EQUILIBRIA OF FROG NERVE WITH DIFFERENT EXTERNAL CONCENTRATIONS OF SODIUM IONS

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In work on the effect of lack of sodium upon the excitability of nerve fibers different results have been obtained by different authors. Working with intact nerve trunks Overton (18) and Lorente de Nó (12, 13) found that the development of inexcitability is a slow process, so that in a sodium-free medium total inexcitability of frog sciatic nerve requires several hours to develop. On the other hand Hodgkin and Katz (8) working with the giant axon of the squid, and Huxley and Stampfli (10) working with single nerve fibers dissected out of a nerve trunk found that inexcitability develops without appreciable delay, as soon as the concentration of sodium ions outside the nerve fibers falls below a certain value, and Feng and Liu (2) working with frog nerves deprived of epineurium found that in these nerves inexcitability develops much faster than in intact nerve trunks.

Two alternative explanations have been offered for the discrepancy between the results of the two types of experiments. (a) With the intact nerve trunks diffusion of sodium ions, through the epineurium into the external sodium-free medium, is an exceedingly slow process, so that retention of salt in the interstitial spaces of the nerve trunk keeps the nerve fibers excitable for considerable periods of time (2, 7-9). (b) According to the results of work on diffusion through nerve carried out by Fenn (3) and Hill (6), and to observations made by the present writer (12), diffusion of solutes through the epineurium and through the interstitial spaces of the nerve trunk is a rapid process; consequently, removal of the epineurium and dissection of single nerve fibers are procedures that markedly increase the sensitivity of the nerve fibers to the lack of sodium (14).

Corroborative evidence that diffusion of sodium ions through the epineurium is a rapid process has already been presented (14), and Gallego (4) has shown that the temporal course of the development of inexcitability of frog nerve in a sodium-free medium is incompatible with the assumption of retention of salt inside the epineurium. On the other hand it was observed by Lundberg (quoted in 14) that after removal of the epineurium the C fibers are more sensitive to the effect of lack of sodium than the A fibers, while in the intact nerve trunk the opposite is true (13) and the present writer (17) has presented a brief analysis.
of some of the changes in the properties of the nerve fibers which result from the removal of the epineurium. A more detailed analysis is being prepared for publication; here it will be sufficient to mention that the changes in properties are indeed spectacular, and that therefore there is no real contradiction between the results obtained by Overton (18) and Lorente de Nó (12, 13) and the results obtained by Hodgkin and Katz (8), Huxley and Stämpfli (10), and Feng and Liu (2). The effect of lack of sodium to a large extent depends upon the state of the nerve fibers, and the fibers of nerve deprived of epineurium as well as the fibers dissected out of a nerve trunk find themselves in states which are very different from the state of the fibers in the intact nerve trunk. Moreover, it is possible to sensitize the fibers of intact trunks to the effect of lack of sodium so far that they become inexcitable in a sodium-free medium as rapidly as single dissected out nerve fibers (see below, Fig. 8).

This paper presents analyses of the development of inexcitability of intact frog nerve trunks in a sodium-free medium, and of the restoration of inexcitable nerve trunks by added sodium ions. The effect of lack of sodium upon nerves deprived of epineurium will be analyzed in a future paper.

I

Technique

The majority of the experiments were done with nerves that had been allowed to become inexcitable in a sodium-free medium and had been restored by means of moderate concentrations of sodium ions.

Since the technique used in this laboratory in work with sodium-deficient nerves has been described in detail elsewhere (4, 13) it will be sufficient to add here a few details.

As a rule the nerves (sciatic-peroneal) were kept in a large volume of a sodium-free medium (0.11 M diethanol dimethylammonium chloride) for 15 to 20 hours; i.e., for several hours after all the nerve fibers had become inexcitable. (During the first 4 hours the sodium-free medium was continuously renewed and stirred.) The nerves were then mounted in humid chambers, in a horizontal position, resting upon the electrodes of the stimulating and recording circuits. The arrangements of the electrodes are given in the diagrams of Fig. 1.

The excitability of the central segment of the nerve (Fig. 1, II, mP) was always restored by means of Ringer’s solution (approximately 0.1 M sodium chloride) that was allowed to act for 1 hour in order to insure recovery of the ability to conduct impulses by all the nerve fibers. After proof had been obtained of the inability of impulses initiated in the central segment to propagate themselves into the peripheral segment (Fig. 1, II, mP), this segment was placed in contact with a restoring solution containing a moderate amount of sodium ions and when the recovery had reached the desired stage a new restoring solution was applied to the nerve; later, the external concentration of sodium was varied again one or more times in order to produce changes in the properties of the nerve fibers. The different solutions were prepared by mixing in the appropriate proportions Ringer’s solution and a 0.11 M solution (of course, sodium-free) of diethanol dimethylammonium chloride.
In all the experiments the nerves were taken from large bullfrogs (*R. catesbiana*, *R. griffio*) so that the diameter of the peripheral segment of the nerve (the peroneal trunk) approached 1 mm. The impulses were always initiated by rectangular pulses of current (100 μA) of duration sufficient to stimulate either all the A or all the C fibers.

When the observations on the effect of the solution applied to the nerve had to be made at brief intervals of time (5 seconds) the restoring solution was applied to the nerve by means of a soft brush soaked in the solution. Thereby care had to be exerted not to leave on the nerve an excess of the solution, since this excess, by lowering the
resistance of the external conductor of the nerve fibers, would decrease the height of the recorded spike. Only in a few instances did an excess of solution left on the nerve cause serious distortion of records. A greater difficulty was experienced in keeping the d.c. amplifier in balance during the first 15 seconds after a restoring solution had been applied to the nerve. The application of the solution caused an upward shift of the base line and since the sweeps of the oscillograph occurred at 5 second intervals there was hardly time to adjust the base line before the next sweep had occurred. For this reason the crest of the restored spike sometimes went beyond the margin of the screen of the oscillograph (cf. Fig. 3, 15).

When the observations on the effect of the solution applied to the nerve had to be made at relatively long intervals of time (several minutes) thin strips of filter paper were placed alongside the peripheral segment of the nerve and drops of the solution were frequently deposited on the strip and the segment of nerve enclosed between them.

II

Restoration of Nerve Deprived of Sodium by Sodium Ions at Different Concentrations

The temporal course of the restoration by sodium ions of the excitability of nerve deprived of sodium to a large extent depends upon the length of time during which the nerve has been deprived of sodium. In order to understand this situation it is necessary to take into account the effects that lack of external sodium has upon the properties of the nerve fibers.

In bullfrog sciatic nerve in a sodium-free medium the development of inexcitability of all the A fibers requires a considerable length of time; the least resistant A fibers begin to become inexcitable after 2 or 3 hours, and the most resistant A fibers become inexcitable after 8 to 10 hours. Oscillographic analysis reveals that the following two changes in the properties of the nerve fibers precede the onset of inexcitability (4, 12, 13): (a) progressive decrease in the speed of conduction of impulses and (b) a progressive decrease in the ability to conduct rhythmic trains of impulses, which is referable to a progressive decrease in the rate of recovery after conduction of impulses, and which indicates that in the absence of sodium the effectiveness of the nerve reaction decreases. The reduction in the effectiveness of the nerve reaction also is demonstrable by analysis of the electrotonic potentials: the rate of establishment and the magnitude of the maxima and overshootings of the electrotonic potentials undergo a progressive reduction.

The state of the nerve fibers does not become stationary after inexcitability has developed; analysis of the electrotonic potentials shows that the progressive change in properties continues until the nerve fibers become entirely unable to produce the nerve reaction in response to the effect of applied currents, and further until applied currents become entirely unable to create slow electrotonus. Such an advanced stage is not reached until the nerve has been kept
in the sodium-free medium for 20 to 30 hours. At this stage the effect of the lack of sodium still is reversible, at least in a majority of the nerve fibers, but if the lack of sodium is maintained for a longer period of time a progressively increasing number of A fibers become irreversibly altered.

When sodium ions are made available to the nerve fibers immediately after these have become inexcitable, i.e. at a time when the changes in other properties of the fibers are relatively unimportant, the recovery of the ability to conduct impulses is a very rapid process. If, however, sodium ions are made available to the nerve after 15 to 20 hours of lack of sodium the recovery of the ability to conduct impulses begins after a considerable delay, because it must be preceded by the reversal of those changes in the properties of the nerve fibers which had taken place after the onset of inexcitability. This reversal can be followed by oscillographic analysis of the electrotonic potential (13). Thus it is clear that the temporal course of the restoration of excitability of sodium-deficient nerve must depend upon the past history of the nerve. The following remarks will serve to describe situations that are encountered in experimental work.

If the nerve has been deprived of sodium only for the relatively short period of time (3 or 4 hours) that is necessary to render a small number of A fibers inexcitable Ringer’s solution or 0.1 N sodium chloride usually begins to restore excitability within 2 seconds; the restoration of excitability can also be effected by means of moderate concentrations of sodium ions (0.015, 0.02 N), in which case the recovery is usually observed to begin 10 or 15 seconds, and rarely 1 minute, after the restoring solution has been applied to the nerve. If the nerve has been maintained in the sodium-free medium for 7 or 8 hours so that the majority of the A fibers have become inexcitable, restoration of excitability to a number of A fibers can still be effected by means of moderate concentrations of sodium ions, but the restoration cannot be expected to begin in less than 1 minute or 2, and even 0.1 M sodium chloride may require 10 or 15 seconds to initiate the recovery, which will progress at a relatively low rate (cf. 4, Fig. 2); moreover, only 0.1 M sodium chloride will be able to effect a total recovery. If, finally, the nerve has been kept in the sodium-free medium for 15 to 20 hours, i.e. for several hours after all the A fibers have become inexcitable, moderate concentrations of sodium ions (0.015, 0.02 N) are either ineffective or are able to restore the excitability of only a few A fibers, and only after they have been allowed to act for 1 hour or longer. Even at the 0.1 N concentration sodium ions are not able to restore the excitability of any A fiber in less than 8 or 10 minutes.

Fig. 2 illustrates the results of a typical experiment in which the peripheral segment of the nerve was placed in contact with Ringer’s solution after it had been deprived of sodium for 17 hours. Record 1 was obtained with the nerve in the sodium-free medium and record 2 approximately 4 seconds after placing
Fig. 2. Recovery of excitability in Ringer's solution of the peripheral segment of a nerve that had become inexcitable in a sodium-free medium. Ringer's solution was applied 4 seconds before record 2 was obtained (R. 4*). The times at which the individual records were obtained are given with the records. When the intervals between successive records were small the duration of the interval is given in seconds in the upper left corner of the records. In this and following figures the amplification (A 10, A 0.5, A 2.8, etc.) is given in millimeter deflection per millivolt input when the distance between consecutive vertical lines separating records measures 50 mm. A red. denotes a reduction in the amplification. R. denotes Ringer's solution.
the nerve in contact with the restoring solution. As is shown by the series of records 2 to 7 no nerve fiber had recovered the ability to conduct impulses 9 minutes afterwards. After 5 additional minutes, however, a few A fibers had become able to conduct impulses (record 8) and from then on the conducted spike increased steadily in height (records 9 to 17). This increase made it necessary to reduce the amplification 4 times, in the intervals between records 17, 18; 22, 23; 27, 28, and 29, 30. The recovery became nearly complete after 58 minutes of the action of sodium ions (record 32). It should be noted that in the early phases of the recovery (records 8 to 16) the speed of conduction of impulses was markedly subnormal, since the shock spike time was abnormally long and the alpha and beta fibers contributed two distinct elevations to the compound spike, while at the end of the recovery (record 32) the shock spike time was brief and the compound spike had normal shape; the recovery of the speed of conduction, therefore, lagged behind the recovery of the ability to conduct impulses.

If they were considered in isolation, the experimental results presented in Fig. 2 would admit of two different interpretations: (a) that the penetration of sodium ions across the epineurium is an exceedingly slow process, the rate of which determines the rate of the recovery, and (b) that the diffusion across the epineurium is rapid enough to make a high concentration of sodium ions available to the nerve fibers within a short time but the restoration of the ability to conduct impulses is a process which takes place at a low rate.

A decision between the two alternatives can be reached by appropriate experiment. In the first place using the arrangement of electrodes indicated in Fig. 1, I a direct analysis can be made of the effect of sodium ions upon the nerve fibers (cf. 13). It is found that the recovery by A fibers of the ability to conduct impulses is a late effect of the presence of sodium ions. Earlier effects are detectable without delay; usually within less than 1 minute the polarizability of the membrane by the anodal current undergoes a significant increase and soon the A fibers begin to produce unconducted impulses in response to the break of the anodal current; then the A fibers produce unconducted impulses also in response to the closure of the cathodal current, and finally the A fibers begin to become able to conduct impulses. On the other hand if using the arrangement of electrodes indicated in Fig. 1, III the recovery is followed by determining the ability of C fibers to conduct impulses it is found that the recovery begins without delay, since under the conditions prevailing in the experiment illustrated by Fig. 2 C fibers usually begin to conduct impulses within 1 minute or 2. Thus it appears that when the observations are made with an appropriate technique the penetration of sodium ions across the epineurium proves to be a rapid process.

A second procedure that can be used to demonstrate the rapid penetration of sodium ions across the epineurium is the following. After the nerve has been
left in the sodium-free medium for 15 to 20 hours the recovery is initiated by a moderate concentration of sodium ions (0.015, 0.02 N) which by itself is not sufficient to restore the ability to conduct impulses to more than a few A fibers but is sufficient to reverse part of the changes that have taken place in the A fibers after the onset of inexcitability; thereby many A fibers are brought into such a state that they can rapidly recover their excitability when the outside concentration of sodium ions is increased. After the moderate concentration of sodium ions has acted for an appropriate length of time it is found that a minimal increase in the concentration of sodium ions outside the epineurium produces a spectacularly rapid restoration of the ability of A fibers to conduct impulses.

Fig. 3 illustrates the results of an experiment of this type. The nerve was kept in the sodium-free medium for slightly over 17 hours. The recovery was initiated by a solution containing sodium ions at the concentration 0.02 N (20 parts Ringer's solution, 80 parts 0.11 M diethanoldimethylammonium chloride). During the first 32 minutes of the action of the restoring solution no unmistakable signs of conducted spikes were observed (records 1 to 5) but after 11 additional minutes a number of C fibers were able to conduct impulses (record 6); this number increased rapidly (record 7) and after 66 minutes of the action of 0.02 N sodium ions, if not all, at least a large majority of the C fibers were conducting impulses (record 10). A readily detectable A spike was first observed after 56 minutes (record 8); the A spike increased progressively in height but at a rate so low that after 67 minutes of the action of sodium ions less than one-tenth of the A fibers had recovered the ability to conduct impulses (records 9, 11).

The series of records 11 to 13 of Fig. 3 prove that the progress of the recovery of the A fibers was so slow that no appreciable increase in the spike height was observed in a 15 second interval; as a matter of fact, the increase in the spike height was hardly detectable in responses elicited at 1 minute intervals. Immediately after the response reproduced in record 13 had been seen on the screen of the oscillograph, the nerve was brushed with a soft brush soaked in a solution containing sodium ions at the concentration 0.025 N (25 parts Ringer's solution, 75 parts 0.11 M diethanoldimethylammonium chloride) which resulted in a spectacular increase in the number of conducting fibers.

Although record 14 was obtained 5 seconds (sic, 5 seconds) after record 13, i.e. hardly more than 4 seconds after the new solution had come in contact with the nerve, the spike in record 14 is considerably higher than that in record 13, and as record 15 shows a further important increment in the spike height occurred during the following 5 second interval. (The exact height of the spike in record 15 cannot be ascertained because a displacement of the amplifier base line had occurred while the restoring solution was being placed in contact.
Fig. 3. Initiation of the recovery of excitability of nerve deprived of sodium by sodium ions at the concentration 0.02 N (records 1 to 13) and enhancement of the recovery by sodium ions at the concentration 0.025 N (records 14 to 26). The 1000 cycles time line applies to the records of A spikes; the 60 cycles time line, to the records of C spikes. The C spikes were recorded at constant amplification, 172 mm. per mv.
with the nerve, but there can be no doubt that the spike in record 15 is considerably higher than the spike in record 14.) After 5 additional seconds the spike displayed the great height with which it appears in record 16. The continuous and rapid increase in the spike height (records 17 to 19) made it necessary to reduce the amplification that was being used. Also at the smaller amplification the increases in the spike height which were observed at 5 second intervals appeared to be large (records 20 to 22).

After record 22 had been obtained the amplification was reduced by an amount that was not noted in the protocol of the experiment; probably it was decreased to one-half the amplification used for record 22. Record 24 was obtained at the new amplification after 4 minutes of the action of 0.025 N sodium ions. Thereafter the increase in the spike height progressed at a low rate and at the time when record 26 was obtained, after 13 minutes of the action of 0.025 N sodium ions, the height of the spike had become practically stationary, thus indicating that 0.025 N sodium ions had already restored the excitability to all those A fibers (about one-third of the A fibers) that 0.02 N sodium ions had prepared for a rapid restoration. This fact indicates that diffusion equilibrium of the nerve trunk with the outside solution was being approached not later than 13 minutes after this solution had been changed. It should be noted that the restored fibers were conducting impulses at a markedly subnormal speed, since in record 26 of Fig. 3 the shock spike time is about twice as long as the time that would have been observed after total restoration by Ringer's solution. Sodium ions at the concentration 0.025 N would not have effected a total restoration of the speed of conduction, even though if they had been allowed to act for 1 or 2 additional hours they would have restored the ability to conduct impulses to an additional number of A fibers (see below, section 5). In this connection it is important to note that the C spike did not undergo an increase in height when the concentration of sodium ions was raised to 0.025 N (Fig. 3, 10, 23, 25; note that in records 23 and 25 the C spike is superposed upon a large A negative after-potential), which shows that 0.02 N sodium ions had been able to restore to all the C fibers the ability to conduct impulses.

In so far as the rate of penetration of sodium ions across the epineurium is concerned the results of this experiment are perfectly reproducible; in point of fact they have been reproduced in 12 different experiments. Another experiment of this type will be described below (Fig. 4), and in another paper (16, Fig. 6) an experiment will be illustrated in which the preliminary restoration was effected by 0.015 N sodium ions and in which raising the concentration of sodium ions to 0.025 N resulted within less than 5 seconds in an even more spectacular increase in the number of conducting fibers than in the experiment illustrated by Fig. 3 As should be expected, however, differences are observed from experiment to experiment in the number of fibers restored by the initial
moderate concentration of sodium ions and in the rate of the recovery after
the external concentration of sodium ions has been raised. These differences are
referable to technical details, such as the length of time during which the nerve
has been deprived of sodium, the concentration of sodium ions used to initiate
the recovery, the length of time allowed for the preliminary recovery, etc.,
but in all cases the same fundamental fact is observed: with nerve pre-
treated with a moderate concentration of sodium ions, raising the concentra-
tion of sodium ions outside the epineurium by 0.005 or 0.01 \text{x} results within
less than 5 seconds in a marked increase in the number of conducting A
fibers.

This fact eliminates the possibility of considering the epineurium as a diffusion
barrier. To be sure, the rapidity of the restoration of pretreated nerve proves
directly only that a few sodium ions have been able to diffuse rapidly across the
epineurium, but it is clear that if some sodium ions pass rapidly across the
epineurium other sodium ions will quickly follow the same path. Indeed, the
rate at which diffusion takes place during the initial phases can serve to calculate
the diffusion constant of sodium ions in the epineurium. By the use of very
moderate assumptions it is found that that constant cannot be less
than $0.75 \times 10^{-4}$ cm.$^2$/min. (14, p. 212). In all probability the true value of
the diffusion constant is considerably higher, but the minimal value is already
so high and the epineurium is so thin (about 40 \mu in bullfrog peroneal nerve)
that the epineurium cannot be a significant obstacle to the penetration of
sodium ions into the nerve trunk. For this reason the existence of the epi-
neurium may be ignored in the interpretation of the ordinary experiment.

The fact that 13 minutes after the concentration of sodium ions outside the
epineurium had been raised, the spike had reached a practically constant height
indicates that 13 minutes is an upper limit for the interval of time that is
required to approach diffusion equilibrium between the nerve trunk and an
outside solution. This result also is reproducible. For example, in the experiment
illustrated by Fig. 4 the spike height remained practically stationary (records
19 to 21) 15 minutes after the external concentration of sodium ions had been
raised to 0.025 N, and later in the experiment after the external concentration
had been raised to 0.03 N the spike height became stationary after 6 minutes
(Fig. 4, 24, 25). In another similar experiment the spike height became station-
ary 11 minutes after the first raise in the external concentration and 4 minutes
after the second raise, and comparable results have been obtained in several
other experiments.

In order to obtain a more accurate view of the situation let us take 13 minutes
as the time that is required for 90 per cent of diffusion equilibrium to be es-
tablished between the nerve trunk and an outside solution. If we assume that
diffusion through the interstitial spaces of the nerve trunk is comparable to
diffusion in a uniform cylinder, which in a first approximation is probably
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correct (cf. 14), we find by means of the theoretical data given by Hill (6, Fig. 5, II) that the diffusion constant of sodium ions is $0.62 \times 10^{-4}$ cm$^2$/min., since the diameter of the peroneal nerve is about 1 mm. and for 90 per cent of diffusion equilibrium at the axis of the cylinder $kt/r^2 = 0.333$. From this value of the diffusion constant it follows that in the two branches of the peroneal nerve, which are about 0.5 mm. in diameter, 90 per cent of diffusion equilibrium should be reached in 3 minutes. Experiment, however, has repeatedly and conclusively shown that the rates of recovery of the peroneal trunk and of its two branches are identical. Consequently, the rate of the recovery is not determined solely by the rate of diffusion of sodium ions in the epineurium and in the interstitial spaces of the nerve trunk, it is determined chiefly by the rate of processes taking place in the nerve fibers themselves.

Under conditions such as these the conclusion to be drawn from the experimental results mentioned above is that diffusion equilibrium of the peroneal trunk with an external solution is approached within about 6 minutes, which yields for the diffusion constant of sodium ions the value $1.4 \times 10^{-4}$ cm$^2$/min. As a matter of fact, however, since total recovery in Ringer's solution is not obtained in less than 1 hour (Fig. 2) it makes hardly any difference whether diffusion equilibrium is reached after 6 or after 13 minutes; in any case the diffusion is far more rapid than the recovery. Sodium ions therefore, do not restore the excitability of the nerve fibers solely by virtue of their presence outside the nerve fibers at a given concentration. The restoration is the result of processes which take place in the nerve fibers after sodium ions have been made available to them (cf. 13).

III

Loss of Excitability of Frog Nerves Deprived of Sodium

To a large extent the interval of time that must elapse before frog nerve becomes inexcitable in a sodium-free medium depends upon the past history of the nerve. As a rule, after a freshly excised bullfrog sciatic nerve has been placed in a sodium-free medium, 8 to 10 hours must elapse before all the A fibers become unable to conduct impulses, and 14 to 16 hours before all the C fibers become inexcitable (cf. 13, section 2). If, however, the inexcitable nerve is restored by sodium ions it again loses its excitability in a sodium-free medium far more rapidly than freshly excised nerve, with the noteworthy peculiarity that the rate of the loss of excitability largely depends upon the concentration of sodium ions that has been used to effect the recovery and upon the duration of the action of the restoring solution (Figs. 4 to 7).

For a correct understanding of the experimental results it is necessary to take into account that to render the nerve fibers inexcitable it is sufficient to reduce the concentration of sodium ions below a minimal or "maintenance" concentration (12, 18) which varies with different fibers. With untreated nerve
the fibers of the A group lose their excitability in much the same manner in the presence of 0.006 N sodium ions as in a sodium-free medium, but 0.01 N sodium ions are sufficient to maintain all the A fibers excitable for 6 or 8 hours; nevertheless, if the nerves are kept in the sodium-deficient solution for 15 to 20 hours a small number of A fibers lose the excitability if the concentration of sodium ions is below 0.03 N and less than one-third of the A fibers remain excitable in the presence of 0.01 N sodium ions. If the outside concentration is below 0.009 N all the A fibers become inexcitable. The maintenance concentration of the C fibers is smaller. All the C fibers or at least a large majority of them remain excitable in the presence of 0.01 N sodium ions, but all become inexcitable in the presence of 0.008 N sodium ions.

In nerve that has been rendered inexcitable in a sodium-free medium and has been restored by a moderate concentration of sodium ions the A fibers again become inexcitable if the concentration of sodium ions is reduced below the maintenance concentration, but with the nerves in such a state considerable differences in the rate of development of inexcitability are observed, depending upon the outside concentration of sodium ions. In a sodium-free medium the nerve fibers lose their excitability at a significantly higher rate than in the presence of concentrations of sodium ions which are below the maintenance concentration. For example after the restoration with 0.03 N sodium ions has reached a practically stationary state the A fibers become inexcitable in a sodium-free medium with remarkable rapidity; a relatively large number of A fibers become inexcitable within a few minutes and total A inexcitability develops within about 30 minutes (Fig. 4, 26 to 35). In the presence of 0.005 N sodium ions the decrease in the spike height does not become perceptible in less than 10 to 15 minutes and total A inexcitability does not develop in less than 60 to 80 minutes. This detail is important for the interpretation of the experimental results presented in Fig. 4.

The first part of the experiment illustrated by Fig. 4 reproduces the experiment illustrated by Fig. 3. After the nerve had been kept in the sodium-free medium for 17 hours the restoration was initiated with 0.02 N sodium ions. No A fiber had recovered the ability to conduct impulses after 34 minutes (Fig. 4, 3), but a few C fibers were able to conduct impulses after 35 minutes (records 4, 5); the C spike increased in height rather rapidly and after 53 minutes of the action of sodium ions a large majority of, probably all, the C fibers were conducting impulses (record 7), since the C spike did not display a readily detectable increase during the following 10 minutes (record 9); only the speed of conduction increased. At that time only a small number of A fibers were able to conduct impulses (records 8 and 10). Appropriate tests showed that the rate of increase of the A spike was so low that no readily detectable change in height could be observed in responses elicited 30 seconds or even 1 minute apart.

The rate of the recovery of the A fibers was then enhanced by raising the outside concentration of sodium ions to 0.025 N (Fig. 4, 11 to 21). A practically stationary height of the spike was reached after 15 minutes, as is shown by the slight difference
Fig. 4. Initiation of the recovery of excitability of nerve deprived of sodium by sodium ions at the concentration 0.02 N (records 1 to 10), enhancement of the recovery by sodium ions at the concentration 0.025 N (records 11 to 25), and development of inexcitability in a sodium-free medium (records 26 to 35). A inc. denotes an increase in the amplification. Note that some of the intervals between successive records are given in minutes.
between the spikes in records 20 and 21, which were elicited 4 minutes apart. The rate of the recovery was then increased again by raising the outside concentration of sodium ions to 0.03 N (records 22 to 25). The height of the spike became practically constant after 6 minutes (cf. records 24 and 25) in spite of the fact that only about one-half of the A fibers had recovered the ability to conduct impulses. As already indicated this observation means that within 6 minutes diffusion equilibrium had been approached and that 0.03 N sodium ions had completed the recovery of all those fibers which had been prepared for a rapid restoration by 0.025 N sodium ions. Neither the 0.025 nor the 0.03 N concentrations of sodium ions increased the number of conducting C fibers, which shows that 0.02 N sodium ions had been able to restore conduction by all the C fibers.

The nerve was deprived of sodium. An important decrease in the height of the A spike was observed after 4 minutes (Fig. 4, 26). The decrease in the height of the A spike continued rather rapidly (records 27, 28, 30) and, as can readily be noted, the decrease in the number of conducting fibers was accompanied by a marked reduction in the speed of conduction of the still conducting fibers (cf. especially the beta elevation of the spike). The majority of the A fibers had lost the ability to conduct impulses after 24 minutes of lack of sodium ions outside the epineurium (record 30) but a few more resistant A fibers were able to conduct impulses during the following 14 minutes (records 31, 33, 35). Emphasis must be placed upon the fact that lack of sodium did not render the C fibers inexcitable (records 29, 32, 34); it only resulted in a moderate decrease in the speed of conduction by C fibers. This observation proves that also in nerve restored by a moderate amount of sodium ions the C fibers are far more resistant to the effect of lack of sodium than the A fibers.

In regard to the problem of the outwards diffusion of sodium ions from the nerve trunk into the external sodium-free medium the results of this experiment are in a certain respect conclusive. A decrease in the spike height comparable to that which was observed within 4 minutes (Fig. 4, 26) would not have been observed within less than 20 minutes if the outside concentration of sodium ions had been 0.005 N; consequently there can be hardly any doubt that within 4 minutes the interstitial concentration of sodium ions had decreased from 0.03 N to not more than 0.005 N, i.e. to not more than 16 per cent of the initial value, so that after 4 additional minutes the interstitial concentration must have decreased to a negligible value. This results leaves no doubt that not only C fibers (Fig. 4, 34) but also A fibers (Fig. 4, 27, etc.) can conduct impulses in the absence of sodium ions in their external medium. If the attempt is made, however, to calculate the diffusion constant of sodium ions a value is obtained which is considerably higher than that which has been calculated above on the basis of observations on inward diffusion of sodium ions. The explanation of the discrepancy is that even though in nerve restored by a moderate concentration of sodium ions the fibers are very sensitive to the effect
of lack of sodium, the excitability of the nerve fibers is not an accurate indicator of the interstitial concentration of sodium ions. A satisfactory solution of the problem of outward diffusion will be presented later on the basis of observations made with spinal roots.

The difference in sensitivity to the lack of sodium of untreated nerve and of nerve restored by a moderate amount of sodium ions can be correlated with other differences in properties, among which the difference in the constitution of the membrane potential deserves mention here, because it explains an otherwise enigmatic phenomenon. With untreated nerve in a sodium-free medium the nerve fibers lose their ability to conduct rhythmic trains of impulses earlier than the ability to conduct single impulses (4, 12), while with nerve restored by a moderate concentration of sodium ions the nerve fibers may conduct trains of impulses while they fail to conduct single impulses.

Fig. 5 illustrates this remarkable phenomenon. Immediately after record 35 of Fig. 4 had been obtained the nerve was placed again in contact with 0.03 N sodium ions, that were allowed to act for 16 minutes. Record 1 of Fig. 5 presents the restored spike. The nerve was then deprived of sodium. No decrease in the height of the conducted spike was observed after 2 minutes (record 2) but after 3 additional minutes a significant decrease in the number of conducting fibers was observed (record 3); in addition it was observed that the speed of conduction had undergone a marked decrease (cf. especially the beta elevation). The nerve was then stimulated with a train of shocks, synchronized with the sweep of the oscillograph, at the frequency of approximately 40 per second, with the result that during the tetanus both the number of conducting fibers and the speed of conduction increased (record 4). The great magnitude of the negative after-potential should be noted. After 15 minutes of lack of sodium outside the epineurium the speed of conduction had undergone a further decrease (record 5), and the number of A fibers capable of conducting single impulses had decreased to approximately one-third of the number of originally conducting fibers, but about twice as many fibers were able to conduct impulses at a higher speed during tetanic stimulation (record 6). Seven minutes later only a few A fibers were able to conduct impulses (record 7) but tetanization again resulted in an increase in the number of conducting fibers (record 8). After 7 additional minutes, i.e. after 30 minutes of lack of sodium outside the epineurium no A fiber was able to conduct impulses (record 9).

The excitability of the nerve was again restored, first with 0.04 N sodium ions (Fig. 5, 10 to 12) and then with Ringer's solution (records 13 to 24). The important detail should be noted that the recovery progressed at a much lower rate than at the start of the experiment (Fig. 4, 11 to 25), after the inexcitable nerve had been pre-treated with a moderate concentration of sodium ions. During the early part of the recovery tetanization still resulted in an increase in the speed of conduction and in the number of conducting fibers, as well as in the production of a large negative after-potential (records 20 and 21), but after the nerve had been in contact with Ringer's solution for 60 minutes, and the recovery had become virtually complete, tetanization produced only a small negative after-potential and did not result in an increase in the speed of conduction or in a significant increase in the spike height (records 23 and 24).
In detail the explanation of the difference in the behaviors of untreated nerve and of nerve restored by a moderate amount of sodium ions is the following. With untreated nerve in a sodium-free medium and in an atmosphere of air or oxygen the L fraction of the membrane potential initially has a low value and decreases progressively in magnitude with advancing time. Tetanization cannot produce a large negative after-potential, for which reason it can result only in an increase in the threshold of stimulation, and as soon as lack of sodium begins to decrease the rate of recovery after
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Conduction the nerve fibers fail to conduct rhythmic trains of impulses (cf. 4, Fig. 1). On the other hand, at the time when all the A fibers become inexcitable the constitution of the membrane potential still is not far from normal, so that the restoration of excitability by sodium ions does not involve a large increase in the value of the L fraction; therefore, removal of the L fraction by repetitive stimulation cannot produce a significant decrease in the threshold of stimulation, with the result that until the rate of recovery after conduction again becomes normal the nerve fibers are unable to conduct trains of impulses (cf. 4, Fig. 2).

A different situation prevails if the nerve is left in the sodium-free medium for several hours after all the A fibers have become inexcitable. The changes in the constitution of the membrane potential become progressively deeper and after 5 or 6 hours both the L fraction and the polarizability of the membrane by the applied anodal current become very small (cf. 13). The restoration of the nerve fibers by sodium ions occurs in two steps. The first step consists in a large increase in the L fraction of the membrane potential, and the second in the transformation of part of the increment of the L fraction into Q fraction. Sodium ions at moderate concentrations are not able to produce the second step.

Consequently, in nerves restored by a moderate concentration of sodium ions, as well as in nerves during the initial phase of the restoration by 0.1 N sodium ions, the L/Q ratio has a high value, for which reason the threshold of stimulation is high and repetitive conduction of impulses, by causing the collapse of the L fraction, i.e. by creating a large negative after-potential, produces a marked decrease in the stimulation threshold. Thus the relief of conduction blocks which appears in Fig. 5, 4, 6, 8, 21 is analogous to the relief of the anodal block or of the calcium block by repetitive stimulation (12, sections VII, 9 and VII, 10). If the frequency of stimulation had been increased the blocks would have been strengthened, for the loss of L fraction would not have been able to compensate for the rise in threshold resulting from repetitive activity at high frequency.

When a nerve, which has been rendered inexcitable in a sodium-free medium, is restored by 0.1 N sodium ions it again acquires a considerable resistance to the lack of sodium, which moreover increases markedly with the duration of the interval of time during which 0.1 N sodium ions are allowed to act. Fig. 6 illustrates the observations that were made with the nerve used to obtain the records of Figs. 4 and 5. The nerve was deprived of sodium after restoration by Ringer's solution acting for 70 minutes.

Whether or not a decrease in the number of conducting fibers occurred during the first 5 minutes of lack of sodium ions outside the epineurium cannot be stated with certainty because the difference between the conductivities of 0.11 M diethanoldimethylammonium chloride and of Ringer's solution is sufficient to produce a slight increase in the spike height when the nerve is placed in contact with the sodium-free medium, and this change cannot become maximal until diffusion equilibrium is approached; i.e. within 4 or 5 minutes. Record 2 of Fig. 6, however, leaves no doubt that after 23 minutes of lack of sodium ions outside the epineurium a number of fibers had lost the ability to
conduct single impulses. This is a short time in relation to the time that is required to make the least resistant A fibers of untreated nerve unable to conduct impulses, but a long time in relation to the time that is required to abolish the ability to conduct impulses by the least resistant A fibers of nerve restored by a moderate amount of sodium ions. Total inexcitability was not observed until the nerve had been deprived of sodium for 2 hours (Fig. 6, 11, 12). This is again a short time in relation to the time that is required to render inexcitable all the A fibers of untreated nerve (8 to 10 hours), but a long time in relation to the time that is required to render inexcitable all the A fibers that have

been restored by 0.03 N sodium ions (36 minutes, Fig. 4, 25 to 35; 28 minutes, Fig. 5, 1 to 9).

The results of this experiment are perfectly reproducible; restoration by 0.1 N sodium ions always results in a marked increase in the resistance of the nerve fibers to the effect of lack of sodium, the increase being the greater the longer 0.1 N sodium ions have been allowed to act. For example in one experiment of this type, in which the nerve was restored by Ringer's solution acting for 2 hours, the resistance of the nerve fibers became so great that after 2 hours in the sodium-free medium less than one-half of the A fibers had lost the ability to conduct impulses; the experiment was discontinued because it was obvious that total inexcitability could not develop in less than 3 or 4 hours.

A feature of the temporal course of the development of inexcitability deserves

Fig. 6. (Continuation of Fig. 5.) Development of inexcitability in a sodium-free medium. Records 4, 6, and 8 were obtained with the use of repetitive stimulation.
emphasize. It will be noted in Fig. 6 that the heights of the spikes in records 1, 4, and 5 are approximately equal, which shows that although during the first hour of lack of sodium a discrete number of A fibers had lost the ability to conduct single impulses (records 2, 3, 5) all or practically all the A fibers were still able to conduct trains of impulses. During the first hour the speed of conduction underwent a noticeable decrease and when the speed reached the low value that had been observed with the nerve restored by a moderate concentration of sodium ions (cf. Fig. 6, 7 and Fig. 5, 1; note the difference in amplification), the decrease in the number of conducting fibers (Fig. 6, 7 to 12) became as fast as that which had been observed earlier (Fig. 4, 25 to 35; Fig. 5, 1 to 0), with the nerve restored by 0.03 N sodium ions. Thus it appears that during the first hour the effect of lack of sodium ions consisted in a progressive change in the properties of the nerve fibers; it was only after the change had reached a certain magnitude that the nerve fibers rapidly began to become inexcitable. The same 2 steps in the development of inexcitability can be observed with untreated nerve (4), although not so sharply delimited as in the case illustrated by Fig. 6.

In the continuation of the experiment the nerve was kept in contact with the sodium-free medium for 16 minutes after record 12 of Fig. 6 had been obtained, and was then restored with 0.02 N sodium ions. Although at the beginning of the experiment this concentration of sodium ions had been able to restore the excitability of only a few A fibers, and only at an exceedingly low rate (Fig. 4, 3, 10), at the stage of the experiment now under consideration 0.02 N sodium ions produced a spectacularly rapid recovery. Record 1 (Fig. 7) presents the spike that was observed after 4 minutes of the action of the restoring solution and records 3 to 6 illustrate the rapid increase in the spike height during the following 9 minutes. It should be noted that the spike in Fig. 7, 6 is as high as the spike previously observed after restoration by 0.03 N sodium ions (Fig. 5, 1).

The interpretation of these observations lies at hand. At the start of the experiment (Fig. 4, 1 to 8) 0.02 N sodium ions had to reverse the profound changes that had taken place in the nerve fibers during the 5 or 6 hours that followed after the development of inexcitability in the sodium-free medium. In the stage of the experiment which is now under consideration (Fig. 7, 1 to 6) 0.02 N sodium ions were made available to the nerve after this had been restored by 0.1 N sodium ions (Fig. 5), and only about 20 minutes after total inexcitability had developed in the sodium-free medium; consequently, the nerve fibers were in such a state that a moderate concentration of sodium ions could effect a rapid restoration of excitability. A proof that the inexcitable fibers were in a state different from that in which they had previously been is that tetanization resulted in rapid decrease of the spike height (Fig. 7, 2); this record was taken with a low intensity electron beam, so that only the tracing
Fig. 7. (Continuation of Fig. 6.) Restoration of excitability by sodium ions at the concentration 0.02 N (records 1 to 6; record 2 was obtained with the use of repetitive stimulation), development of inexcitability in a sodium-free medium (records 7 to 13), restoration of excitability by sodium ions at the concentrations 0.015 N (records 14 to 18) and 0.02 N (records 19 to 22), and development of inexcitability in the presence of sodium ions at the concentration 0.005 N (records 23, 24). Record 2 was obtained with the use of repetitive stimulation.
of the superposed, repetitive spikes was photographed; the height of the single spike is given by record 1), while previously tetanization had resulted in an increase in the spike height (Fig. 5, 4, 6, 8, 21).

The recovery of excitability was still in progress when the nerve was again deprived of sodium, which undoubtedly is the reason why during the first 3 minutes of lack of external sodium the spike height still underwent a slight increase in height (Fig. 7, 7); nevertheless, the effect of sodium lack developed rapidly (records 8 to 12) and within 20 minutes the inexcitability became practically total (records 12, 13); i.e., inexcitability developed much faster this time than the preceding time (Fig. 6).

Immediately after inexcitability had set in the nerve was treated with 0.015 N sodium ions and since the nerve fibers had just become inexcitable, this small concentration of sodium ions was sufficient to effect a fairly rapid recovery of excitability (Fig. 7, 14 to 18). The rate of the recovery was markedly enhanced by raising the concentration of sodium ions outside the epineurium to 0.02 N (Fig. 7, 19 to 22) and after this solution had been allowed to act for 125 seconds the concentration of sodium ions outside the epineurium was lowered to 0.005 N, which resulted in a rapid decrease in the number of conducting fibers; a significant decrease in the spike height was observed after 3 minutes (record 23) and a much greater decrease after 3 additional minutes (record 24). Clearly, the nerve fibers were in such a state that their excitability rapidly followed the changes in the interstitial concentration of sodium ions, with the result that any change in the concentration of sodium ions outside the epineurium was immediately followed by a change in the number of conducting fibers.

The experiment was discontinued. During 8 consecutive hours the concentration of sodium ions outside the epineurium had been changed a number of times and the observations that had been made had convincingly shown that the rate of diffusion of sodium ions could never have been an important factor in determining the rate of change in the excitability of the nerve fibers. The manner in which variations in the concentration of sodium ions outside the epineurium modified the excitability of the nerve fibers had proven to depend primarily upon the state of the nerve fibers, for which reason the rate of establishment of the excitability changes could be made high or low by judiciously altering the state of the nerve fibers.

As already mentioned, the experimental data on outwards diffusion of sodium ions are not suitable for the evaluation of the diffusion constant. To be sure, there is no reason for believing that the constant should be different for outwards and for inwards diffusion, but it is desirable to confirm the validity of the results obtained in experiments on inward diffusion.

A satisfactory evaluation of the diffusion constant for outwards diffusion can be made on the basis of results obtained with spinal motor roots (cf. 14).
In the first place the fibers of the roots are more sensitive to the lack of sodium than the fibers of the trunk, and on the other hand, by repeated removal and addition of sodium ions the fibers of the roots can readily be brought into such a state that inexcitability of a number of A fibers develops within a few seconds after the removal of sodium from the surface of the root.

The observations illustrated by Fig. 8 were made with a spinal motor root that had been dissected together with a segment of spinal cord and with a long segment of the corresponding nerve trunk. The length of the root was 33 mm. The impulses were initiated in the trunk; the first recording electrode was placed on the root at 16 mm. from the cord and the second electrode was in contact with the spinal cord at the point of emergence of the root. In the course of a long experiment, in which the fibers of the root were rendered inexcitable in a sodium-free medium and restored by sodium ions several times, the fibers
became highly sensitized to the effect of lack of sodium. \(^1\) A clear concept of the degree of sensitization can be obtained by considering that with a freshly excised motor root the segment of the root next to the spinal cord does not become inexcitable in a sodium-free medium in less than 50 to 60 minutes (cf. 15), while at the stage of the experiment illustrated by Fig. 8 total inexcitability of that segment developed in 1 minute (records 7, 8) or in 2 minutes (records 15, 16). At the same time that the sensitization was being produced about one-half of the fibers of the root became irreversibly inexcitable.

Fig. 8 presents two series of records (1 to 8 and 9 to 19), which illustrate the development of inexcitability under two different conditions. At the beginning of the series 1 to 8 the root was in diffusion equilibrium with 0.04 N sodium ions. A number of nerve fibers became inexcitable within less than 5 seconds after the root had been washed with the sodium-free solution (record 2), and the development of inexcitability advanced so rapidly (records 3 to 6) that after 1 minute of lack of sodium the recorded spike became a monophasic deflection and displayed the small height with which it appears in records 7 and 8. That the spike became a monophasic deflection proved that conduction of impulses was blocked in all the fibers at no less than 3 or 4 mm. ahead of the spinal cord. The height of the spike in records 7 and 8 is about one-third of the height of the spike in record 1, but since in the case of record 1 the height of the first phase of the spike was reduced by the arrival of impulses at the second recording electrode, it is clear that less than one-third of the initially conducting fibers were still able to conduct impulses up to point 16 mm.

At the beginning of the series 9 to 19 (Fig. 8) the root was in diffusion equilibrium with 0.1 N sodium ions (Ringer's solution). Removal of sodium again caused inexcitability of a number of fibers within less than 5 seconds (record 10) but as had to be expected the development of inexcitability (records 11 to 19) progressed at a somewhat lower rate than in the preceding case. This time the stage illustrated by record 6 was reached after 95 seconds of lack of sodium (record 15), and a stage slightly more advanced than that which is illustrated by record 8 was reached after 140 seconds (record 16). Records 17 to 19 illustrate more advanced stages of the development of inexcitability.

One minute after record 19 had been obtained the root was placed in contact with a solution containing sodium ions at the concentration 0.01 N, which resulted in a rapid recovery of excitability, beginning within less than 10 seconds and reaching a stationary state after 1 minute, which shows that diffusion equilibrium was approached within not more than 1 minute. When its

\(^1\) When a spinal root, particularly a dorsal root, is deprived of sodium for the first time a partial recovery of excitability is observed every time that the washing of the root with the sodium-free medium is discontinued (15, Fig. 2). Such a phenomenon cannot be observed if the root is deprived of sodium after it has been rendered inexcitable and has been restored by sodium ions acting for a relatively short time (10 to 20 minutes).
height became constant the spike had approximately the same magnitude and shape with which it appears in Fig. 8, 6, which proved that at the stages illustrated by Fig. 8, 7, 8 and 15, 16 the interstitial concentration of sodium ions was less than 0.01 N; for the sake of the argument let it be assumed that the interstitial concentration was 0.009 N.

Thus we find that when the root which was in equilibrium with 0.04 N sodium ions was placed in contact with the sodium-free solution 78.5 per cent of diffusion equilibrium was reached in 1 minute, and that when the root which was in equilibrium with 0.1 N sodium ions was placed in contact with the sodium-free solution 91 per cent of diffusion equilibrium was reached at the axis of the root in 2.2 minutes. By means of Hill’s curve (6, Fig. 5, II) we find that for 78.5 per cent of diffusion equilibrium \( k/t^2 = 0.2 \) and for 91 per cent, \( k/t^2 = 0.34 \). If we take the radius of the root to be 0.2 mm. \( (r = 0.02) \) we find for the diffusion constant \( k \) of sodium ions in the interstitial spaces of the root the values \( 0.8 \times 10^{-4} \text{ cm.}^2/\text{min.} \) in the first case, and \( 0.62 \times 10^{-4} \text{ cm.}^2/\text{min.} \) in the second case. If we consider that during restoration by 0.01 N sodium ions diffusion equilibrium was approached within 1 minute we find for \( k \) a higher value, \( 1.75 \times 10^{-4} \text{ cm.}^2/\text{min.} \), if we assume that 91 per cent of diffusion equilibrium had been reached.

It should be noted that the values of \( k \) calculated here agree with the values calculated in section 2.

IV

Comment on Diffusion of Sodium Ions

Before drawing any definitive conclusion on the speed of diffusion of sodium ions through the interstitial spaces of frog nerve it is necessary to consider whether or not the permeability of the connective tissue sheath (epineurium plus perineurium plus endoneurium) for sodium ions changes when the nerve is placed in a sodium-free medium.

The only assumption which is compatible with the experimental results is that in the absence of sodium ions the permeability of the sheath decreases slightly and very slowly. During the early stages of the development of inexcitability of previously untreated nerve, i.e. during the first few hours of lack of sodium, the restoration of excitability by sodium ions takes place with such an amazing rapidity that there can be no doubt that during that time the sheath is highly permeable to sodium ions. The rapidity of the initial phases of the restoration decreases somewhat with advancing time, which might be regarded as a sign that the permeability of the sheath is slowly undergoing a decrease, but even at the time when the inexcitability of the A fibers is becoming total the initial phase of the restoration is still so rapid that the permeability of the sheath must necessarily be high. It is only when finally the stage is reached at which the observations illustrated by Fig. 2 were made that the restoration of A fibers begins after an important latent period; in view of this
fact it would be permissible to assume that the permeability of the sheath has undergone a significant decrease. However, there are cogent reasons for believing that the delay in the onset of the restoration is chiefly due to other causes than a decreased permeability of the sheath, since the restoration of the excitability of C fibers begins much earlier than that of A fibers and since direct analysis of the effect of the added sodium ions upon the A fibers reveals that the restoration of these fibers begins much earlier than is indicated by the appearance of conducted A spikes; nevertheless, the assumption of a slight decrease in the permeability of the sheath would be compatible with the experimental results, because the recovery of the ability of C fibers to conduct impulses begins in about 1 minute or 2, and the effect of the added sodium ions upon the electrotonic potentials of A fibers becomes detectable in about 1 minute.

Thus we find that it would be permissible to assume that in a sodium-free medium the permeability of the sheath decreases slightly while the nerve fibers are becoming inexcitable, i.e. long after the interstitial concentration of sodium ions has become negligible, and that the decrease continues after total inexcitability has set in. But, on the other hand, there can be no doubt that after the inexcitable nerve has been treated with a moderate concentration of sodium ions the sheath is again highly permeable to sodium ions (Fig. 3). Under conditions such as these it is clear that the diffusion constant of sodium ions that has been calculated above is valid for the sheath of (a) normal nerve, (b) nerve that has been kept in a sodium-free medium for several hours only, and (c) nerve that has been kept in the sodium-free medium for many hours but has been treated with a moderate concentration of sodium ions.

In conclusion, whether or not the permeability of the sheath for sodium ions undergoes a decrease when the nerve is kept in a sodium-free medium for an extended period of time is a question that may be left unanswered, because the answer has no practical value. In the first place, it is a priori clear that if lack of sodium ions should decrease the permeability of the sheath the decrease would begin after the interstitial concentration of sodium ions has become negligible, and on the other hand, the experimental evidence has shown that if a decrease should take place, the decrease would be only small and would take place only after the nerve fibers have become inexcitable; moreover, the evidence has shown that the change in permeability would be reversible by sodium ions. Consequently, the change in permeability, if such should occur, would not constitute a cause of error in experiments on the role of sodium in nerve physiology.

With the evaluation of minimal values for the diffusion constant of sodium ions in the connective tissue sheath of nerve the problem of diffusion of sodium ions through nerve has received a solution that for all practical purposes is sufficient. Even the lowest calculated value, $0.62 \times 10^{-4}$ cm.$^2$/min., which
undoubtedly is too low, leads to the conclusion that diffusion of sodium ions is so rapid that only in exceptional instances would it be necessary to take the diffusion time into account. As a rule the experimental results may be interpreted on the basis of the assumption that diffusion of sodium ions is practically instantaneous. For example, using Hill’s curve (6, Fig. 5, II) we find that in the peroneal trunk of the bullfrog (diameter, 1 mm.) 90 per cent of diffusion equilibrium must be reached in 13 minutes; in the two branches of the peroneal nerve of the bullfrog (diameter 0.5 mm.) 90 per cent of diffusion equilibrium must be reached in 3 minutes, and in the peroneal branches of ordinary frogs of small size (diameter 0.25 mm.) 90 per cent of diffusion equilibrium must be reached in 0.75 minute (45 seconds!). The peroneal branches of ordinary frogs do not become totally inexcitable in a sodium-free medium in less than 7 hours, and even when their nerve fibers find themselves in an advanced stage of Wallerian degeneration total inexcitability requires several hours to develop (4).

The calculated minimal value for the diffusion constant of sodium ions is too low because the calculation has been carried out on the basis of assumptions so moderate that they could not overstep the range of validity of the experimental observations. An independent proof of the validity of the results of the calculations can be obtained by evaluating the diffusion constant of sodium chloride, which can be done by means of a simple procedure. Determinations are made of the changes in the electric conductivity of the nerve when the external concentration of sodium chloride is varied from 0.02 M (the solution having been made isotonic by addition of glucose) to 0.1 M or to 0.2 M and conversely. The measurements are carried out by recording at 5 second intervals the potential difference established by rectangular pulses of current between 2 points 5 mm. apart at the center of a 40 mm. long interpolar segment. Initially the changes in conductivity take place with great rapidity, but the rate of change also decreases with great rapidity and after about 4 minutes the change in conductivity becomes imperceptible. Let us assume that the change in conductivity becomes imperceptible when the diffusion process reaches 90 per cent of equilibrium. In Hill’s curve (6, Fig. 5, II) we find that for 90 per cent of diffusion equilibrium $kt/r^2 = 0.333$. Since $r = 0.05$ cm. we find that if 90 per cent of diffusion equilibrium is reached in 4 minutes the diffusion constant $k$ of sodium chloride is $2 \times 10^{-4}$ cm.$^2$/min. and if 50 per cent of equilib-

\[\text{The measurement of changes in the longitudinal conductivity must be made at the center of a long interpolar segment in order to avoid the inclusion of the membrane of the nerve fibers in the measuring circuit. Also, ions must be used to change the conductivity of the interstitial fluid which do not cause a rapid change in the nerve fibers. For example, when the change in conductivity is produced by solutions containing relatively high concentrations of potassium ions, penetration of those ions and of water in the nerve fibers produces seemingly disconcerting results, which will be described in another paper.}\]
rium is reached in 5 minutes $k$ is $1.65 \times 10^{-4}$ cm.$^2$/min. Even if 90 per cent of diffusion equilibrium should not be reached until after 6 minutes the diffusion constant would still be $1.4 \times 10^{-4}$ cm.$^2$/min. As determined by Fenn (3) the diffusion constant of CO$_2$ is $0.71$ to $0.96 \times 10^{-4}$ cm.$^2$/min.

Equilibria of Nerve Fibers with Different Concentrations of Sodium Ions

In experiments on restoration of inexcitable nerve with moderate concentration of sodium ions the observation is customarily made that each increase in the restoring concentration results in a partial restoration, which begins rapidly but tends to reach soon a more or less steady state (Figs. 3 to 7). To be sure, the state of the nerve improves continuously with advancing time but at a rate so low that the nerve seems to have reached an equilibrium with the restoring solution. The equilibrium is characterized by the speed of conduction of impulses and by the number of conducting fibers.

A similar observation can be made when nerves are kept for 20 to 24 hours in the presence of a subnormal concentration of sodium ions. As has been mentioned in section 3 if the concentration of sodium ions in the external medium is below 0.03 N a certain number of A fibers lose the ability to conduct impulses, but this is not the only noticeable change, the speed of conduction of the excitable fibers is subnormal. If the concentration of sodium ions is reduced further both the decrease in the number of conducting fibers and the decrease in the speed of conduction become greater.

If the external concentration of sodium ions is 0.04 N all the A fibers remain excitable and the speed of conduction undergoes only a hardly perceptible reduction. Nevertheless by analysis of the electrotonic potential it can readily be demonstrated that the effectiveness of the nerve reaction undergoes a significant decrease.

The experiment illustrated by Fig. 9 was done with a pair of nerves. One of the nerves (n. I) was kept in the presence of 0.025 N sodium ions for 15 hours, and the other (n. II), in the presence of 0.04 N sodium ions for practically the same length of time. Appropriate tests showed that only a small number of A fibers of nerve I had lost the ability to conduct impulses, while the vast majority if not all the A fibers of nerve II were able to conduct impulses.

Records 1 to 8 of Fig. 9 present the electrotonic potentials observed at 5 mm. from the polarizing electrode with the nerves in the sodium-deficient solutions. Examination of the maxima and overshootings of the slow electrotonus readily shows that the effectiveness of the nerve reaction had decreased in both nerves, and as should be expected, it had decreased more in nerve I than in nerve II. Also the polarizability of the membrane by the anodal current was subnormal.

Treatment of the nerves with Ringer's solution for 60 minutes resulted in a
Fig. 9. Electrotonic potentials that were produced by rectangular pulses of applied currents at 5 mm. from the polarizing electrode, with the nerves in the test solutions (1 to 8) and at two stages of the restoration by Ringer's solution (9 to 16 and 17 to 24).

recovery of the effectiveness of the nerve reaction, since the maxima and overshootings of the slow electrotonus became greater and sharper (Fig. 9, 9 to 16).
The recovery of nerve II was complete after 60 minutes but that of nerve I did not reach completion until the nerve had been kept in Ringer's solution for 160 minutes (Fig. 9, 17 to 24).

Emphasis must be placed upon the low rate of the recovery of the effectiveness of the nerve reaction. (To this low rate corresponds the low rate of the recovery of the ability to conduct rhythmic trains of impulses, cf. 4 and 12, Fig. XV, 48.) All the inexcitable fibers of nerve I recovered the ability to conduct impulses in less than 10 minutes, but full restoration of the properties of the nerve fibers required 160 minutes. This observation shows once more that the restoration of sodium-deficient nerves involves long lasting processes which take place in the nerve fibers after the normal external concentration of sodium ions has been made available to them.

Experiments have not been done with nerves kept in concentrations of sodium ions between 0.04 and 0.1 N, but since the changes in properties observed in nerves kept in 0.04 N sodium ions are rather profound, and since nerves that have been deprived of sodium ions for an extended period of time cannot be fully restored by concentrations below 0.1 N, it seems justifiable to reach this general conclusion. The nerve fibers can retain the ability to conduct impulses in the presence of concentrations of sodium ions above 0.03 N, but the nerve fibers can retain full functional ability only when the external concentration of sodium ions approaches the normal concentration (0.1 N). If the concentration of sodium ions is reduced the effectiveness of the nerve reaction, the speed of conduction, and finally the number of conducting fibers decrease. It would be consistent with the experimental results, and reasonable, to assume that the spike height decreases before the nerve fibers become inexcitable, since this is the observation that has been made by Hodgkin and Katz (8) with the giant axon of the squid, but working with multifibered nerves it is as a rule impossible to ascertain the presence of changes in the height of the individual fiber spikes.\footnote{When isotonic sugar solutions are used as sodium-free media the height of the spike undergoes a progressive increase during the first few hours of lack of sodium; later, when the nerve fibers begin to become inexcitable the spike height undergoes a progressive decrease, which, in view of the observations made by Hodgkin and Katz (8), may be in part referable to a decrease in the height of the individual fiber spikes.}

In a strict sense to speak of equilibrium of a nerve with a subnormal concentration of sodium ions is incorrect, for the change in the properties of the nerve fibers is progressive, but since after 15 or 20 hours the rate of change in properties is exceedingly small, it may be said that practically an equilibrium has been established at that time.

An important detail is this. The state of equilibrium with a given concentration of sodium ions depends upon the past history of the nerve (cf. Figs. 4 to 7). For example, if a nerve is maintained in a sodium-free medium for 15 to 20
hours and the nerve is then restored by a moderate concentration of sodium ions, 0.015 or 0.02 N, the nerve will not reach the state in which it would find itself if it had been kept in the restoring concentration all the time, at least it will not reach such a state in several hours. Similarly, if the restoring concentration is raised to 0.03 or 0.04 N the state of the nerve will rapidly improve for a few minutes, but later the recovery will progress at a very low rate, so that in all probability the nerve would never reach the state in which it would find itself if it had been kept in the presence of 0.03 or 0.04 N sodium ions all the time. If, however, the nerve is restored by 0.1 N sodium ions and is again rendered inexcitable in a sodium-free medium, a moderate concentration of sodium ions will rapidly bring the nerve into or nearly into the state in which it would find itself, had it been kept in the presence of that moderate concentration of sodium ions all the time (Fig. 7).

A similar situation prevails in reference to the resistance of the nerve fibers to the effect of the lack of sodium. A nerve that has been kept in the presence of a moderate amount of sodium ions for more than 15 to 20 hours may contain a number of inexcitable fibers, but the majority of the still excitable fibers will prove to be considerably more resistant to the effect of lack of sodium than the fibers of nerve which, after having become inexcitable in a sodium-free medium, has been restored by a moderate concentration of sodium ions. For example, in one experiment a nerve was kept in the presence of 0.025 N sodium ions for 23 hours. A small number of A fibers had become inexcitable, but the majority of the still conducting fibers were so resistant to the effect of lack of sodium that after 2 hours in the sodium-free medium more than two-thirds of the initially conducting fibers were still able to conduct impulses. It will be remembered that in the experiment illustrated by Fig. 4, all the A fibers that had been restored by 0.03 N sodium ions became inexcitable in the sodium-free medium in 38 minutes. As was described in section 3, if the inexcitable nerve is restored by 0.1 N sodium ions the nerve fibers acquire a high resistance to the effect of lack of sodium (Fig. 6).

It seems, therefore, that prolonged lack of sodium produces in the nerve fibers changes which cannot be fully reversed by small concentrations of sodium ions. To be sure, the effect of a small concentration of sodium ions increases steadily with advancing time tending to bring the nerve into the state which corresponds to equilibrium with that concentration, but this state will not be reached, at least not in several hours, unless a higher concentration of sodium ions has acted temporarily upon the nerve.

VI
DISCUSSION

It will contribute to the clarity of the discussion to summarize what has become known about the role of sodium in nerve physiology.
(a) Sodium ions together with chloride ions play the main role in determining the concentration of water outside the nerve fibers. In this respect the role of sodium is not specific since sugars (18) and the chlorides of a number of quaternary ammonium ions (12, 13) can substitute for sodium chloride.

(b) The presence of sodium ions in the external medium of the nerve fibers plays a role in determining the total value of the resting membrane potential, since this potential undergoes a large increase when the nerve is kept in an isotonic (0.22 M) sugar solution. The increase is established in a progressive manner; it begins gradually and continues for a number of hours. This fact indicates that the increase in the membrane potential is not immediately referable to the lack of external sodium ions; on the other hand, abundant evidence is available to show that the increase in the membrane potential is produced by the activity of the metabolic mechanisms of the nerve fibers (12, chapter IV).

In this respect the role of sodium is not specific, since the resting membrane potential keeps approximately normal value in the presence of isotonic solutions of the chlorides of certain quaternary ammonium ions (13), some of which are of the restoring type (for example, tetraethylammonium), and some of which are of the inert type (for example, choline).

(c) Sodium plays a role in maintaining the excitability of the nerve fibers, since in the absence of external sodium the nerve fibers become inexcitable (18).

In this respect the role of sodium has a high degree of specificity, but is not entirely specific. Lithium can replace sodium and maintain the excitability of the nerve fibers (8, 18); lithium also can restore the excitability of A and C fibers that have become inexcitable in a sodium-free medium (5). A substance (probably a quaternary ammonium compound) extracted from the oxbrain restores to fibers of the A and Et classes the ability to produce impulses, and a number of quaternary ammonium ions can restore to Et fibers the ability to conduct impulses (13). Cocaine can also substitute for sodium and temporarily restore to A fibers the ability to conduct impulses (16).

(d) Sodium plays a role in maintaining the ability of the nerve fibers to establish the nerve reaction; i.e., those processes by means of which the nerve fibers respond to changes in the value of the membrane potential (12, 13).

In this respect the role of sodium has the highest degree of specificity. Lithium cannot substitute for sodium (5) and the quaternary ammonium ions of the restoring type can restore only to a limited degree the ability of the nerve fibers to establish the nerve reaction (13).

From the four aspects of the role of sodium only aspects (a) and (b) are directly dependent upon the concentration of sodium ions, or of substituting ions, outside the nerve fibers. The results presented in this and in preceding papers (12, 13) leave no doubt that the nerve fibers can conduct impulses and establish the nerve reaction for extended periods of time after the external
concentration of sodium ions has become negligible. Therefore only the sodium that is present inside the nerve fibers can play a role in the production of the nerve impulse and of the nerve reaction, and there are reasons for believing that the role is largely indirect (13).

Under conditions such as these, since nerve fibers kept in a sodium-free medium lose, if not all, at least an important part of their internal sodium content (12, pt. 1, p. 111), and since muscle fibers kept in a sodium-free medium must lose their internal sodium (cf. 11, with references to the literature), it seems logical to conclude that, in so far as the production of the nerve impulse and the nerve reaction are concerned, the presence of sodium ions outside the nerve fibers is necessary only because in the absence of external sodium the nerve fibers lose part of their internal sodium. This conclusion is in contradiction with the hypothesis put forward by Hodgkin and Katz (8) to explain the participation of sodium ions in the production of the nerve impulse.

That the function of sodium is quite complex follows immediately from the fact that so many different substances can substitute for sodium, each in its own manner and none being able to play completely the role of sodium. In view of this situation it seems logical to believe that sodium takes part, directly or indirectly, in a number of chemical reactions, so that different substances may substitute for sodium in different manners and degrees.

An important problem that future research must solve is that of the changes in the internal sodium content of the nerve fibers which result from changes in the external concentration of sodium. The technical difficulties encountered in research of this kind are great, but an important part of the difficulties has been removed by the evaluation of the diffusion constants of sodium ions and of sodium chloride in the interstitial spaces of the nerve trunk. On the basis of the information that is now available it may be stated with certainty that within less than 10 minutes after placing the nerve in a sodium-free medium no interstitial sodium can exist in the nerve, except for that amount of internal sodium which is leaking into the interstitial spaces. There remains, however, a very great difficulty; the internal sodium will be present both in the nerve fibers and in the fibrils of the connective tissue sheath, and since these fibrils make up an important part of the nerve (cf. 14, Fig. 1), chemical analysis cannot lead to definitive conclusions regarding the exchange of sodium by the nerve fibers until a procedure has been elaborated to differentiate between the sodium which is present in the nerve fibers and the sodium which is present in the fibrils of the connective tissue sheath.

The experimental observations presented in this paper lead to an interesting working hypothesis. The internal sodium content of the nerve fibers and the external concentration of sodium ions are in equilibrium in the sense that a different internal sodium content corresponds to each external concentration; the properties of the nerve fibers are determined by the internal sodium content. A decrease in the external concentration causes a decrease in the internal
sodium content, which results in a change in the properties of the nerve fibers. Conversely, when a nerve that has been kept in a sodium-free medium for 15 to 20 hours is restored by a moderate concentration of sodium ions the internal sodium content reaches only a moderate value; therefore, the changes that had taken place in the chemical structure of the nerve fibers are reversed only in a partial manner; it is only when the normal concentration of sodium ions (0.1 N) is made available to the nerve fibers that the internal sodium content may reach its normal value and consequently the changes in the properties of the nerve fibers may be fully reversed.

The rapidity with which nerve fibers pretreated with a moderate concentration of sodium ions regain their ability to conduct impulses after the external concentration has been increased by a small amount indicates that nerve fibers may take up sodium ions from their external medium with great rapidity, but this observation does not necessarily indicate that those additional sodium ions, which effect the last step of the restoration, directly participate in the production of the nerve impulse. As a matter of fact such an assumption is made unnecessary by certain experimental observations.

In pretreated nerve the last step of the restoration of the ability to conduct impulses can be effected by cocaine (16), while it seems quite unlikely that a strong base like sodium could be directly replaced by a weak base like cocaine; therefore, it seems probable that sodium ions effect the last step of the restoration through an indirect mechanism. On the other hand, the last step of the restoration of the excitability by sodium ions can be effected by the applied anodal current, since there is during the restoration by sodium ions a stage, during which the nerve fibers are not excitable to cathodal currents of any magnitude and cannot conduct impulses, but during which the nerve fibers can produce impulses in response to the break of the anodal current (13, Fig. 6, 1; Fig. 43, 6 to 11; Fig. 48, 9 to 16). The effect of the anodal current cannot be explained by assuming that the flow of the current results in a rapid inward transport of sodium ions by the current, since the restoration is not permanent, and since the effect can also be observed during the last stages of the development of inexcitability in a sodium-free medium (13, Fig. 9, 25 to 27; Fig. 13, 13 to 24). The likely explanation of the effect of the anodal current is that during its brief flow this current temporarily establishes certain electrochemical changes in the nerve fibers, which can also be produced and be made permanent by sodium ions acting for a longer period of time. (For a similar situation, which is encountered in the analysis of the restoration of veratrinized nerve by the anodal current and by CO₂, cf. 12, Fig. II, 17.)

SUMMARY

Using the ability of the nerve fibers to conduct impulses as indicator of changes in the concentration of sodium ions in the interstitial spaces of nerve
an evaluation has been made of the diffusion constant of sodium ions. The calculated minimal value ($0.62 \times 10^{-4}$ cm.$^2$/min.) undoubtedly is much too low; nevertheless, it is still so high that as a rule the diffusion of sodium ions is far more rapid than the establishment of excitability changes; therefore, diffusion times need not be taken into account in the interpretation of ordinary experiments.

By measurements of the changes in the longitudinal conductivity of nerve which result from changes in the external concentration of sodium chloride an evaluation has been made of the diffusion constant of sodium chloride in the interstitial spaces of nerve. A minimal value for this constant is $1.4 \times 10^{-4}$ cm.$^2$/min.

The evidence presented would be compatible with the assumption that the permeability of the connective tissue sheath for sodium ions decreases slightly after the concentration of sodium ions in the interstitial spaces of the nerve has become negligible; the evidence, however, shows that changes in the permeability of the sheath cannot play a significant role in determining the temporal courses of the development of inexcitability in a sodium-free medium and of the restoration of excitability by added sodium ions. If a decrease in the permeability of the sheath should take place in a sodium-free medium, the change would be small and would occur after the nerve fibers have become inexcitable; on the other hand the action of a moderate concentration of sodium ions would be sufficient to restore the permeability of the sheath.

As measured by the recovery by A fibers of the ability to conduct impulses the restoration by 0.1 N sodium ions of nerve that has been deprived of sodium for 15 to 20 hours, i.e. for several hours after the nerve fibers have become inexcitable, begins after a significant delay, since no A fiber begins to conduct impulses in less than 8 or 10 minutes. The delay is referable to the fact that, before the A fibers can regain the ability to conduct impulses, those changes in their properties have to be reversed, which have taken place in the absence of sodium ions. Usually within 1 minute after sodium ions are made available to the nerve the polarizability of the membrane by the anodal current begins to increase; the A fibers soon begin to produce unconducted impulses in response to the break of the anodal current; then, they produce unconducted impulses in response to the closure of the cathodal current, and finally they become able to conduct impulses, although at a markedly reduced speed. The C fibers, that become inexcitable in a sodium-free medium later than the A fibers, begin to conduct impulses within 1 minute or 2 after 0.1 N sodium ions are made available to the nerve.

Treatment of a nerve, that has been kept in a sodium-free medium for 15 to 20 hours, with a moderate concentration of sodium ions (0.015, 0.02 N), acting for 1 hour or 2, is not sufficient to restore the ability to conduct impulses to more than a few A fibers, but it produces in a relatively large number of
fibers a partial restoration, so that when the concentration of sodium ions outside the epineurium is increased by 0.005 or 0.01 N a significant number of A fibers begin to conduct impulses within less than 5 seconds. Initially the recovery progresses with great rapidity, but after a small number of minutes the height of the conducted spike remains practically stationary. Increase of the external concentration of sodium ions by a small amount again causes a rapid enhancement of the recovery, but once more, after a few minutes the height of the spike remains practically stationary, etc. A subnormal concentration of sodium ions may restore to all the A fibers the ability to conduct impulses, but only 0.1 N sodium ions are able to produce a complete restoration of the speed of conduction, and only after they have been allowed to act for a considerable period of time. The ability of all the C fibers to conduct impulses may be restored by relatively small concentrations of sodium ions, 0.02 to 0.025 N.

Nerve fibers that have become inexcitable in a sodium-free medium and have been restored by sodium ions are far more sensitive to the effect of the lack of sodium than the fibers of untreated nerve. Repeated removal and addition of sodium ions may bring the nerve fibers, especially those of spinal roots, to a state in which the sensitivity to the lack of sodium is exceedingly great; spinal root fibers may then begin to become inexcitable in a sodium-free medium within a few seconds. Treatment of the nerve with 0.1 N sodium ions for 1 hour or 2 is sufficient to bring about a marked increase in the resistance to the lack of sodium. On the other hand keeping a nerve in Ringer's solution or in the presence of 0.04 N sodium ions does not produce a readily detectable increase in the sensitivity to the lack of sodium. Even the resistance of nerve kept in the presence of 0.025 N sodium ions for 23 hours is very high, since after 2 hours in a sodium-free medium more than two-thirds of the initially conducting fibers will be able to conduct impulses.

Frog nerve reaches different states of equilibrium with different external concentrations of sodium ions. The states are characterized by the degree of effectiveness of the nerve reaction, the speed of conduction of impulses, and the number of conducting fibers. Approximately the same equilibrium state may be reached by (a) leaving the nerve for 20 to 24 hours in the presence of a subnormal concentration of sodium ions and (b) by leaving the nerve in a sodium-free medium for 15 to 20 hours, restoring it with 0.1 N sodium ions acting for a short period of time, rendering it inexcitable again in a sodium-free medium, and finally restoring it with a moderate concentration of sodium ions. If, however, the nerve that has been kept in a sodium-free medium for 15 to 20 hours is restored directly by a moderate concentration of sodium ions the state will not be reached, at least not for several hours, which corresponds to equilibrium with that concentration.

The role of sodium in nerve physiology is discussed. Sodium participates in
at least four processes. (a) The regulation of the concentration of water outside the nerve fibers; (b) the regulation of the total value of the membrane potential; (c) the production of the nerve impulse, and (d) the establishment of the nerve reaction. In so far as processes (c) and (d) are concerned only the sodium present inside the nerve fibers plays a role; the presence of sodium ions outside the nerve fibers is important only because in the absence of interstitial sodium ions the nerve fibers lose a part of their internal sodium content. The nerve impulse and the nerve reaction may be produced for long periods of time after the concentration of sodium ions outside the nerve fibers has become negligible.

A working hypothesis is put forward according to which the internal sodium content and the interstitial concentration of sodium ions are in equilibrium in so far as a different internal sodium content corresponds to each interstitial concentration. The properties of the nerve fibers are determined by the internal sodium content. The change in properties, i.e. in the state of the nerve fibers, results from processes that take place inside the nerve fibers after the interstitial concentration of sodium ions and consequently also the internal sodium content have been changed.

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