LOSS AND RECOVERY OF EXCITABILITY BY NORMAL AND BY DEGENERATING NERVES DEPRIVED OF SODIUM

By A. GALLEGO

(From the Laboratories of The Rockefeller Institute for Medical Research)

(Received for publication, April 17, 1950)

According to observations made by Overton (13) frog nerve deprived of sodium does not become inexcitable until after it has been kept in a sodium-free medium for several hours. This observation was confirmed by Lorente de Nó (6) who later presented the results of an analysis of the progressive changes in the properties of the nerve fibers which precede the development of inexcitability as well as of those changes which follow after the onset of total inexcitability (7–11, *cf.* also 4). In bullfrog sciatic nerve fibers of the A group do not begin to become inexcitable until after the nerve has been kept in the sodium-free medium for 2 or 3 hours and the number of inexcitable fibers increases progressively with advancing time until after 8 or 10 hours of lack of sodium all the A fibers are inexcitable; at that time, however, there are many fibers of the B and C groups which are still able to conduct impulses; inexcitability of all the C fibers does not develop in less than 14 to 16 hours. The onset of inexcitability is preceded by progressive decreases in the speed of conduction and in the ability of the nerve fibers to conduct rhythmic trains of impulses.

This paper describes certain aspects of the temporal courses of (*a*) the loss of excitability of the A fibers of frog nerve deprived of sodium and (*b*) the recovery of excitability after sodium ions are made available to the nerve. Observations were made with normal nerves and with nerves in various stages of degeneration after interruption of the continuity of the nerve fibers (Wallerian degeneration).

I

Technique

During the experiments recordings were made of the spike of the action potential of impulses that were initiated in an untreated segment of the nerve and that propagated themselves into a segment treated first with a sodium-free medium and then with Ringer's solution (approximately 0.1 M sodium chloride).

Immediately after excision the nerves (peroneal with its two main branches dissected up to the level of the ankle) were mounted in humid chambers in a horizontal position, resting upon the electrodes of the stimulating and recording circuits, under slight tension so as to maintain their natural length. The impulses were always initiated near the central end of the nerve; the distance from the stimulating cathode

(s_1) to the first recording electrode (r_1) was about 20 mm., and the distance from electrode r_1 to the second recording electrode (r_2) was 15 to 20 mm. Electrode r_2 was in contact with the peripheral end of the nerve which was crushed again with strong forceps shortly before the start of the observations. With untreated nerve this procedure yields a record of the conducted spike having a large diphasic artifact that increases in magnitude with advancing time. The fact, to be described later, that with sodium-deficient nerve the diphasic artifact of the spike progressively decreases shows that the end segment of the nerve, *i.e.* the segment of nerve which supplies the demarcation current, is more sensitive to the effect of lack of sodium than the rest of the nerve.

About 1 hour after the nerve had been mounted the segment of nerve extending from the peripheral crushed end to about 2 mm. from the stimulating cathode (electrode s_1) was placed in contact with the sodium-free medium. For this purpose 2 thin strips of filter paper were placed alongside the nerve and drops of the sodium-free medium were deposited upon the strips and upon the segment of nerve enclosed by them, very frequently at the beginning of the observations and less frequently in later stages. This procedure was equivalent to placing the nerve in a large and constantly stirred volume of the sodium-free medium. The strips of paper were removed of course during the brief periods of recording. In order to avoid changes at the point of the nerve at which the impulses were initiated drops of Ringer's solution were deposited upon the point in contact with the stimulating electrode and upon the next 2 mm. of nerve several times during the experiment.

A 0.11 M solution of diethanoldimethylammonium chloride was used as sodium-free medium because isotonic solutions of saccharose or glucose are known to produce changes in the resting membrane potential and in the action potential, which are referable to the lack of ions in the external medium of the nerve fibers (7). These changes are prevented by the quaternary ammonium ions of the inert type, of which diethanoldimethylammonium is an outstanding example, as well as by certain other ions (8).

After the desired degree of inexcitability had been reached the nerve fibers were restored by means of sodium ions (Ringer's solution or 0.11 M sodium chloride; for the purpose of the experiments both solutions are interchangeable). The nerve was stimulated at 5 second intervals by means of shocks synchronized with the sweep of the oscillograph. After one arbitrarily chosen response had been seen on the oscillograph's screen the nerve was brushed with a soft brush soaked in the restoring solution and for several minutes the same procedure was repeated during each one of the successive 5 second intervals between sweeps. Care was exerted not to leave an excess of fluid on the nerve, because an excess of fluid, by lowering the resistance of the external conductor of the nerve fibers, reduces the height of the recorded response. In spite of all care the shortness of the available intervals of time did not make it possible to avoid the occasional presence of a slight excess of fluid during the recording of the restored responses. When the restoration is rapid a slight reduction in the resistance of the external medium of the nerve fibers does not interfere with the observation of the growth of the spike, but when the restoration is relatively slow, as was the case in the experiment illustrated by Fig. 2, the initial phases of the restoration (Fig. 2, *1* to *7*) are difficult to follow. During more advanced stages of the

restoration by sodium, when the observations did not need to be done at brief intervals of time the restoring solution was applied to the nerve using strips of filter paper.

Often the experiments were done with the two peroneal nerves of one frog. One nerve was normal and the other was undergoing Wallerian degeneration after interruption of its continuity a number of days before. All the experiments reported in this paper were done with nerves of ordinary frogs (*R. pipiens*).

The stimuli used to initiate the nerve impulses were shocks resulting from the thyratron-controlled discharge of a small condenser through the primary of a transformer. In all cases the shocks were supramaximal for the fibers labelled A in Erlanger and Gasser's classification (*cf.* Erlanger, 2). The shocks were chosen to be supramaximal in order to insure that in the untreated segment of the nerve all the A fibers would be able to follow rhythmic stimulation. The stimulating shocks were initiated by the sweep circuit of the oscillograph at a fixed point of the sweep; repetitive stimulation resulted from repetitive sweep deflections at the frequency of about 100 per second in the case of Figs. 1 to 3 and of about 60 per second in the case of Figs. 4 and 5. The repetitive stimulation was maintained for approximately one-fifth of a second and the responses were photographed with standing film.

II

Normal Nerves

It will contribute to the clarity of the presentation to repeat here that the sensitivity to the lack of sodium is not the same with all the A fibers, for which reason the A fibers do not all become inexcitable simultaneously. As a rule, the most resistant A fibers do not become inexcitable in less than 7 or 8 hours; *i.e.*, they become inexcitable several hours later than the least resistant ones. The degree of sensitivity is not in a readily detectable relationship to the diameter of the fibers. By varying the strength of the stimulating shock it can be demonstrated that among the more resistant fibers there are both fibers with low threshold and fibers with high threshold. Even at very advanced stages of the development of inexcitability some of the responding fibers are fibers of very low threshold. Apparently, within the A group of fibers the degrees of sensitivity to the lack of sodium are distributed more or less at random.

The development of inexcitability is illustrated by the series of records reproduced in Fig. 1. The records of the first and third columns were obtained with the use of single shocks, and the records of the second (except record *2*) and fourth columns with the use of repetitive stimulation. Record *1* presents the spike that was recorded 33 minutes after the nerve had been placed in contact with the sodium-free medium, and before any readily detectable change in the conducted spike could be observed; record *2* presents the spike that was recorded 145 minutes after placing the nerve in contact with the sodium-free medium; at this time the effect of lack of sodium had already become apparent.

The spike height had decreased slightly, which in all probability indicates that a number of nerve fibers had already lost the ability to conduct impulses. The shock spike time had increased, which proves that the speed of conduction of the fastest fibers had decreased. The shape of the spike had changed, in particular the diphasic artifact had become much smaller. Since the end of the nerve had only been injured by sharp crushing the only possible interpretation of this change is that the end segment of the nerve fibers had a greater sensitivity to the lack of sodium than the rest of the nerve and had already become inexcitable, with the result that impulses that had propagated themselves past the first recording electrode were blocked at some distance from the end of the nerve.

Slightly more advanced stages of the development of inexcitability are illustrated by records *3* to *8*. The continuous decrease in the spike height indicated that the number of conducting fibers was decreasing steadily, the continuous increase in the shock spike time showed that the speed of conduction of the responding fibers was undergoing a progressive reduction, and the progressive change in the spike towards monophasicity demonstrated that the conducted impulses were being blocked at progressively greater distances from the end of the nerve; nevertheless, records *4*, *6*, and *8* prove that at least the majority of the excitable fibers still were able to follow rhythmic stimulation without obvious signs of failure.

Inability of nerve fibers to follow the stimulus rhythm was easily detected at the stage illustrated by records *9* and *10*. During repetitive stimulation (record *10*) the spike height rapidly decreased during the early part of the stimulation period to remain practically constant during the rest of the stimulation. The immediate assumption to be made is that, while the majority of the conducting fibers still were able to follow rhythmic stimulation, the fibers of a certain small group which were on the verge of total inexcitability were able to respond only to the first few shocks of the rhythmic train. Indeed, at a later stage (records *11* and *12*) the majority of the responding fibers were able to follow rhythmic stimulation without obvious signs of failure, which proves that in the intervening interval of time those fibers which in the case of record *10* had failed to follow the stimulus rhythm had become unable to conduct single impulses.

After the stage illustrated by records *11* and *12* had been reached, since only the more resistant fibers of the nerve were able to conduct impulses, the decrease in the number of conducting fibers again became more or less continuous; *i.e.*, fibers dropped out of the conducted response individually or in very small groups. The records of the series *13* to *27* show that during the final stages of the development of inexcitability the speed of conduction underwent a further decrease; at the same time the ability of the conducting fibers to follow rhythmic stimulation became progressively smaller. At the end of the

observations (records *25* to *27*) the speed of conduction was approximately one-fifth of the speed that had been measured at the beginning of the experiment (record *1*), and only a very few fibers were able to conduct more than the first impulse of a rhythmic train (record *26*).

The nerve was placed in contact with Ringer's solution after it had been deprived of sodium for 7 hours. The recovery of excitability (Fig. 2) did not take place so rapidly as it would have if sodium ions had been made available to the nerve 1 or 2 hours earlier. If Ringer's solution is applied to nerve deprived of sodium before the height of the conducted spike has become less than one-fourth of the initial height a significant increase in the spike height is customarily observed within 2 or 3 seconds. In the present instance, however, the effect of lack of sodium had progressed so far that even 0.1 N sodium ions were able to effect only a relatively slow recovery (*cf.* 10). Since the spike in record *4* is higher than the spike in record *1*, it is obvious that a certain number of nerve fibers had recovered their ability to conduct impulses within 15 seconds, but since the number of restored fibers was small, the decrease in the resistance of the external conductor of the nerve fibers, which was brought about by the drops of Ringer's solution that was being deposited on the nerve (see above, Technique), was sufficient to mask the progress of the recovery (records *5* and *6*) until after 1 minute of the action of sodium ions (record *7*), when also the speed of conduction was found to have undergone a significant increase. After one additional minute the increases both in the spike height and in the speed of conduction became important (record *10*).

The recovery progressed steadily with advancing time, the successive stages of the restoration of excitability being in reverse order of those which had been observed during the development of inexcitability. Beginning with records *11* and *12* the nerve was stimulated alternatively with single shocks (records *11* and *13* and all the following records in the first and in the third columns of records) and rhythmic trains of impulses (records *10* and *12* and all the following records of the second and fourth columns of records). At the time when record *12* was obtained only a small number of fibers were able to conduct more than the first impulse of the train and no fiber was able to conduct more than the first 5 impulses, which of course indicated that the ability to conduct trains of impulses corresponds to a higher degree of recovery than the ability to conduct single impulses. Also the ability to conduct impulses at high speed corresponds to a higher degree of recovery than the ability to conduct impulses at low speed. In examining records *11*, *12* to *35*, *36* of Fig. 2 it should be noted that during the initial stages of the recovery the restored fibers conducted impulses at a very low speed and that the speed of conduction increased progressively at the same time that the number of conducting fibers increased. At the end of the observations (Fig. 2, *35*, *36*), although the restored fibers were able to conduct trains of impulses without marked signs of failure the speed of

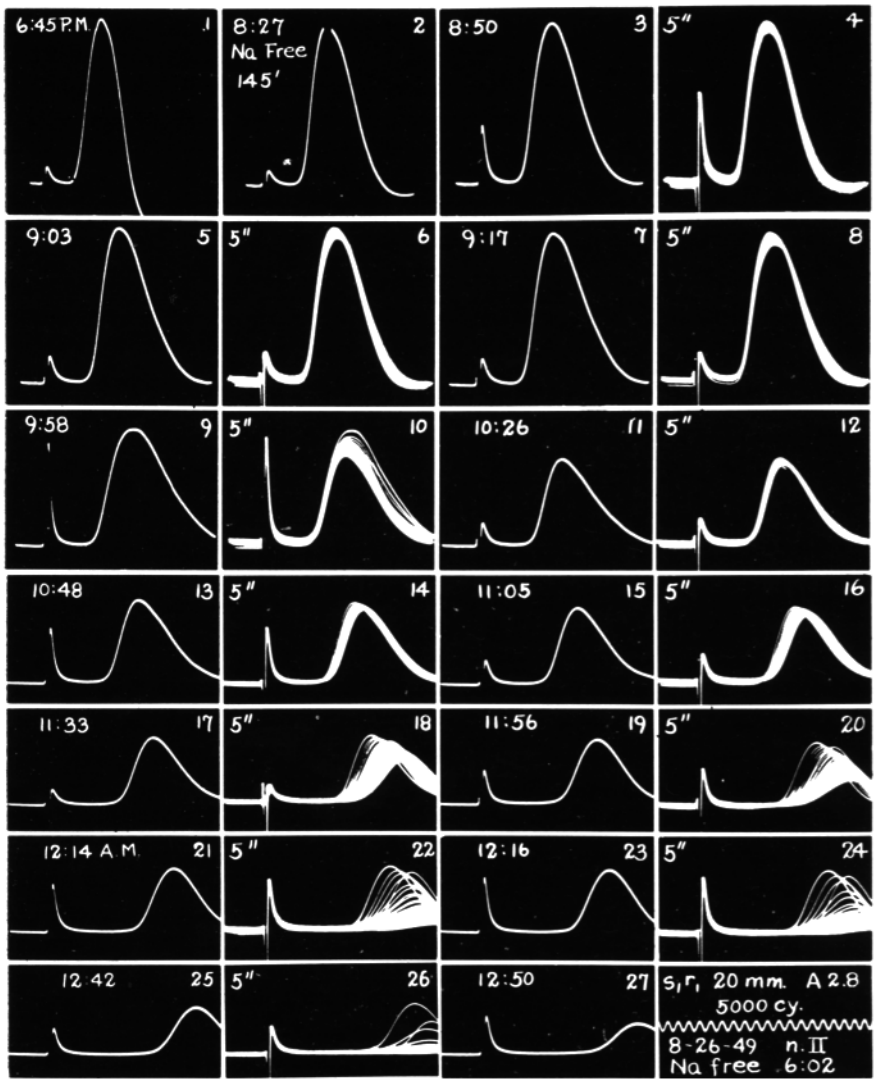

FIG. 1. Spikes of maximal A volleys of impulses recorded at 20 mm. from the stimulating cathode (s_1r_1 distance, 20 mm.) during the development of inexcitability in a sodium-free medium. In this and in the following figures the amplification (*A 2.8*, *A 10*, etc.) is given in millimeter deflection per millivolt input, when the distance between consecutive vertical lines separating records measures 50 mm. The nerve was placed in contact with the sodium-free medium at 6:02 p.m. The times at which the individual records were obtained are given with the records; when the intervals between successive records were short, the duration of the intervals is given in seconds on the upper left corner of the records. The records of the first and of the third columns and record *2* were obtained with the use of single shocks, the other records with the use of repetitive stimulation. Normal frog (*R. pipiens*) nerve.

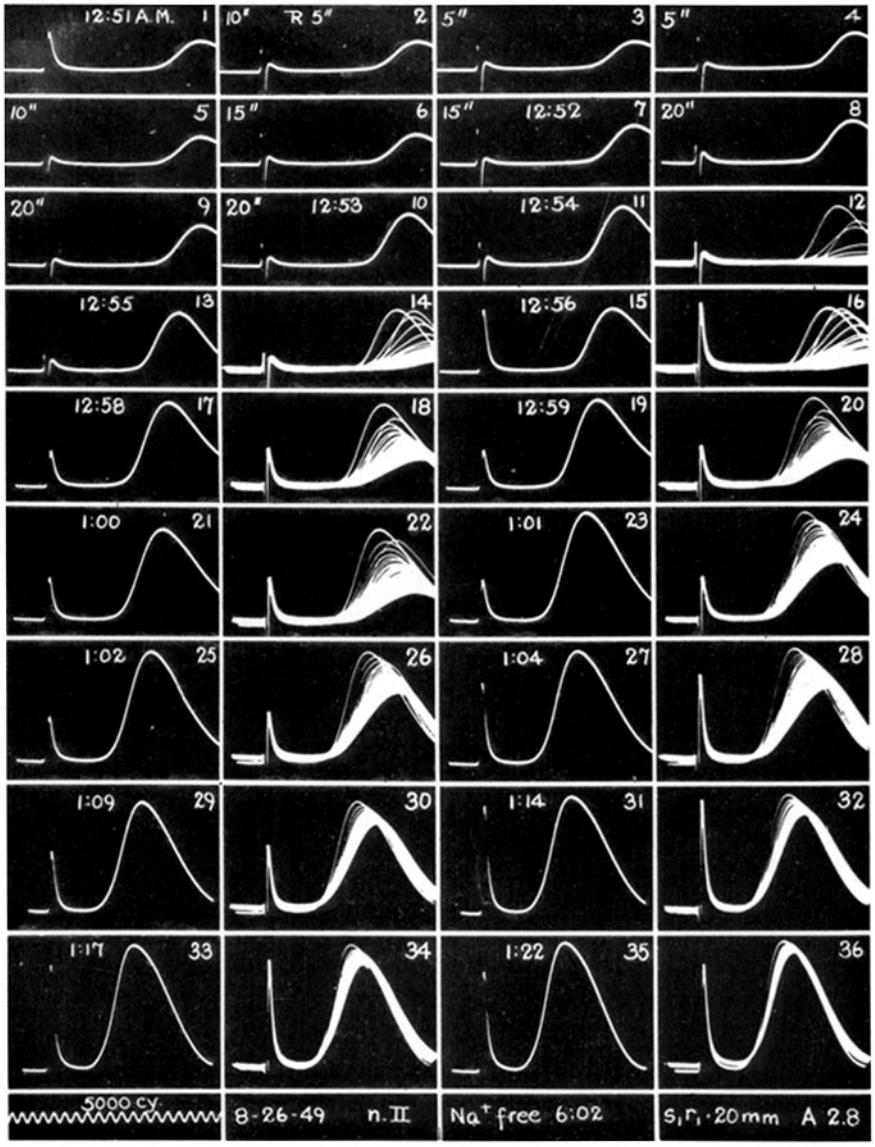

FIG. 2. (Continuation of Fig. 1.) Restoration of the excitability of the nerve fibers by Ringer's solution (*R.*). During the early part of the recovery (records *1* to *10*) the intervals between successive records are given in seconds on the upper left corner of the records. Record *2* was obtained 5 seconds after the application of Ringer's solution to the nerve. Beginning with the pair of records *11* and *12* stimulation was effected alternatively by single shocks and by rhythmic trains of shocks.

conduction still was markedly subnormal (*cf.* Fig. *1*, *1*); also the height of the spike was subnormal (*cf.* Figs. 1, *1* and 2, *35*).

On the basis of the results of other similar experiments it may be stated that the recovery would have progressed for no less than an additional hour but probably would not have become complete. The nerves of the ordinary frog (*R. pipiens*) are less resistant to the effects of the lack of sodium than the nerves of the bullfrog (*R. catesbiana*, *R. gryllio*). With bullfrog sciatic-peroneal nerves complete recovery in the presence of sodium ions can be obtained even after the nerves have been left in the sodium-free medium for *15* to *20* hours; *i.e.*, for several hours after all the A fibers have become inexcitable. With frog sciatic-peroneal nerves it is usually found that a number of fibers become irreversibly inexcitable in the sodium-free medium within 7 to 8 hours; *i.e.*, before or just after the most resistant fibers have lost their ability to conduct impulses. Knowledge of this difference between the properties of nerves of different frog species might prove to be useful in the planning of experiments.

III

Degenerating Nerves

The left sciatic nerves of a number of frogs were sharply cut with scissors on the same day. After the operation the frogs were kept at an average temperature of 22°C. The study of the degenerating nerves was begun 6 days after the operation and was continued for 9 additional days; *i.e.*, until the height of the conducted A spike had decreased to about 1/40 of the normal height.

The following observations concerning Wallerian degeneration may be mentioned here (*cf.* 12, with references to the literature). With frog peroneal nerve all the points of the nerve fibers, at least up to the level of the ankle, degenerate practically simultaneously, since the height of the spike initiated near the point at which the nerve had been severed does not decrease during conduction by any greater amount than that which should be expected to result from temporal dispersion of the individual fiber spikes and from the presence of cut branches, and since except for the effect of cut branches the spike initiated near the peripheral end and conducted toward the central end has the same height as the spike conducted in the opposite direction, a result which is in agreement with anatomical studies of degenerating nerves (Cajal, 1). It should also be noted that neither the threshold of stimulation nor the speed of conduction changes by a considerable amount during Wallerian degeneration. For example, in the two advanced stages of degeneration illustrated by Figs. 3 to 5 the threshold of stimulation of the fastest fibers was only slightly higher, and the speed of conduction of the fastest fibers only slightly smaller than in the companion normal nerve.

Except for a quantitative difference, consisting in that with degenerating nerve total inexcitability develops in a sodium-free medium faster than with

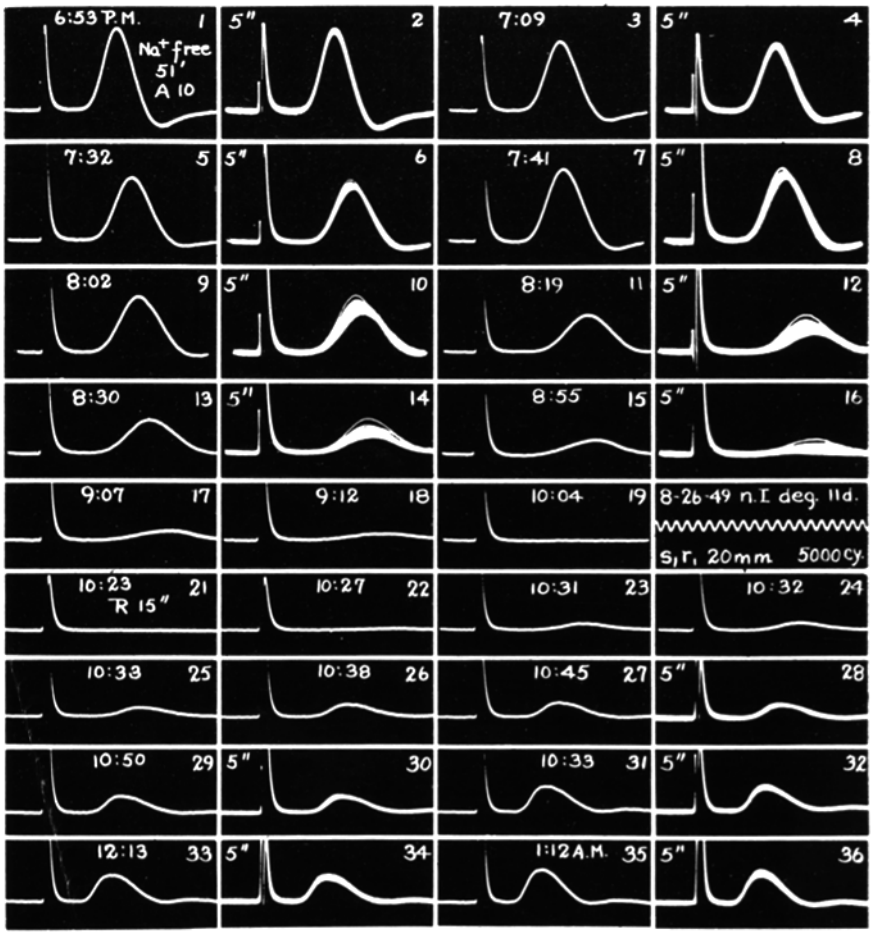

FIG. 3. Development of inexcitability in a sodium-free medium (records *1* to *19*) and recovery of excitability in Ringer's solution (records *21* to *36*) observed with a nerve that was in an advanced stage of Wallerian degeneration 11 days after the interruption of its continuity. Records *17* to *26* were obtained with the use of single shocks; all the other records were obtained alternatively with the use of single shocks (first and third columns) and of rhythmic trains of shocks (second and fourth columns).

normal nerve, the phenomena observed with degenerating nerve deprived of sodium closely resemble the phenomena that are observed with normal nerve.

In the experiments illustrated by Fig. 3 the observations were made 11 days after interrupting the continuity of the nerve. A comparison of record *1* of Fig. 3 with record *1* of Fig. 1 reveals that with the degenerating nerve the maximal A spike had only approximately one-fifth of the normal height, which indicates

that a large fraction of the A fibers had become inexcitable. Records *1* and *2* of Fig. 3 were obtained 51 minutes after the nerve had been placed in contact with the sodium-free medium. No effect of the lack of sodium in the external medium was detectable at this time; the spike of the single volley of impulses

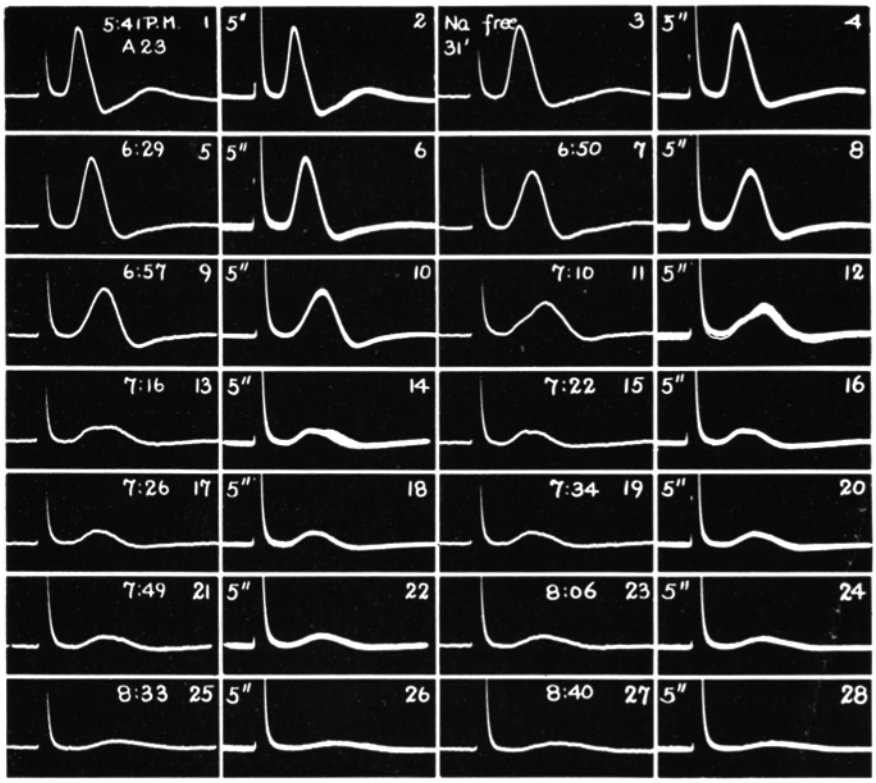

FIG. 4. Development of inexcitability in a sodium-free medium observed with a nerve the continuity of which had been interrupted 15 days before. Note the high amplification (*A 23*) that was used. The records of the first and third columns were obtained with the use of single shocks; the records of the second and fourth columns, with the use of repetitive stimulation.

(record *1*) still had the initial height and the fibers followed rhythmic stimulation without failure (record *2*).

The development of inexcitability is illustrated by the series of records *3* to *19*. (In examining the records attention should be given to the fact that the strength of the stimulating shock was increased in the interval between records *5*, *6* and *7*, *8*; the increase was necessitated by the presence of a bad contact in the stimulating circuit inside the nerve chamber.) Qualitatively the

sequence of changes that appear in the series of records *1* to *19* of Fig. 3 is equal to the sequence that appears in Fig. 1, for which reason no detailed description need be given. It will be sufficient to emphasize that with degenerating nerve the sequence of changes developed at a higher rate than with the normal nerve. The stages of inexcitability illustrated by records *27* of Fig. 1 and *18* of Fig. 3 are comparable, since the recorded spikes were approximately

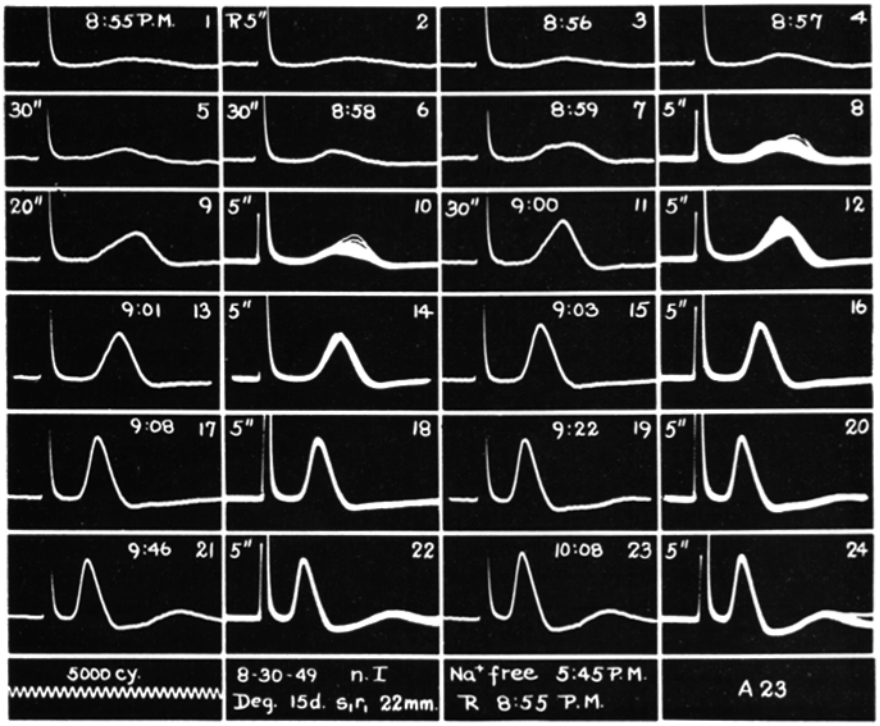

FIG. 5. (Continuation of Fig. 4.) Record *2* was obtained 5 seconds after the nerve had been placed in contact with Ringer's solution (*R.5"*). Records *1* to *6* were obtained with the use of single shocks; the other records, alternatively with single shocks (first and third columns) and with repetitive stimulation (second and fourth columns).

equal fractions of the initial spikes (records *1* in Figs. 1 and 3); that stage was reached with normal nerve after practically 7 hours, with the degenerating nerve, after slightly over 3 hours. In the degenerating nerve all the nerve fibers had been inexcitable for a number of minutes when record *19* of Fig. 3 was obtained; at that time a large fraction of the fibers of the normal nerve were still excitable (Fig. 1, *9*).

The nerve was left in the sodium-free medium for practically 1 hour after all the nerve fibers had become inexcitable and was then placed in contact

with Ringer's solution. As was expected the recovery (Fig. 3, *21* to *36*) did not begin immediately, but after a significant delay. Record *22* that was obtained after 4 minutes of the action of sodium ions and record *23* that was obtained 4 minutes later present only small conducted spikes. As was also expected, the speed of conduction was small during the initial stages of the recovery but the speed of conduction increased later, at the same time that the number of responding fibers increased.

The observations were continued until 1:12 a.m. (Fig. 3, *35*, *36*) at which time the spike height and the speed of conduction had reached stationary values. From a comparison of records *1* and *2* with records *35* and *36* it follows that a number of nerve fibers had become irreversibly inexcitable in the sodium-free medium. That with the degenerating nerve a certain number of fibers had become irreversibly inexcitable in the sodium-free medium is not remarkable; the remarkable fact is that a relatively large number of fibers recovered so far that they were able to conduct impulses with the same speed as at the beginning of the experiment (records *35* and *36*). If the nerve had not been kept in the sodium-free medium after all the fibers had lost the ability to conduct impulses the recovery in Ringer's solution would have been nearly total. At least this is the observation that has been made in other experiments.

The experiment illustrated by Figs. 4 and 5 was done with the use of a nerve in an advanced stage of Wallerian degeneration, 15 days after the interruption of its continuity. Parallel observations were made with the companion normal nerve. The height of the maximal spike in the degenerating nerve was only about 1/40 of the height of the spike in the normal nerve, which indicated that only a small number of fibers were still able to conduct impulses; nevertheless, the threshold of stimulation of the degenerating nerve was hardly more than 1.25 times the threshold of the normal nerve, and the speed of conduction of the fastest fibers in the degenerating nerve was not less than 80 per cent of the speed of conduction in the normal nerve. On the other hand, the spike initiated in the degenerating nerve by a maximal A shock became fractionated into two elevations, which indicated that the excitable A fibers included both fibers of fast conduction and fibers of slow conduction.

The development of inexcitability in the sodium-free medium is illustrated by the series of records reproduced in Fig. 4. Except for the fact that the fibers of slower conduction became inexcitable quite rapidly, Fig. 4 presents the same sequence of changes that have been described above in reference to Fig. 1.

The nerve was placed in contact with Ringer's solution after only 3 hours of lack of sodium and before all the nerve fibers had become inexcitable. For this reason the recovery of excitability (Fig. 5) took place more rapidly than in the experiment illustrated by Fig. 3. Readily detectable increases in the conducted responses were observed after 1 minute (Fig. 5, *3*) and after 2 minutes (Fig. 5, *4*) of the action of sodium ions, and with advancing time the conducted response increased continuously (Fig. 5, *5* to *22*); the recovery became practically total

in slightly more than 2 hours (Fig. 5, *23* and *24*). The sequence of changes to be observed in Fig. 5 is essentially that which has been described above in reference to Fig. 2. The only detail to be added is that an important fraction of the fibers of slow conduction began to recover the excitability much later than fibers of fast conduction, since definite evidence of the existence of a late elevation in the spike did not begin to appear until the nerve had been in contact with Ringer's solution for 27 minutes (Fig. 5, *19*, *20*). This observation confirms a general rule that summarizes numerous observations that have been made in this laboratory with nerves deprived of sodium: those fibers which become inexcitable first when the nerve is deprived of sodium, recover their excitability last after sodium ions are made available to the nerve.

IV

DISCUSSION

Although overwhelming evidence is available to show that sodium ions diffuse with great rapidity through the epineurium and that diffusion equilibrium of the nerve with an external test solution is approached within a few minutes (Lorente de Nó, 9 to 11; Gallego and Lorente de Nó, 4), it is pertinent to emphasize that the observations presented in this paper are incompatible with an assumption recently put forward by Hodgkin and Katz (5) to explain the fact that frog nerve does not become totally inexcitable until it has been maintained in a sodium-free medium for several hours. According to Hodgkin and Katz (5, p. 44), the delay in the onset of total inexcitability is referable to "retention of salt in the interstitial spaces of the nerve trunk."

The assumption of retention of salt inside the epineurium is incompatible with the observation (Fig. 1) that a considerable interval of time—not less than 5 to 6 hours—elapses between the onset of partial inexcitability and total inexcitability. Since the minimal concentration of sodium ions which is necessary to maintain excitability of all the A fibers for 8 or 10 hours is about 0.01 N it is clear that inexcitability of a few A fibers is sufficient proof that not later than 2 or 3 hours after the nerve has been placed in contact with the sodium-free medium the concentration of sodium ions in the interstitial spaces of the nerve trunk has fallen below the minimal maintenance concentration, and it is also clear that the interstitial concentration of sodium ions must steadily decrease with advancing time. Consequently, the only possible explanation of the experimental facts (Fig. 1) is that at least many A fibers can conduct impulses for a considerable period of time in the presence of negligible concentrations of sodium ions in their external medium. Actually, the interstitial concentration of sodium ions becomes negligible before any A fiber loses its ability to conduct impulses (4, 9–11).

Since the presence of sodium ions in the external medium of the nerve fibers at more than a minimal concentration is not immediately necessary for the

production of the nerve impulse, the logical assumption to be made is that inexcitability results from changes which take place in the nerve fibers at a relatively low rate after the external concentration of sodium ions has fallen below a certain value. In support of this assumption there is the fact that the onset of inexcitability is preceded by progressively advancing decreases in the speed of conduction and in the ability of the nerve fibers to conduct rhythmic trains of impulses. On the other hand, the fact that the rate of the recovery in the presence of sodium (Figs. 2, 3, 5) depends upon the length of time during which the nerve fibers have been kept in the inexcitable state, proves that the changes in properties induced in the nerve fibers by the lack of sodium continue and become progressively greater after inexcitability has set in. Finally, the fact that during the recovery in the presence of sodium ions the sequence of changes in the conducted response duplicates in the reverse order the sequence that is observed during the development of inexcitability, unavoidably leads to the conclusion that the restoration of excitability in the presence of sodium is referable to the reversal of those changes in the properties of the nerve fibers that had resulted from the lack of external sodium.

Degenerating fibers are less resistant to the effect of the lack of sodium than are the fibers of normal nerve. That this would be the case was expected, what was not expected is that degenerating fibers, even in a very advanced stage of Wallerian degeneration, may fully recover from inexcitability resulting from the lack of sodium. The conclusion to be drawn from this observation is that degenerating nerve fibers retain a great deal of functional ability up to the time when they become inexcitable, and that the development of inexcitability takes place as an almost explosive process throughout the entire length of the peroneal nerve *i.e.*, at least up to the level of the ankle. Beginning with the 7th day after section of the nerve the conducted A spike loses every day an important fraction of its height until on the 15th day the height of the A spike is only about 1/40 of the normal height. Every day the conducting fibers are found to be able to conduct impulses throughout their entire length and every day the conducting fibers are able to recover from inexcitability resulting from the lack of sodium. Consequently, the daily decrease in the height of the conducted spike means that every day a number of fibers become inexcitable which up to that day had retained a great deal of functional ability.

Recently Feng and Liu (3) have observed that after removal of the epineurium the nerve fibers become inexcitable in a sodium-free medium much earlier than in nerves with intact epineurium. The correct interpretation of Feng and Liu's observations has been given by Lorente de Nó (9). Since the epineurium is not a diffusion barrier, and therefore the epineurium does not significantly delay the diffusion of sodium ions out of the nerve, the correct interpretation of Feng and Liu's experimental results is that removal of the epineurium results in profound changes in the properties of the nerve fibers,

one of which consists in an increase of the sensitivity of the nerve fibers to the lack of sodium. In the experiments reported by Feng and Liu after removal of the epineurium total inexcitability developed much earlier than in the experiments with degenerating nerves illustrated by Figs. 3 and 4. Consequently, the increase in the sensitivity to the lack of sodium, which results from the removal of the epineurium is even greater than that which results from a prolonged period of Wallerian degeneration.

There is still one experimental fact that deserves consideration. It has appeared that points of the end segments of the nerve fibers become inexcitable in a sodium-free medium earlier than points at a greater distance from the killed end of the nerve, this being the reason why during the development of inexcitability the recorded spike becomes a monophasic deflection (Figs. 1, 3, 4). The only possible explanation of the difference seems to be that cathodal polarization of the nerve fibers by their own demarcation current results in an increase in the rate of development of inexcitability in a sodium-free medium. Lorente de Nó (7, pt. 1, p. 386) found that applied cathodal polarization did not increase the effect of lack of sodium in a readily detectable amount, but during an applied cathodal polarization, which by itself does not produce a conduction block, the current that flows through the membrane of the nerve fibers is smaller than the demarcation current that flows across the membrane in the segments of the nerve fibers in the immediate neighborhood of the killed end of the nerve.

V

SUMMARY

A study has been made of the loss of excitability in a sodium-free medium and of the recovery of excitability in Ringer's solution by A fibers of normal frog nerves and of nerves in advanced stages of Wallerian degeneration.

With normal nerves that are being kept in a sodium-free medium the number of conducting fibers does not undergo a readily detectable decrease in less than 1 to 2 hours; inexcitability of all the A fibers does not develop in less than 7 to 8 hours. During the development of inexcitability the speed of conduction of the still conducting fibers undergoes a progressive decrease; in advanced stages the speed of conduction is not more than one-fifth of the normal speed. The nerve fibers lose the ability to conduct rhythmic trains of impulses earlier than the ability to conduct single impulses.

The recovery of excitability in Ringer's solution duplicates in a reverse order the sequence of changes that have been previously observed during the development of inexcitability. The rate of the recovery of excitability in Ringer's solution is higher than the rate of the loss of excitability in the sodium-free medium.

With degenerating nerves the effect of the lack of sodium develops qualita-

tively in the same manner in which it develops with normal nerves. Degenerating nerve fibers, however, become inexcitable in a sodium-free medium earlier than normal fibers. The recovery of the excitability in Ringer's solution takes place in much the same manner in normal and in degenerating nerve fibers.

The loss of excitability during Wallerian degeneration is a process that develops simultaneously, or practically so, throughout the entire length of the fibers. The nerve fibers retain a great deal of functional ability throughout the several days which precede the onset of inexcitability and then suddenly become inexcitable.

LITERATURE CITED

1. Cajal, S. R., Degeneration and Regeneration of the Nervous System, translated and edited by R. M. May, London, Oxford University Press, 1928, **1.**
2. Erlanger, J., The analysis of the compound action potential in nerve, chapter I, *in* Erlanger, J., and Gasser, H. S., Electrical Signs of Nervous Activity, Philadelphia, University of Pennsylvania Press, 1937, 1.
3. Feng, T. P., and Liu, Y. M., The connective tissue sheath of nerve as effective diffusion barrier, *J. Cell. and Comp. Physiol.*, 1949, **34,** 1.
4. Gallego, A., and Lorente de Nó, R., On the effect of ammonium and lithium ions upon frog nerve deprived of sodium, *J. Gen. Physiol.*, 1951, in press.
5. Hodgkin, A. L., and Katz, B., The effect of sodium ions on the electrical activity of the giant axon of the squid, *J. Physiol.*, 1949, **108,** 37.
6. Lorente de Nó, R., Effect of choline and acetylcholine chloride upon peripheral nerve fibers, *J. Cell. and Comp. Physiol.*, 1944, **24,** 85.
7. Lorente de Nó, R., A Study of Nerve Physiology, *Studies from The Rockefeller Institute for Medical Research*, 1947. Parts 1 and 2.
8. Lorente de Nó, R., On the effect of certain quaternary ammonium ions upon frog nerve, *J. Cell. and Comp. Physiol.*, 1943, **33,** suppl., 1.
9. Lorente de Nó, R., The ineffectiveness of the connective tissue sheath of nerve as a diffusion barrier, *J. Cell and Comp. Physiol.*, 1950, **35,** 195.
10. Lorente de Nó, R., Equilibria of frog nerve with different external concentrations of sodium ions, *J. Gen. Physiol.*, 1951, **35,** 145.
11. Lorente de Nó, R., On the existence of a gradient of sensitivity to the lack of sodium in the spinal roots of the bullfrog, *J. Gen. Physiol.*, 1951, in press.
12. Lorente de Nó, R., Electrotonus in degenerating frog nerve fibers, *J. Neurophysiol.*, in press.
13. Overton, E., Beiträge zur allgemeinen Muskel- und Nervenphysiologie. II. Ueber die Unentbehrlickkeit von Natrium- (oder Lithium-) Ionen für den Contractionsact des Muskels, *Arch. ges. Physiol.*, 1902, **92,** 346.