of the electrotonic potential decreased; the ratio of the height of the fast component to the height of the slow component of course remained practically unchanged. The absolute magnitudes of the deflections may be compared without danger of significant error in the records obtained after the application of the lithium solution (Fig. 4, *13* to *24* and Figs. 5, 6).

One hour later, when a significant number of nerve fibers had become inexcitable (*cf.* Fig. 2, *15*, *16*), the progressive change in the properties of the nerve fibers had reached an advanced stage. The catelectrotonus (Fig. 5, *1*, *5*, *9*) did not display a slow component, which shows that the L fraction of the membrane potential had decreased to a negligible value. Also the polarizability of the membrane by the anodal current had decreased markedly, since the 0.5 μa. current was hardly able to produce slow anelectrotonus (Fig. 5, *2*) and even the 2.5 μa. current created only a small anelectrotonus (Fig. 5, *6*) that increased continuously during the flow of the applied current. The fact that the anelectrotonus increased continuously during the polarization proves that the continued presence of lithium ions had deprived the nerve fibers of their ability to establish the nerve reaction.

In spite of the low polarizability of the membrane the 4.5 μa. current was able to create a large slow anelectrotonus; the manner, however, in which the anelectrotonus was established was highly abnormal and closely resembled the manner in which the anelectrotonus is established by large current in nerves

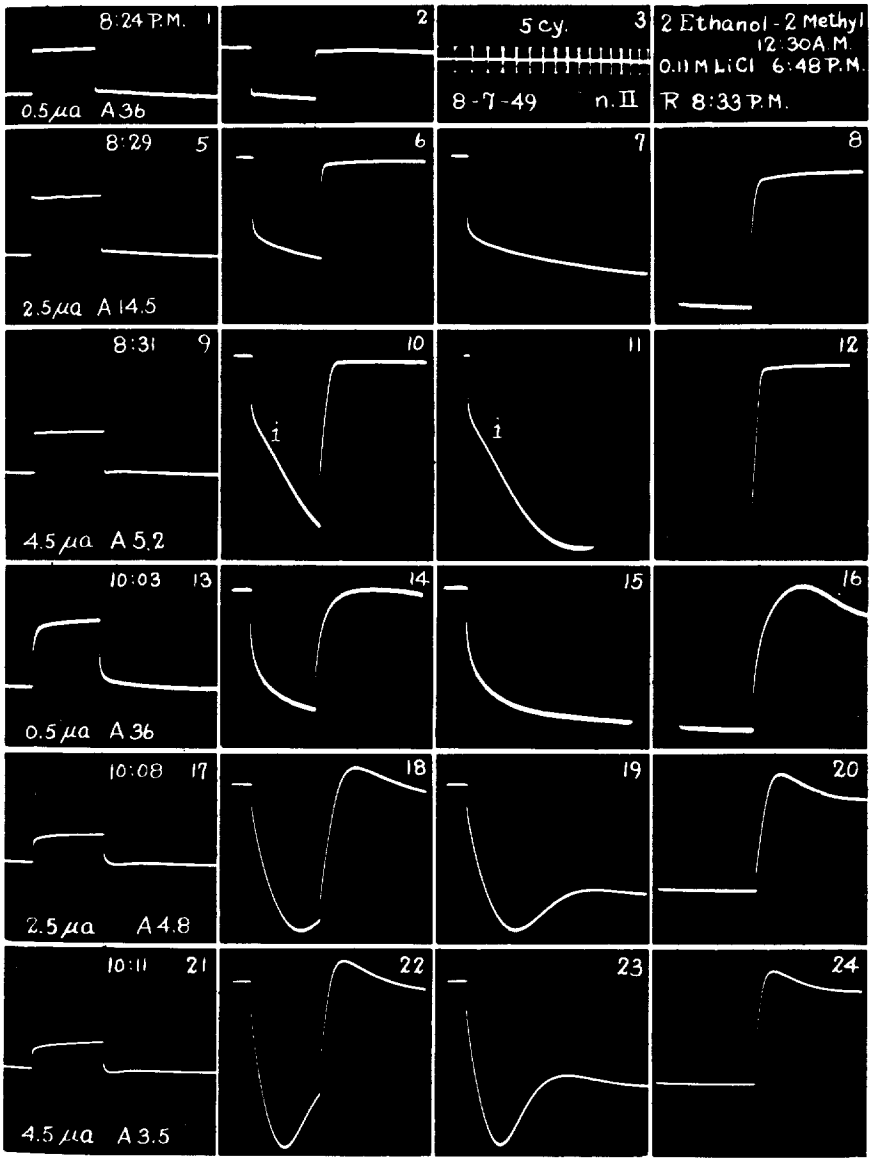

FIG. 5 (continuation of Fig. 4). Electrotonic potentials recorded after 96 minutes of the action of 0.11 M lithium chloride (records *1* to *12*) and after restoration by Ringer's solution acting for 90 minutes (records *13* to *24*).

in an advanced stage of the effect of lack of sodium (*cf.* reference 8, page 103). During the establishment of the slow anelectrotonus the tracing displayed an

inflection point, marked by the letters (*i*) in Fig. 5, *10*, *11*, which indicated that the flow of the anodal current up to the inflection point had been sufficient partially to restore the properties of the membrane, whereby the polarizability of the membrane by the anodal current, or otherwise stated the ability of the anodal current to increase the L fraction of the membrane potential had been enhanced.

Thus it appears that lithium ions acting upon nerve deprived of sodium produce in the electrotonic potentials precisely those changes which would ultimately have developed if the nerve had been left in the inert sodium-free medium (0.11 M diethanoldimethylammonium chloride) for a longer period of time. Therefore, the effect of lithium ions upon nerve deprived of sodium is very remarkable. In so far as the production of the nerve impulse is concerned lithium ions substitute for sodium ions and restore the excitability of the nerve fibers, but in regard to the constitution of the membrane potential and to the regulation of changes in the value of this potential, which is the operation of the nerve reaction, lithium ions cannot substitute for sodium ions, indeed, in the presence of lithium ions the nerve fibers undergo at an increased rate those changes which would have taken place in an inert sodium-free medium. The assumption is likely that those changes, rather than the decrease in the total value of the membrane potential (Fig. 3), are the direct cause of the inexcitability that develops if the presence of lithium ions is maintained after the restoration of excitability has been effected.

The changes in the properties of the nerve fibers which are demonstrated by the analysis of the electrotonic potentials are fully reversible by sodium ions, provided only that these ions are made available to the nerve before the changes have advanced very far. Records *13* to *24* of Fig. 5 were obtained 90 minutes after the nerve had been placed in contact with Ringer's solution. At the end of this time the L fraction of the membrane potential had acquired a relatively large value, as is shown by the height of the slow component of the catelectrotonus (Fig. 5, *13*, *17*, *21*); the polarizability of the membrane by the anodal current had been largely restored, since the anelectrotonus displayed large slow components (Fig. 5, *14* to *16*, *18* to *20*, and *22* to *24*), and the nerve fibers had regained the ability to produce the nerve reaction with a great deal of efficiency, since in all cases the electrotonic potential displayed an overshooting after the end of the applied current, and in the cases of the 2.5 μa. and 4.5 μa. currents the slow anelectrotonus displayed sharp maxima during the flow of the applied current (Fig. 5, *18*, *19; 22*, *23*). Only the lack of a maximum in the anelectrotonus created by the 0.5 μa. current (Fig. 5, *14* to *16*) was a sign that the nerve reaction had not recovered its full efficiency. Nevertheless, at that time the recovery of excitability was approaching completion (Fig. 2, *17*, *19;* note the times on the records).

After 150 minutes of the action of sodium ions the recovery was already

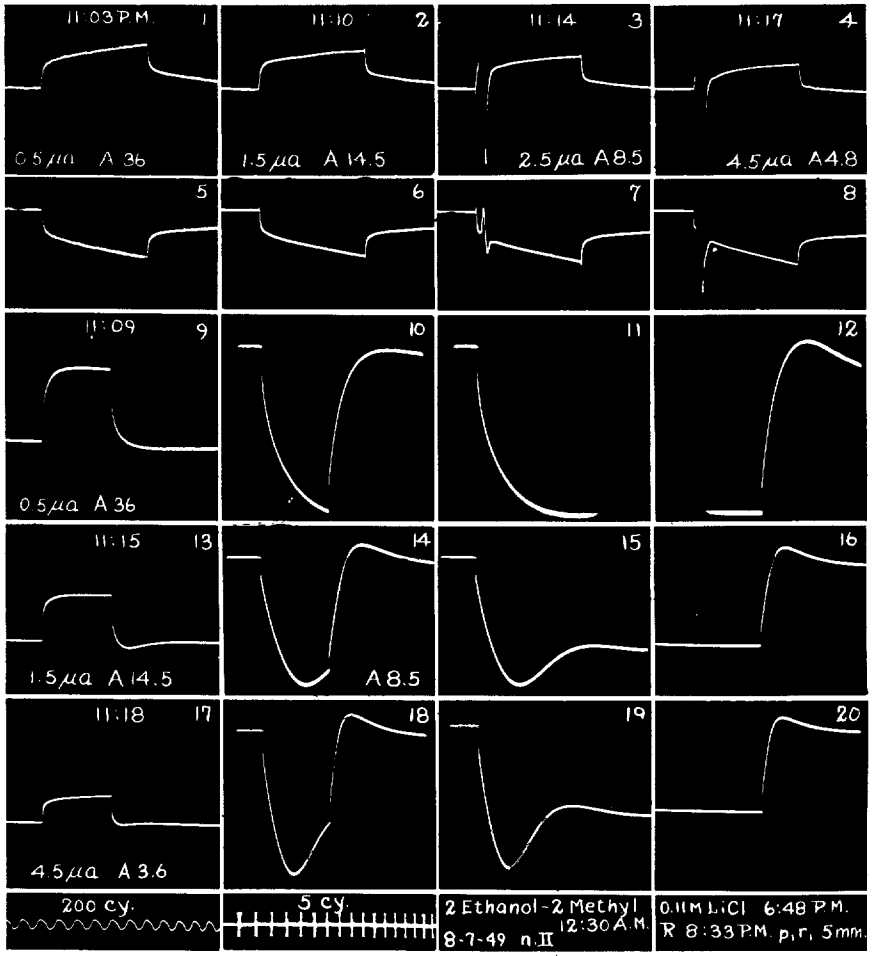

FIG. 6 (continuation of Fig. 5). Electronic potentials recorded after the completion of the recovery by Ringer's solution. The 200 cycles time line applies to records *1* to *8*. Shortly after the make and shortly after the break of the applied current the deflections in records *1* to *8* display a sudden change in slope; the heights of the deflections at the times when the changes occurred are equal to the heights of the discontinuities in records *9* to *20;* they measure the fast electrotonus. Note that in the case of records *3*, *4* and *7*, *8* the make of the current initiated impulses at the cathode of the polarizing circuit.

complete, since the electrotonic potentials (Fig. 6) were such as are regularly observed with nerves that have been kept in Ringer's solution in an atmosphere of oxygen for 20 to 24 hours. The large slow components in the catelectrotonus (Fig. 6, *9*, *13*, 17) corresponded to the large value that the L fraction of the

membrane potential had acquired. The increase in the efficiency of the nerve reaction revealed itself in the greater magnitude of the postcathodal overshootings (Fig. 6, *9*, *13*, *17*) and in the increased sharpness of the maxima and overshootings of the anelectrotonus (Fig. 6, *10* to *12*, *14* to *16*, *18* to *20*). Even when the 0.5 μa. current was used the anelectrotonus passed through a maximum, which although of small magnitude was unmistakable (Fig. 6, *11*, *12*).

IV

DISCUSSION

The fact that ammonium ions cannot substitute for sodium ions and restore the excitability of nerve fibers needs no comment. It only confirms the general rule that, at least among the simple ones, only quaternary ammonium ions that have two or more ethyl groups can substitute for sodium (8). The effect of lithium, however, deserves detailed analysis.

In the first place, the fact deserves consideration that, when acting upon a nerve that has been totally inexcitable in a sodium-free medium for several hours, lithium ions do not begin to restore the excitability of the nerve fibers until about 10 minutes after the test solution has been placed in contact with the nerve (Fig. 1, *6*, *7*). In this respect lithium ions are not different from sodium or from quaternary ammonium ions of the restoring type (8). That the latent period of the restoration is not referable to slow diffusion of the restoring ions through the epineurium into the nerve is made quite unlikely by the fact that C fibers recover their excitability earlier than A fibers (Fig. 1, *6*, *7*). On the other hand, no difficulty has been found in demonstrating that lithium ions penetrate into the nerve, of course across the epineurium, with the same dramatic rapidity that has been demonstrated for sodium ions (9, 10).

In the experiment 8-6-'49 the nerve, that had been kept in the sodium-free medium for 20 hours, was treated with a solution containing sodium chloride at the concentration 0.015 M for 1 hour. At the end of this time only a few A fibers had recovered their ability to conduct impulses and the rate of the recovery was so low that the conducted spike did not show an appreciable increase in intervals of time as long as 1 or 2 minutes. In order to test the effect of lithium ions the nerve was stimulated at 5 second intervals by maximal A shocks synchronized with the sweep of the oscillograph. Immediately after one arbitrarily chosen conducted spike had been observed on the screen of the oscillograph the nerve was placed in contact with a solution containing lithium chloride at the concentration 0.022 M and sodium chloride at the concentration 0.012 M. The following spike was elicited of course less than 5 seconds after the nerve had come in contact with the test solution; nevertheless, it showed a marked increase in height; further spectacular increases were observed with each following sweep of the oscillograph. Thus there can be no doubt (*a*) that

lithium ions penetrate across the epineurium without hindrance and with great rapidity and (*b*) that when the nerve fibers find themselves in the appropriate state lithium ions can restore their excitability almost instantly.

Under conditions such as these the delay in the beginning of the restoration, that is observed with nerve that has been left in the sodium-free medium for several hours after it has become inexcitable, must be interpreted as the duration of the interval of time which is necessary for lithium ions to reverse certain changes that in the absence of sodium have taken place in the nerve fibers. That the restoration of excitability by lithium ions is the result of processes taking place in the nerve fibers at a relatively low rate is also proven by the fact that initially the restored nerve fibers conduct impulses at a very low speed, which becomes progressively greater at the same time that the number of restored fibers increases (Figs. 1 and 2). In this respect lithium and sodium ions have the same effect upon sodium-deficient nerve (*cf.* references 3 and 10).

The extensive information that is now available on the role of sodium in nerve function (7, 8, 10) leaves no doubt that this role is quite complex, since sodium is necessary for the production of the two physiological responses of nerve, the nerve impulse and the nerve reaction. (The nerve reaction may be said to be the process by means of which the nerve fibers regulate the relative value of the various fractions of their membrane potential. The recovery of losses of membrane potential that occur during conduction of impulses is the result of the operation of the nerve reaction. For a detailed discussion, *cf.* reference 7, chapters VIII and XV; reference 8, section 9.) The differences between the actions of sodium and of lithium upon nerve deprived of sodium establish a sharp difference between the mechanism underlying the production of the nerve impulse and the mechanism underlying the establishment of the nerve reaction. Lithium can substitute for sodium in the production of the nerve impulse but lithium is not able to substitute for sodium in the establishment of the nerve reaction. Interestingly enough, a similar division of functions appears during the restoration of anoxic nerve by an applied anodal current; the artificially repolarized nerve fibers can produce nerve impulses, but they remain unable to establish the nerve reaction (reference 7, chapter XIII).

The difference between the actions of sodium and of lithium upon nerve deprived of sodium cannot be explained without assuming that sodium plays a role in the operation of at least two different chemical systems in the nerve fibers. And if consideration is given to the differences that exist between the actions of the various quaternary ammonium ions of the restoring type it becomes obvious that each of those two chemical systems must include a number of links which can be differently modified by different agents. For example, there are quaternary ammonium ions that can substitute for sodium in the establishment of the nerve reaction by A fibers, even though in an imperfect manner, but those ions cannot substitute for sodium in the production

of impulses by A fibers (*cf.* reference 8, section 9); while certain quaternary ammonium ions can substitute for sodium in the production of impulses by fibers of slow conduction, the function of the restored fibers occurs with characteristic abnormalities; the action of each quaternary ammonium ion has features of its own, etc. Clearly, the nerve fibers are tremendously complex electrochemical systems and no attempt should be made to explain their function in terms of hypotheses based on arbitrarily postulated analogies with simple models.

The fact that nerves kept in ionized inert sodium-free media can maintain their membrane potential at the same level as nerves kept in Ringer's solution leads to the conclusion that sodium does not play a specific role in determining the total value of the membrane potential. Sodium, however, plays a rather specific role in determining the constitution of the membrane potential, *i.e.* the relative values of the various fractions of the membrane potential, since in nerve deprived of sodium the L fraction of the membrane potential decreases to a negligible value, and since the restoration by sodium and by quaternary ammonium ions of the restoring type begins with an increase in the value of the L fraction. In this process lithium cannot substitute for sodium; indeed, in the presence of lithium ions the L fraction decreases more rapidly than in the presence of inert quaternary ammonium ions. Remarkably enough, diethylpiperidine, which restores to fibers of slow conduction the ability to conduct impulses, has upon the L fraction of the membrane potential of A fibers an effect similar to that of lithium (*cf.* 8, page 176).

To what extent the depolarizing action of lithium is referable to a poisoning by lithium of those chemical reactions which underlie the creation of the L fraction is a question that must be left open; indeed, it must be emphasized that the mechanism of the depolarization of the nerve fibers by certain inorganic ions and by certain organic poisons still is far from being understood.

SUMMARY

An analysis has been made of the effect of ammonium and of lithium ions upon frog nerve deprived of sodium.

Ammonium ions cannot substitute for sodium ions and restore the excitability of the nerve fibers; nor can they increase the L fraction of the membrane potential and the efficiency of the nerve reaction. Certain observations, however, indicate that the presence of ammonium ions outside the nerve fibers may delay the development of inexcitability in a sodium-free medium of nerve fibers restored by a moderate amount of sodium ions.

Lithium ions can substitute for sodium and restore to nerve fibers of the A and C groups the ability to conduct impulses; the effect upon B fibers has not been investigated. Lithium cannot substitute for sodium in the role that sodium plays in the creation of the L fraction and in the establishment of the nerve

reaction. In this respect lithium and sodium have opposite effects. This fact establishes an important difference between the two physiological responses that the nerve fibers can produce, the nerve impulse and the nerve reaction.

With untreated nerve the depolarization of nerve by lithium ions at high concentrations is preceded by a phase of hyperpolarization; with nerve deprived of sodium the depolarization begins without delay.

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