

ON THE EXISTENCE OF A GRADIENT OF SENSITIVITY TO THE
LACK OF SODIUM IN THE SPINAL ROOTS OF
THE BULLFROG

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In a paper (14) dealing with work done with nerves that had become inexcitable in a sodium-free medium evidence was presented to show that certain quaternary ammonium ions, of which tetraethyl-ammonium is the prototype, can substitute for sodium and restore the excitability of nerve fibers belonging to the groups labelled B and C in Erlanger and Gasser's classification (*cf.* Erlanger, 2). The evidence was sufficient to conclude that a vast majority of the B and C fibers are restored by tetraethyl-ammonium, but it was not sufficient to decide whether or not *all* the B and C fibers can be restored by tetraethyl-ammonium. For this reason the fibers restored by tetraethyl-ammonium were grouped in a special class, that was labelled Et, until it should become possible to decide whether or not the Et class includes all the B and C fibers.

The most promising method to begin the attack on this particularly difficult problem seemed to be this, to ascertain whether or not the C fibers of dorsal root origin belong to the Et class. Gasser (5, 6) has recently divided the C fibers into two main groups s.C (sympathetic C) and d.r.C (dorsal root C) and therefore it seemed reasonable to expect that if there were C fibers which are not included in the Et class, those fibers would belong to one of Gasser's two C groups (s.C and d.r.C).

A number of experiments were done with the use of bullfrog spinal roots that had been kept in a sodium-free medium for several hours, and the remarkable observation was made that the fibers of the spinal roots (A as well as C) become irreversibly inexcitable in a sodium-free medium long before all the A fibers in the sciatic-peroneal nerve have lost their ability to conduct impulses. Evidence was obtained in one experiment, in which the roots were kept in the sodium-free medium for a short period of time only, that at least a part of the d.r.C fibers belong to the Et class, but the complete analysis of this question was postponed; immediate attention was given to the differences in the properties of A fibers in the spinal roots and in the nerve trunks.

It has been found (*a*) that the sensitivity to the lack of sodium of the fibers of the dorsal root is greater than the sensitivity of the fibers of the ventral root, (*b*) that in both the dorsal and the ventral roots the sensitivity to the

lack of sodium decreases with increasing distance from the cord, and (*c*) that the gradient of sensitivity continues in the nerve trunk for about 10 mm. beyond the trunk-roots margin.

I

Technique

The experiments were done with nerves from large bullfrogs. The spinal cord was divided through the symmetry plane and on each side one pair of roots (dorsal and ventral roots) was prepared together with a segment of the corresponding half of the spinal cord and with a long segment of the nerve trunk that results from the fusion of the two roots. In this manner the flow of demarcation currents in the spinal roots was avoided.

In some experiments the preparations were kept in a large and frequently stirred volume of a sodium-free solution (0.11 M diethanoldimethylammonium chloride) and were then mounted in humid chambers in air. In other experiments the preparations were mounted in the humid chambers immediately after excision; the sodium-free solution was then applied by placing two thin strips of filter paper alongside the segment that was going to be deprived of sodium and depositing drops of the sodium-free solution upon the strips of filter paper at short intervals of time. The restoring solutions were applied by repeatedly brushing the preparation, in the intervals between the sweeps of the oscillograph, with a soft brush soaked with the restoring solution (for further details *cf.* reference 16).

The impulses were always initiated near the peripheral end of the nerve trunk. The second recording electrode was always placed in contact with the spinal cord at the origin of the roots. The first recording electrode was placed at points of the trunk or of the roots which will be given during the description of the individual experiments.

It will contribute to the clarity of the discussion to mention here that with the bullfrog the spinal dorsal root consists mainly of the "central" processes of the axons of the ganglion cells; the peripheral margin of the ganglion is usually at about 1 mm. from the point at which the fusion of the dorsal and the ventral roots into a nerve trunk becomes complete. This point will be called the trunk-roots margin. The distance between the trunk-roots margin and the spinal cord measured between 21 and 32 mm.

II

Gradient of Sensitivity to the Lack of Sodium in the Spinal Roots

The results obtained in a considerable number of experiments have been constant. The dorsal root becomes inexcitable in a sodium-free medium earlier than the ventral root and in both roots points at a short distance from the cord become inexcitable earlier than points at a greater distance. At no time during the development of inexcitability does a demarcation potential appear between points of the roots or between points of the roots and points of the nerve trunk, which indicates that, as is the case with the fibers of nerve trunks, the inexcitability of root fibers develops in the sodium-free medium without a readily detectable change in the total value of the resting membrane potential.

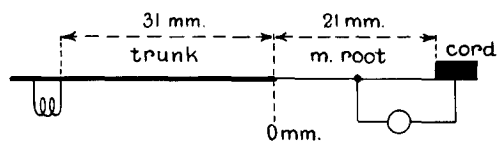
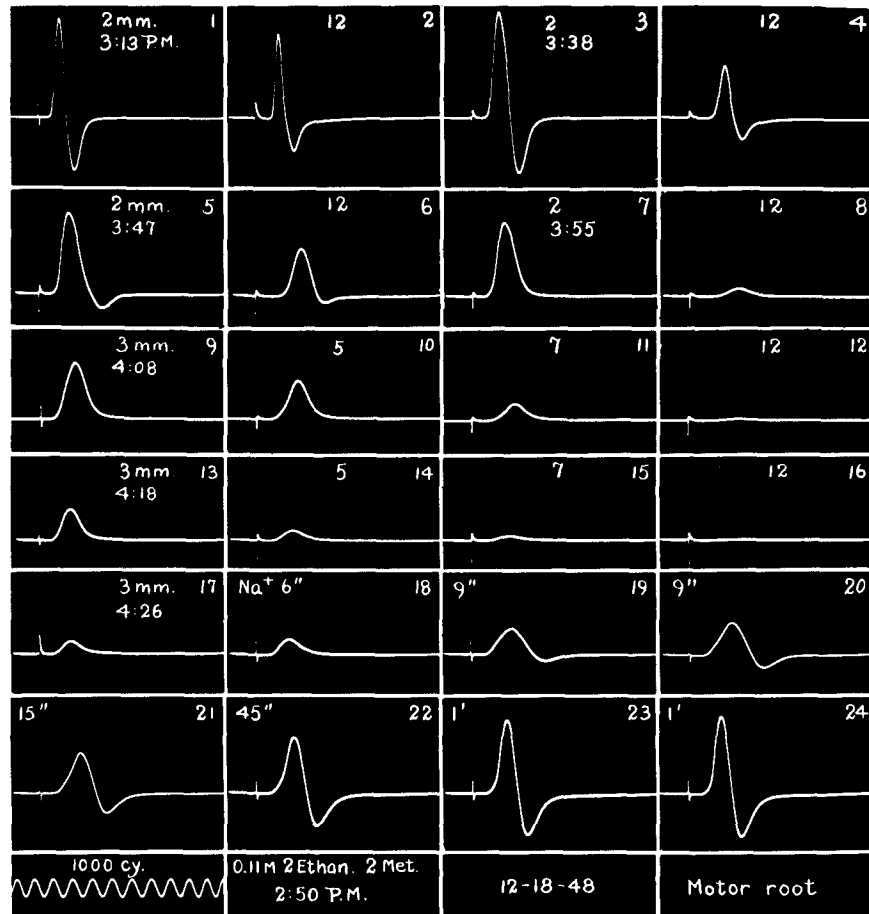


FIG. 1. Development of inexcitability in a sodium-free medium of the fibers of a spinal motor root (records 1 to 17), and restoration of excitability by Ringer's solution (18 to 24). The arrangement of electrodes is indicated in the diagram below. The numbers on the records indicate the distances in millimeters from the first recording electrode to the trunk-root margin (point 0). The times at which the records were obtained are indicated with the records. When they are relevant the intervals between successive records are given in seconds or minutes in the upper left corner of the records.

The presentation of the experimental observations will be done by describing in detail 2 typical experiments one of which was done with the use of the ventral root and the other with the use of the dorsal root.

Fig. 1 illustrates the results of depriving of sodium the fibers of the ventral root. The impulses were initiated in the nerve trunk at 31 mm. from the trunk-root margin. The first recording electrode was placed on the root at the distances from this margin indicated in millimeters on the records. The second recording electrode was in contact with the spinal cord at the point of emergence of the root, for which reason the recorded spikes were diphasic until a conduction block was established at several millimeters from the spinal cord.

Records 1 and 2 were obtained with the first recording electrode at 2 mm. and 12 mm. respectively from the trunk-root margin, after 23 minutes of lack of sodium. At that time all the nerve fibers were able to conduct impulses throughout their entire length and the speed of conduction was normal. After 25 additional minutes, however, a number of fibers failed to conduct impulses up to point 12 mm. since the spike in record 4 is considerably smaller than the spike in record 2; in addition, the speed of conduction was subnormal. The spike recorded at 2 mm. from the margin (record 3) was higher than it had previously been (record 1). The explanation of this interesting fact is not difficult to find; owing to the relatively short distance (19 mm.) between the 2 recording electrodes the impulses began to reach the second electrode before the spike had reached its crest at the first electrode; the decrease in the speed of conduction resulted in an increase in the temporal separation of the 2 phases of the spike, which caused an increase in the height of the first phase of the record; also the failure of a number of impulses to reach the second recording electrode contributed to increasing the height of the first phase of the record.

After 9 additional minutes also the spike recorded at 2 mm. from the margin (record 5) had decreased in height, thus indicating that a number of impulses had failed to reach the first recording electrode; the small size of the second phase of the spike was a sign that few impulses were reaching the spinal cord. The spike at point 12 mm. (record 6) also had a small second phase, which was the reason why the first phase was not markedly lower than the first phase of the spike in record 4.

At this time the development of inexcitability was advancing rapidly. Record 7 presents the spike at point 2 mm. as a strictly monophasic deflection, which shows that all the impulses were being blocked several millimeters ahead of the spinal cord, and record 8 proves that only a few impulses were being conducted up to point 12 mm.

Records 9 to 12, that were obtained at 3, 5, 7, and 12 mm. from the trunk-root margin respectively, illustrate the state of the block after 78 minutes of lack of sodium. It will be noted that the number of conducting fibers markedly decreased with decreasing distance from the spinal cord, and that, if any, only a few impulses reached point 12 mm. Probably the deflection recorded at this

point was referable to electrotonic spread of the action potential of impulses blocked ahead of this point. The conduction block became progressively deeper with advancing time. Records *13* to *16* illustrate the state of the block after 88 minutes of lack of sodium; it is obvious that in all the fibers of the root the margin of the block was undergoing a progressive displacement toward the trunk-root margin.

Finally, after 96 minutes of lack of sodium, when the spike at point 3 mm. had decreased to the small height with which it appears in record *17*, the root was brushed with a soft brush soaked in Ringer's solution, which operation was repeated during the intervals (2 seconds) between successive sweeps of the oscillograph. In view of the rapidity of the recovery it was not deemed advisable to change the position of the first recording electrode, but if records *18* to *24* are examined with some care it will be found that at the beginning of the recovery the spike was either monophasic (record *18*) or had only a relatively small second phase (records *19*, *20*), which indicated that the recovery was taking place at different points of the root with a rapidity that was greater at points at some distance from the spinal cord than at points near the cord, or otherwise stated that those points of the nerve fibers which had lost the excitability first recovered the excitability last. The progressive changes in the shape of the spikes in records *18* to *24* also prove that initially the recovered fibers conducted impulses at a subnormal speed, and that the speed of conduction increased progressively paralleling the increase in the number of conducting fibers.

Fig. 2 illustrates the results of an experiment done with the use of a dorsal root of unusual length (32 mm.). The first recording electrode was placed at those distances from the central margin of the spinal ganglion which are given in millimeters with the records. The second recording electrode was in contact with the cord at the point of entrance of the root. As is regularly observed with dorsal roots the development of inexcitability was very rapid. The spike in record *5*, which was elicited after 2 minutes of lack of sodium is considerably smaller than the spike in record *4*, and further decreases in the spike height took place in the intervals between records *6* to *8*. It will be noted that the development of inexcitability was accompanied by a marked reduction in the speed of conduction of the excitable fibers.

Records *9* to *12* were obtained in order to analyze the state of the conduction block at different points of the root. The application of the sodium-free medium was discontinued while the records were being obtained. That the conduction block was relatively more important at points near to the spinal cord than at points far from the cord was obvious, but records *9* to *12* do not give a correct view of the longitudinal distribution of the block, since a comparison of records *8* and *12* clearly shows that a partial recovery of excitability took place after the washing of the root with the sodium-free medium had been discontinued.

The root was then repeatedly washed with the sodium-free medium during

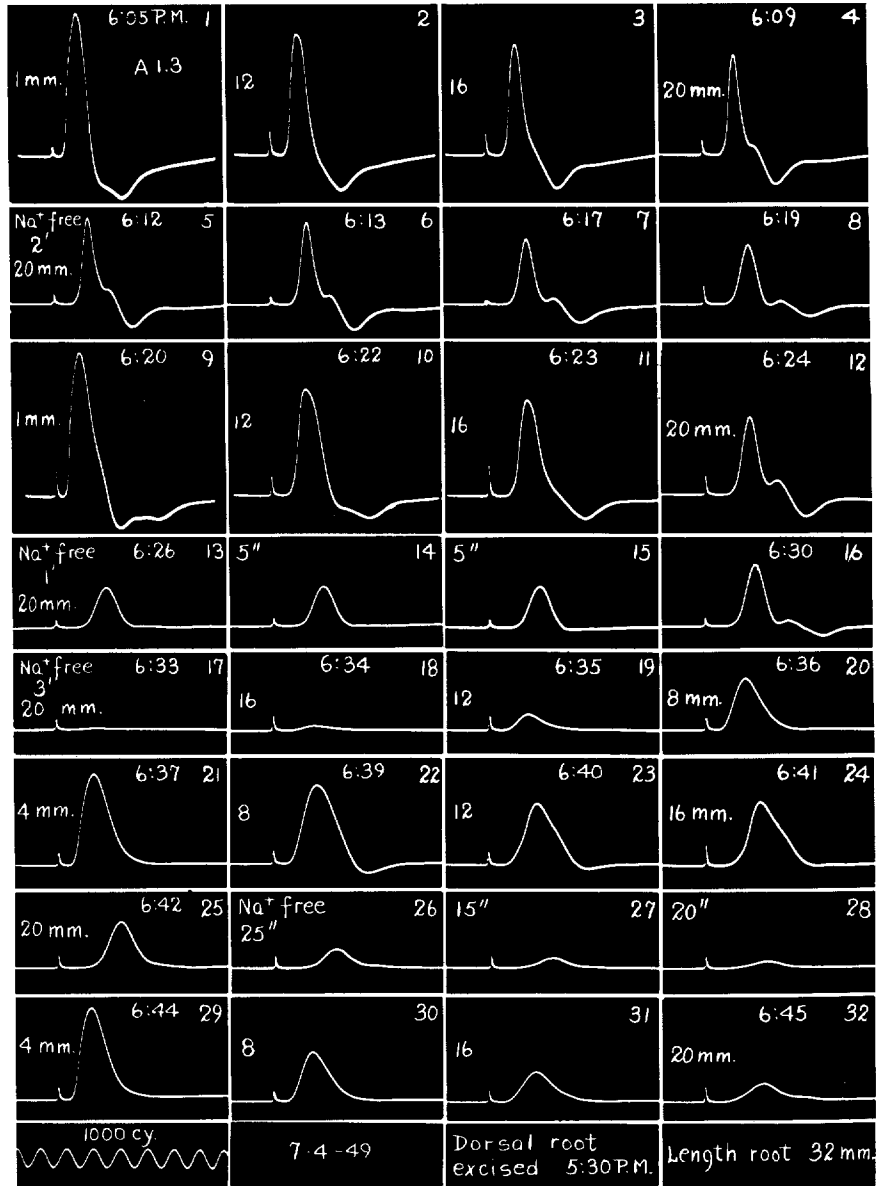


FIG. 2. Development of inexcitability in a sodium-free medium of the fibers of a spinal dorsal root. The distances of the first recording electrode to the trunk-root margin (point 0) are given in millimeters with the records. The washing of the root with the sodium-free solution was discontinued several times: immediately after record 8 had been obtained, immediately before obtaining record 13, immediately before obtaining record 17, and immediately after record 28 had been obtained.

1 minute and after the end of this time the spike at point 20 mm. was found to have the small height with which it appears in record 13 and to be an almost monophasic deflection. Discontinuing the washing of the root with the sodium-free solution again resulted in a partial recovery, which became detectable after 15 seconds (record 14) and obvious after 10 seconds (record 15). The recovery ceased to progress after 4 minutes (record 16).

Once more the root was repeatedly washed with the sodium-free solution, this time during 3 minutes. Discontinuing the washing of the root with the sodium-free solution again resulted in a partial recovery, but at this stage of the experiment the rate of the recovery was so low that records 17 to 21 may be regarded as giving a correct view of the longitudinal distribution of the conduction block. It will be noted that the height of the spike, and therefore the number of conducting fibers markedly increased with increasing distance from the spinal cord. Records 22 to 25 illustrate the longitudinal distribution of the block after the partial recovery had become practically complete in the proximity of the spinal cord.

Immediately after record 25 had been obtained the root was repeatedly washed with the sodium-free solution, the washing being continued throughout the intervals between records 25 to 28. It was discontinued after record 28 had been obtained. The rapid development of inexcitability is placed in evidence by records 25 to 28, and records 29 to 32 show that the number of conducting fibers rapidly decreased with decreasing distance from the spinal cord. A comparison of records 28 and 32 reveals that at this stage of the experiment only a very slight degree of recovery took place at point 20 mm. after the washing of the root with the sodium-free solution had been discontinued. Consequently, records 29 to 32 give a fairly accurate idea of the longitudinal distribution of the conduction block at this stage of the experiment.

In the continuation of the observations it was found that after an additional 5 minute period of washing no impulse was able to propagate itself beyond point 20 mm., and that no recovery took place after the washing was discontinued. After an additional 20 minute period of washing the margin of the total block was found to be at point 8 mm., and after an additional 40 minute period, at point 4 mm. The meaning of these observations is, of course, that in the sensory root the resistance to the effect of lack of sodium markedly increases with increasing distance from the spinal cord.

In the continuation of the experiment the excitability of the root was restored first with solutions containing moderate amounts of sodium ions and then with Ringer's solution. The margin of the total block was observed to move progressively towards the spinal cord indicating that those segments of the root, which had become inexcitable first, recovered their excitability last.

The results of the experiments illustrated by Figs. 1 and 2 are perfectly reproducible except in one respect. The fibers of the spinal roots are far more sen-

sitive to the effect of lack of CO₂ than the fibers of the sciatic-peroneal trunk, for which reason the spinal roots undergo a significant deterioration when they are kept in an atmosphere of air or of oxygen for several hours. This spontaneous deterioration increases the sensitivity of the root fibers to lack of sodium, so that the conduction block develops more rapidly in stale than in freshly excised roots. In all cases, however, the block develops in the manner illustrated by Figs. 1 and 2.

Under conditions such as these the conclusion is unavoidable that a gradient of sensitivity to the lack of sodium exists in the fibers of the spinal roots. In both roots the gradient is such that the sensitivity to the lack of sodium decreases with increasing distance from the spinal cord. Therefore the gradients in the motor root and in the central segment of the dorsal root, *i.e.* the segment between the ganglion and the cord, have opposite signs when they are referred to the cell bodies of the neurons, and in reference to the position of the neuron body the gradient in the dorsal root has opposite signs in the central and in the peripheral segments of the root.

III

Extension of the Gradient of Sensitivity to the Lack of Sodium in the Nerve Trunk

The gradient of sensitivity to the lack of sodium does not cease abruptly at the trunk-roots margin; it continues in the trunk, but only for some 10 to 12 mm. beyond the trunk-roots margin; in the segment of the nerve trunk peripheral to the point at which the gradient ceases to be readily demonstrable, the sensitivity to the lack of sodium seems to be more or less uniform. In view of the importance of this fact a considerable number of experiments have been done. The consistent results that have been obtained will be presented by describing in detail a typical experiment (Figs. 3 and 4).

The observations illustrated by Fig. 3 were made after the trunk and the two spinal roots had been kept in a large volume of the sodium-free medium for approximately 4 hours, at the end of which time the preparation was mounted in a humid chamber. The peripheral end segment of the trunk where the impulses were to be initiated was treated with Ringer's solution, to insure that no change in the state of the nerve fibers would take place during the experiment. Then the spike of maximal A volleys initiated in that segment was recorded at a number of points of the nerve trunk and of the spinal roots.

The records of the first and of the third columns of Fig. 3 illustrate the observations that were made with the preparation in the sodium-free medium. The numbers on the upper left corner of the records give the distance in millimeters of the first recording electrode to the trunk-roots margin. The records of the first column and records 3 and 7 were taken at points peripheral to the trunk-roots margin, and records 11, 15, 19, and 23 at points central to the

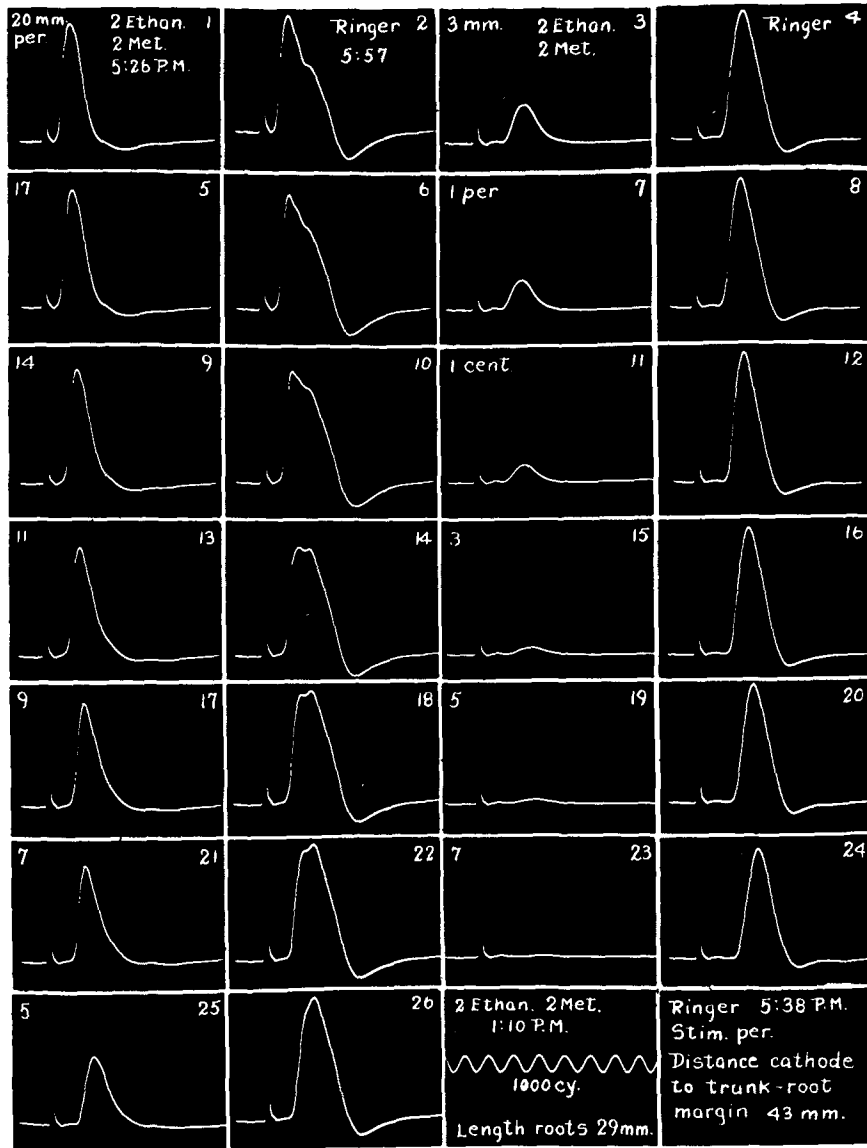


FIG. 3. Longitudinal distribution of the conduction block in a trunk-roots preparation, with the preparation in a sodium-free medium (records of the first and third columns) and after restoration by Ringer's solution (records of the second and fourth columns). Further explanation in the text.

margin. In all cases the second recording electrode was in contact with the spinal cord at the zone of origin of the roots.

A comparison of records 1 and 2 and 5 and 6 will prove that at the time when the observations were begun all the fibers of the nerve trunk were still able to conduct impulses. On the other hand, if the records of the first column are examined in succession from above downward it will be noted that between points 20 mm. peripheral to 14 mm. peripheral (records 1, 5, and 9) the spike height underwent only the small decrement that should be expected to result from increased temporal dispersion of the individual fiber spikes; between points 14 mm. and 11 mm. (records 9 and 13), however, the spike height underwent a more marked decrease, and from point 9 mm. on (record 17) the decrease in the spike height became rapid (*cf.* records 21, 25, and records of the third column). This finding was a proof that a certain number of impulses were blocked between points 14 mm. and 11 mm. and that the block became progressively greater with decreasing distance from the spinal cord. Records 7 and 11 show that only a small number of impulses reached the trunk-roots margin, and record 23, that no impulse was propagated for more than 7 mm. into the spinal roots; since the length of the roots was 29 mm. it is clear that the block became total at 22 mm. from the spinal cord.

The excitability of the nerve fibers was then restored by means of Ringer's solution, that was allowed to act for 30 minutes. The records of the second and fourth columns of Fig. 3 were obtained, after the restoration had become complete, at the same points of the preparation at which the corresponding records of the first and the third columns had been obtained. The amplification was maintained constant throughout the experiment.

Since after the restoration all the impulses were propagated past the dorsal root ganglion the irregularity in the external conductor of the nerve fibers, which is created by the volume of the ganglion, resulted in artifacts in the recorded spikes (Fig. 3, second column) of the type of those which were analyzed by Marmont (18). The artifacts, however, did not prevent a valid comparison of the heights of the spikes in the records of the first and of the second columns. It will be noted that at points 20, 17, and 14 mm. peripheral to the trunk-roots margin the spike height was not increased by the action of sodium ions (records 1, 2; 5, 6; 9, 10), while at all the other points of the nerve, beginning with points 11 mm. (records 13, 14) and 9 mm. (records 17, 18) the spike underwent a significant increase, which of course was spectacular at points of the roots (records 11, 12; 15, 16; 19, 20; 23, 24).

Since, as already mentioned, essentially identical results have been obtained in a number of other similar experiments it may be concluded that although the gradient of sensitivity to lack of sodium is not strictly confined to the spinal roots, the gradient extends peripheralwards only for some 10 to 12 mm. beyond the trunk-roots margin. To be sure, a more detailed study might show that a

slight gradient of sensitivity to the lack of sodium exists in the whole nerve trunk, but if such were the case the gradients in the trunk and in the roots would

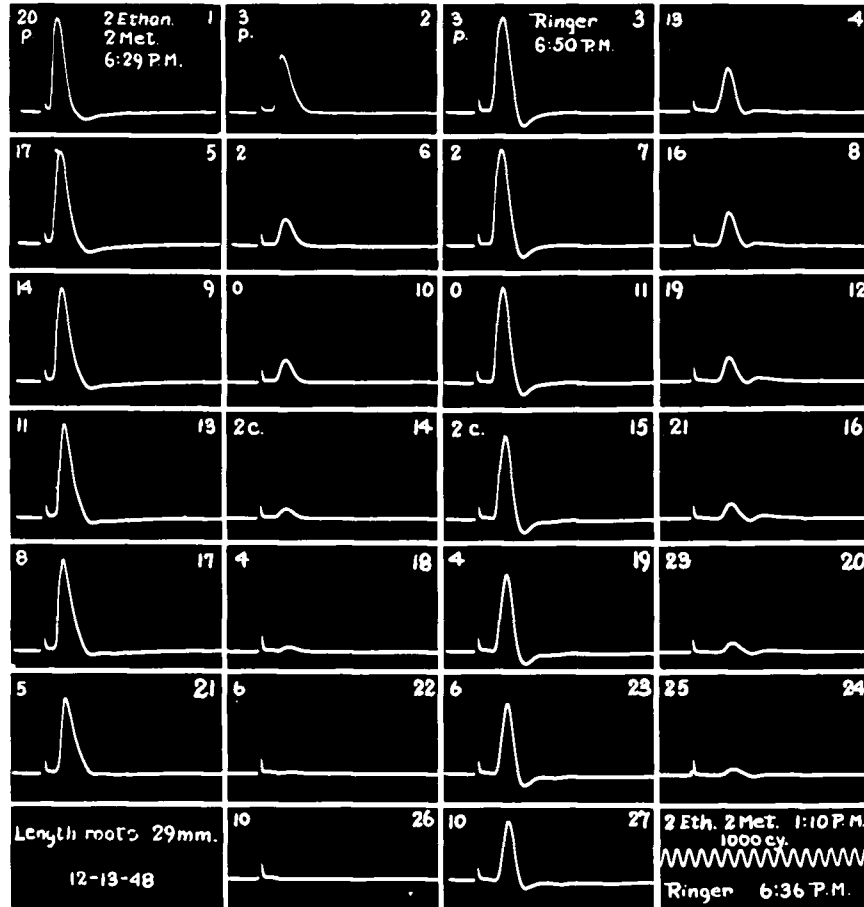


FIG. 4. Longitudinal distribution of the conduction block in a trunk-roots preparation, with the preparation in a sodium-free medium (records of the first and second columns) and after restoration by Ringer's solution (records of the third and fourth columns). Further explanation in the text.

still be of different orders of magnitude and therefore would not be comparable with one another.

The observations illustrated by Fig. 4 were made with a trunk-roots preparation taken from the same bullfrog as the preparation that had been used to obtain the records reproduced in Fig. 3. The preparation, however, was left

in the sodium-free medium for almost 5.5 hours, an interval of time which was sufficient for important segments of the root fibers to become irreversibly inexcitable.

The records of the first and second columns of Fig. 4 present the spikes that were recorded with the preparation in the sodium-free medium at the distances peripheral (*p*) and central (*c*) from the trunk-roots margin which are indicated in millimeters in the upper left corner of the records. After the preparation had been treated with Ringer's solution and the recovery had ceased to progress the records of the third column were obtained at the same points at which the records of the second column had been obtained; in addition, records were obtained at shorter distances from the spinal cord (Fig. 4, fourth column).

At the time when the observations were begun (Fig. 4, 1) a number of fibers of the trunk had already become inexcitable, which is obvious from the fact that the spike in record 3, that after completion of the restoration was obtained at a point 3 mm. peripheral to the trunk-roots margin, is higher than the spike in record 1, that was obtained at a point 20 mm. peripheral to the trunk-roots margin. Nevertheless, if the records of the first column are examined in succession from above downwards it will be found that at points between 20 mm. *p* and 11 mm. *p* (records 1, 5, 9, 13) the spike decreased in height only by those small amounts which should be expected to result from the progressive increase in the temporal dispersion of the individual fiber spikes. Therefore, all the excitable fibers of the trunk were able to conduct impulses up to a point 11 mm. peripheral to the trunk-roots margin.

Between points 11 mm. and 0 the spike underwent a decrease in height (records 13, 17, 21, 2, 6, 10), which indicated that in this segment of the trunk the number of conducting fibers rapidly decreased with decreasing distance from the spinal cord. In the roots themselves the conduction block was so important (records 14, 18, 22, and 26) that at a point 6 mm. central to the trunk-roots margin only a small electrotonic potential was recorded (record 22). It is important to note that the longitudinal distribution of the conduction block in the last 10 mm. of the trunk and in the roots was practically equal to that which had been observed in the companion preparation (Fig. 3, first and third columns of records) 1 hour earlier, which is a proof that the differences between trunks and roots in the sensitivity to the lack of sodium are very important. To the same conclusion lead the observations that were made after treatment of the roots with Ringer's solution.

In the presence of sodium ions all the fibers regained their ability to conduct impulses up to a point 2 mm. peripheral to the trunk-roots margin (Fig. 4, 3, 7), but in the roots the recovery of excitability was only incomplete. Examination of the records of the third and fourth columns of Fig. 4 readily shows that from the trunk-roots margin on (Fig. 4, 11) the spike crest markedly decreased in height with decreasing distance from the spinal cord, which of

course indicated that the number of conducting fibers rapidly decreased with decreasing distance from the spinal cord. Record 12 (Fig. 4) proves that only a small number of fibers were able to conduct impulses up to a point 19 mm. from the trunk-roots margin and 10 mm. from the spinal cord. After completion of the observations illustrated by Fig. 4 and after it had been ascertained that more prolonged treatment of the roots with Ringer's solution could not improve the state of the roots, the two roots were separated by dividing the spinal cord; it was found that the spikes that appear in records 12, 16, 20, and 24 were conducted by fibers of the ventral root, since no spike could be detected in the dorsal root at point 21 mm. *c* and the small spike that was observed at point 19 mm. *c* undoubtedly was an electrotonic potential. This observation proved again that the dorsal root fibers are more sensitive to the lack of sodium than the ventral root fibers.

Thus it appears that the differences in the sensitivity to the lack of sodium of the fibers in the spinal roots and of the fibers in the trunk are so great that important segments of the fibers of the roots become irreversibly inexcitable long before all the fibers of the trunk have lost the ability to conduct impulses.

IV

DISCUSSION

In considering the results presented in this paper the question arises whether or not the presence of the epineurium could be the direct explanation of the greater resistance of the fibers of the trunk to the lack of sodium. For several cogent reasons the answer to the question must be a categorical negative.

(*a*) The longitudinal distribution of the gradient of sensitivity to the lack of sodium is different from the longitudinal distribution of the epineurium. In the first place the gradient extends peripheralwards into the trunk for some 10 to 12 mm. beyond the trunk-roots margin, and on the other hand, the epineurium extends centralwards past the trunk-roots margin for several millimeters. The distance central to the ganglion through which the epineurium extends varies with individual preparations, but in all cases the epineurium is present at the level of the ganglion and for 1 or 2 mm. central to the ganglion. Therefore, since only a part of the roots has no epineurium it is obvious that the difference in the sensitivity to the lack of sodium described in this paper cannot be explained directly in terms of the presence or absence of the epineurium.

(*b*) The epineurium would retard the development of inexcitability in a sodium-free medium if it were a diffusion barrier that could effectively prevent the escape of sodium ions from the nerve into the external sodium-free medium. One of the best established facts in nerve physiology, however, is the fact that the intact epineurium of frog nerve is not a diffusion barrier (15-17). In addition, the results presented in this paper by themselves are sufficient to show that the epineurium does not prevent the diffusion of sodium ions, and that

the decisive factors in determining the excitability changes are the properties of the nerve fibers.

If the epineurium were a diffusion barrier it would delay the penetration of sodium ions into the nerve trunk so that after inexcitability had developed the recovery of the fibers in the trunk would be very slow in relation to the recovery of the root fibers. In point of fact, however, the recovery of the fibers of the trunk begins earlier and is faster than the recovery of the fibers of the roots. In addition, within the roots themselves the rate of recovery depends upon the distance from the spinal cord, and is independent of the presence or absence of the epineurium. Indeed, the root segment extending between the trunk-roots margin and the central margin of the ganglion, which has epineurium, begins to recover earlier and recovers faster than the segments of the root at a shorter distance from the spinal cord, which have no epineurium.

A remarkable fact is that the sign of the gradient is not determined by the position of the cell bodies of the neurons; independently of the position of the cell bodies the gradient is such that the sensitivity to the lack of sodium decreases in all the fibers with increasing distance from the spinal cord. Therefore, the gradient of sensitivity to the lack of sodium could hardly be brought into a relationship to the result of transport in the axons of substances performed in the cell bodies (for recent discussions of the transport problem *cf.* Young, 23, 24; and Weiss and Hiscoe, 22). On the other hand the gradient of sensitivity to the lack of sodium ends more or less abruptly about 10 to 12 mm. peripheral to the trunk-roots margin; therefore it is not comparable to other gradients in peripheral nerve fibers which have been described in the literature (Causey and Werner, 1; Kugelberg, 9; Kugelberg and Skoglund, 10; Lorente de N6, 13, pt. 1, p. 88, with references to the classical literature; Rexed, 20).

Under conditions such as these the most reasonable assumption seems to be that the gradient of sensitivity to the lack of sodium is just one aspect of a transitional gradient, that serves to establish a gradual change between the properties that the axons have inside the spinal cord and the properties that they have inside the nerve trunks.

The situation seems to be this. The three segments of the nerve fibers, (*a*) the segment located inside the spinal cord, (*b*) the segment in the spinal roots, and (*c*) the segment in the nerve trunks have different environments. Histological studies have revealed important differences in the structures of the segments of the nerve roots inside and outside the central nervous system (for a recent, excellent presentation of the question, *cf.* Tarlov, 21). Chemical differences between the two segments also are known to exist (Klüver, 8). On the other hand, the segments of the roots inside the spinal cord are in equilibrium with a milieu which is not identical with blood plasma, since a so called "blood-barrier" exists between blood capillaries and nervous tissue. The seg-

ments of the roots in the spinal canal are in equilibrium with the cerebrospinal fluid, which has a composition of its own, different from that of blood plasma (*cf.* Merrit and Fremont-Smith, 19; Livingston, 11). Finally, the fibers in the nerve trunks probably are in equilibrium with blood plasma, since as Hastings (7, p. 95) has stated "the extracellular phase of tissues . . . is a network of connective tissue diluted . . . with an ultrafiltrate of plasma." Thus it is understandable that a transitional root gradient serves to bridge over the differences in properties which exist between the segment of the fibers in the spinal cord and the segment in the nerve trunk.

The continuation of the gradient for a short distance in the nerve trunk raises an exceedingly interesting question. According to the working hypothesis just stated the fact would indicate that the subdural space continues in the nerve trunk for about 10 to 12 mm., or better stated that in the first 10 to 12 mm. of the nerve trunk the composition of the interstitial fluid changes gradually from that of the cerebrospinal fluid to that of blood plasma. That such an extension of the subdural space actually exists is a conclusion to be drawn from those experimental observations on the circulation of the cerebrospinal fluid which have led to the belief that in part this fluid is reabsorbed at nerve roots (*cf.* Merrit and Fremont-Smith, 19; Livingston, 11).

To assume that the subdural space continues for some distance in the nerve trunk might seem to be in contradiction to the fact that the epineurium is not a diffusion barrier. After a little consideration, however, it is found that no contradiction exists. The exchange of fluid and of solutes between the capillaries and the interstitial spaces of the nerve depends, of course, upon the properties of the connective tissue sheath of the nerve (endoneurium plus perineurium plus epineurium) and it is logical and permissible to assume that the connective tissue sheath of nerve serves among others the purpose of keeping the interstitial spaces of the nerve relatively "insulated" from the interstitial spaces of the neighboring tissues. The connective tissue sheath can play this role as long as the nerve has an intact circulation; in the excised nerve diffusion equilibrium must be established rapidly between the interstitial spaces of the nerve and the medium in which the nerve is being kept.

That the dorsal root is more sensitive to the lack of sodium than the ventral root could hardly be explained in any other way than by assuming that the two roots have partly different chemical constitution. As a matter of fact it is well known that the acetylcholine content of the ventral root is many times greater than the acetylcholine content of the dorsal root (Loewi and Hellauer, 12; for more recent literature *cf.* Feldberg, 3). Whether or not this particular difference in the chemical composition of the two roots is directly related to the difference in the sensitivities to the lack of sodium is a question that cannot be answered at the present state of knowledge; the important fact is that a chemical difference between the two roots exists. Similarly the fact that the

fibers of the spinal roots are more sensitive to the lack of sodium than the fibers of the nerve trunk points to the existence of chemical differences. No information on this problem exists in the literature. The important observations recently made by Causey and Werner (1) with radioactive phosphorus undoubtedly would throw light on the problem if they were extended to include the spinal roots.

The temporal course of the development of inexcitability of the dorsal root in the experiment illustrated by Fig. 2 deserves especial comment. To begin with, be it emphasized that the observations made in this experiment are reproducible, since they have been uniformly repeated in a number of similar experiments.

During the development of inexcitability it was observed several times that after the washing of the root with the sodium-free medium had been discontinued a partial recovery of excitability took place. The washing was discontinued for the first time after record 8, Fig. 2, had been obtained; *i.e.*, after 10 minutes of lack of external sodium. As was demonstrated with the ventral root, later in the experiment, 90 per cent of diffusion equilibrium was established between the root and the external solution within about 1 minute (16). Consequently, the partial recovery of excitability took place after the concentration of sodium ions in the interstitial spaces of the root had become negligible.

A possible explanation of the recovery is this, that sodium present in the connective tissue fibrils of the endoneurium passed into the interfibrillar spaces and was taken up by the nerve fibers. The explanation may be based on the following argument. A transfer of sodium from the connective tissue fibrils to the nerve fibers could not take place to a significant extent during the periods of washing of the root with the sodium-free medium, since the external concentration of sodium ions was permanently zero and therefore the majority of the sodium ions escaping from the connective tissue fibrils were lost by diffusion into the external sodium-free medium. After the washing was discontinued, however, sodium ions leaking from the connective tissue fibrils could remain in the interstitial spaces until they were taken up by the nerve fibers. Only future research can decide whether this explanation is correct or not.

A more likely explanation of the partial recovery may be obtained by following this line of thought. Let it be assumed that sodium is present inside the nerve fibers in 2 forms, tightly and loosely bound sodium. (To be sure, it could also be assumed that the internal sodium is present at various depths, but as a working hypothesis this second assumption is not more useful than the first.) Loss of a certain amount of loosely bound sodium is sufficient to deprive the nerve fibers of their ability to conduct impulses. Release of tightly bound sodium tends to replace the loosely bound sodium that has been lost. While the root is being washed with the sodium-free medium the concentration of sodium

ions in the interstitial spaces is virtually zero and the loss of loosely bound sodium by the nerve fibers is so rapid that release of tightly bound sodium is not sufficient to compensate for the loss. After the washing has been discontinued, as soon as the interstitial concentration of sodium ions reaches a certain small value, the rate of loss of loosely bound sodium by the nerve fibers decreases, with the result that conversion of tightly bound internal sodium into loosely bound sodium becomes sufficient to restore the excitability of the nerve fibers.

In support of this explanation there is a significant fact. The partial restorations that occurred after the interruptions of the washing of the root with the sodium-free solution resulted in an increase in the number of conducting fibers but not in a restoration of the normal speed of conduction (Fig. 2). A similar effect was later obtained by restoring the inexcitable root by means of moderate concentrations of sodium ions, which may be taken as a sign that each one of the successive partial restorations illustrated by Fig. 2 was the result of redistribution inside the nerve fibers of progressively smaller amounts of internal sodium.

V

SUMMARY

The Et class of fibers includes fibers of Gasser's d.r. C group.

The fibers of the dorsal root are more sensitive to the effect of lack of sodium than are the fibers of the ventral root.

In the two roots there is a gradient of sensitivity to the lack of sodium, which is such that in all the root fibers the sensitivity decreases with increasing distance from the spinal cord. The gradient continues in the trunk up to about 10 to 12 mm. peripheral to the trunk-roots margin. No comparable gradient of sensitivity to the lack of sodium has been observed in the rest of the nerve trunk.

The gradient of sensitivity to the lack of sodium has no relationship to the anatomical distribution of the epineurium.

As a working hypothesis it is suggested that the gradient of sensitivity to the lack of sodium is one aspect of a transitional gradient that serves to establish a gradual change between the properties that the axons have inside the spinal cord and the properties that they have inside the nerve trunks.

Details are given of the temporal course of the loss of excitability by root fibers deprived of sodium. It is suggested that sodium is present in the nerve fibers, in 2 forms, loosely and tightly bound sodium and that loss of loosely bound sodium is sufficient to render the nerve fibers unable to conduct impulses. If the rate of loss of loosely bound sodium is decreased, conversion of

tightly bound into loosely bound sodium may temporarily restore the excitability of the nerve fibers.

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