Role of Potential Wave Spreading Along Myelinated Nerve Fiber in Excitation and Conduction

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THE significance of the myelin sheath in electric excitation and in conduction of a nerve impulse has already been elucidated to a great extent by previous investigations (1–3). The results of these investigations are consistent, indicating that the myelin sheath has a distributed capacity and a very high leakage resistance. This capacity and resistance of the myelin sheath are considered to form, together with the axis-cylinder and the surrounding fluid medium, a cable-like electrical network.

In a recent paper, one of us (4) has stressed the importance of the 'potential wave' spreading along this cable-like system in electrical excitation of the nerve fiber. It has been inferred that, when a stimulating voltage pulse is applied to a nerve fiber at a point some distance away from a node of Ranvier, the change in the potential difference across the myelin sheath spreads along the fiber at a finite rate and that this spread of potential wave is the process which precedes initiation of an impulse at the node. It has also been pointed out that the time required for this potential wave to spread from node to node could be the main factor determining the internodal conduction-time in saltatory conduction.

In Lillie's model of the myelinated nerve fiber (5, 6), the myelin sheath was compared to a thick layer of glass through which no capacitative current flow could take place. In this model, as well as in Frank's more refined model (7), the 'impulse' jumps from a 'node' to the next at almost infinite speed, the over-all rate of conduction being determined solely by the rapidity of the process occuring at the nodes. Although the experiment of Huxley and Stämpfli (3) demonstrated the existence of some delay within each internode, the large discontinuity in the time of appearance of the action current that they found at each node might be interpreted as supporting *saltation with respect to time* as opposed to the idea that the capacity of the myelin sheath could be the main factor determining the internodal conduction-time. The evidence brought forward by Tasaki and Takeuchi (1, 2) and others in support of the saltatory theory has dealt mainly with the discontinuity in the physiologically 'active' place of the fiber, namely with *saltation with respect to space* and not directly with saltation with respect to time.

The present investigation was undertaken with a view to determining whether or not saltation with respect to time, besides that with respect to space, occurs in actual nervous conduction. In the light of a series of experiments showing the spread of a potential wave along the myelin sheath at a finite rate (4), we first examined whether or not the latent period of an action current (recorded from the site of stimulation) depends upon the distance from a node of Ranvier. By several different methods we could demonstrate a significant dependence of the latent period

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upon the distance from a node within one internodal segment. We then examined the time-course of the longitudinal action current (associated with a conducted impulse) as a function of distance along a nerve fiber. Finally we examined the dependence of the shape of the action potential of a single nerve fiber upon the distance from a node of Ranvier. The results of all these investigations, which are described in the present paper, showed clearly that the potential wave spreading along the myelin sheath plays an important part in determining the internodal conduction-time in saltatory conduction. Our results indicate also that the idea of saltation with respect to time should primarily be excluded from the concept of saltatory conduction in the myelinated fiber.

Method

All the experiments described in this paper were done on single nerve fibers isolated from sciatic-gastrocnemius or sciatic-sartorius preparations of the frog (*Rana esculenta* or *R. temporaria*).

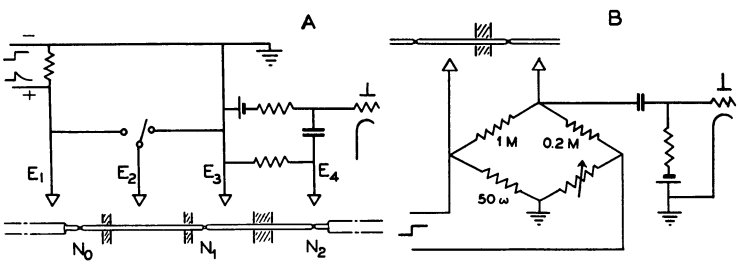

Fig. 1. Experimental arrangements used for determining the latency of action current at varying distances from a node of Ranvier. *A:* Bridge insulator method used for the experiments of figs. 2*A*, 3 and 4. *B:* Oil gap method used for the experiments of figs. 2*B* and 5. Shaded areas are air or oil gaps.

For the measurement of the latency of action currents evoked in response to rectangular voltage pulses or to brief condenser shocks, both the method of bridge-insulator (1) and the oil-gap partition method (3) were used. The use of these two different methods for the investigation of our problem was found to be extremely advantageous. *Diagrams A* and *B* in figure 1 show the experimental arrangements used for this purpose.

Bridge-Insulator (Fig. 1A). The bridge-insulator consisted of four separate pieces of glass plate, each carrying a pool of Ringer's solution. The two plates on each side were large (approximately 5 x 4 cm.) and had convex edges which faced the two small pieces of glass plate in the middle. The smallest of them had a straight edge on one side and a concave edge on the other, the narrowest part being approximately 0.8 mm. wide and 8 cm. long. The other glass piece had also one straight and one concave edge, the minimum width in the middle being 1.5 mm. These four pieces of glass plate were so arranged that there were air gaps, about 0.2 mm. wide, between the glass plates. On each of the glass plates, Ringer's fluid was placed and a single fiber preparation was mounted in such a position that only the internodal stretch of the fiber was immersed in the smallest pool of Ringer's fluid, while the neighboring nodes were located in the adjacent pools. A nonpolarizable electrode of the Ag-AgCl-Ringer type was dipped in each pool of Ringer and the stimulating circuit and the amplifier were connected as shown in the diagram. These arrange-

ments are substantially the same as the tripolar method for leading off action currents from the site of stimulation adopted by Tasaki and Takeuchi (1). By means of the switch shown in the figure, stimulating pulses were applied either between the electrodes E_1 and E_2 (with both E_2 and E_3 grounded) or between E_2 and E_3 (now E_1 and E_2 being directly connected together).

Oil-Gap Partition (Fig. 1B). Two blocks of Perspex, containing each a concavity filled with Ringer's fluid, were electrically separated by a layer of paraffin oil, 0.6 mm. thick. This oil partition was made with two polystyrene foils spaced by a U-shaped piece of celluloid. The gap between the foils could be filled with oil through a borehole in one of the Perspex blocks. Before starting an experiment the blocks with the partition between them were screwed together. With a fine needle both foils were perforated from one side in order to obtain holes of approximately 150 microns diameter exactly facing each other. A nylon fiber was then pushed through the holes and tied to the nerve fiber contained in one of the pools. After extending the fiber horizontally between two insulated watchmaker forceps it could be pulled through the holes by means of a micromanipulator. At this stage the gap was usually filled with oil, though sometimes Ringer's fluid was used as control to show that oil did not affect the shape of the action currents by adhering to the surface of the fiber. The horizontal movement of the fiber could be read with an accuracy of 0.05 mm. from a dial at the micromanipulator. Two electrodes (Ag-AgCl-Ringer type) were immersed in the troughs of Ringer's fluid on both sides of the partition. Rectangular voltage pulses of varying strengths were applied to the fiber through these electrodes and the action currents thus elicited from the fiber were led off with the same electrodes. To balance out the deflection of the electron beam of the oscillograph by the stimulating current, a Wheatstone bridge was used. The resistance across the partition with a fiber through it was generally 2 to 4 megohms. As the resistance of the preparation between the electrodes was found to change slightly when the fiber was shifted through the partition, a constant resistance of 1 megohm was connected between the electrodes; this made balancing of the bridge easy.

Other Experimental Arrangements. Stimuli were usually taken from a Grass Standard Model 3 C Stimulator connected through a Stimulus Isolation Unit (of the same firm) to the preparation. The amplifier was a condenser-coupled two-stage preamplifer coupled to a d.c. amplifier of a Cossor 2-beam oscillograph. The time required to complete 64 per cent of the response to a rectangular pulse was of the order of 10 μsec. For potential measurements, a cathode follower input-stage with a Victoreen VX 41 electrometer tube was used, giving an increase of the input time constant by a factor of 1.5. Other experimental arrangements were frequently used for special investigations and will be mentioned when applied.

Results

Strength-Latency Relation Determined by the Method of the Bridge-Insulator. Prior to each latency determination with the experimental arrangements of figure 1*A*, the following test was made to assure that there was no node of Ranvier between the two stimulating gaps. The electrode E_2 in the figure was connected to the input of the amplifier, all other electrodes were grounded and the action current of the fiber associated with a propagated impulse was recorded. In all cases this procedure gave an action current with two well-defined peaks, typical for an internode (2).

The electrodes were then connected as shown in the figure and a series of action current records were taken to measure the latency at the site of stimulation.

Typical records of the action currents obtained by the experimental arrangements of figure 1*A* are given in figure 2*A*. The stimulating pulses used in this experiment were *brief condenser shocks* decaying with the time constant of 7 μsec. When the shock was applied across the gap remote from the recording stretch, i.e. between the electrodes E_1 and E_2 (figure 1*A*), it was relatively easy to locate the start of the action current even when the shock strength was about 130 per cent of the threshold strength (records in figure 2*A*, far). In this case, the latency of action current, i.e. the time from the start of the stimulating pulse to the start of the response, was very long as compared with the duration of the shock artefact. The configuration of the action current was found to remain almost unchanged when the shock strength was increased.

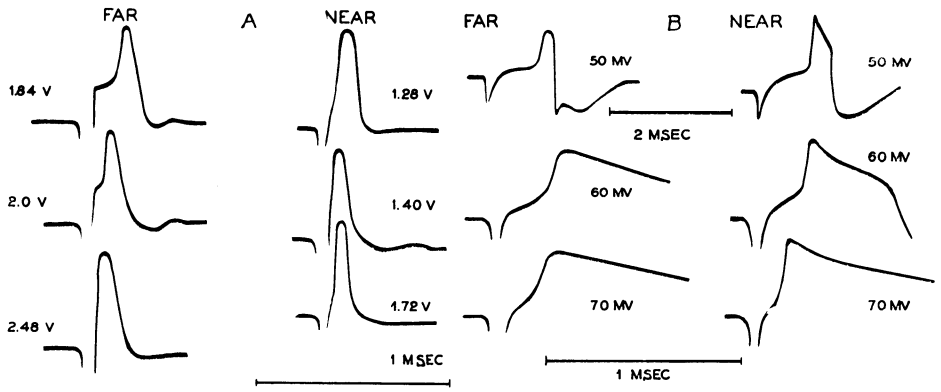

Fig. 2. ACTION CURRENTS recorded at different distances from a node of Ranvier. The terms *near* and *far* represent the relative distance between the site of stimulation and the first node on the cathodal side. Records in *A* were obtained with the arrangements of fig. 1*A* and with brief shocks of 7-μsec. duration at 20°C. Records in *B* were taken with the arrangement of fig. 1*B* at 18°C. Strengths of stimuli are given. Time mark, 1 msec.

When the node of Ranvier, labeled N_1 in figure 1*A*, was located close to the middle gap and brief shocks were applied between the electrodes E_2 and E_3, the action currents were found to start before the end of the shock artefact except when the shock strength was at threshold. On such occasions determination of the starting point of the action current became uncertain and the whole configuration of the current had to be considered for determining latencies. The position of the starting point was inferred from the positions of the peak and the descending limb of the action current curve, under assumption that the shape of the action current is independent of the shock strength.

Figure 3 shows an example of the strength-latency relations obtained by this method. The lower curve represents the latency observed when brief condenser shocks of 7-μsec. duration were applied across the middle gap near the recording stretch (fig. 1*A*). The upper curve shows results obtained with shocks applied across the lateral gap remote from the recording stretch.

At threshold excitation with a brief shock the latency showed considerable play, which is consistent with the observations made by Blair and Erlanger (8, 9) and also with the results of a similar observation on the squid giant axon by Hodgkin, Huxley and Katz (10). For shock strengths slightly above threshold the latency

became stable, and the results obtained at the beginning of a series of measurements were always the same as the ones obtained at the end. The longest latency observed in threshold excitation was about 350 μsec. at 20°C. At any shock strength above threshold, the latency for the stimulus applied far from the recording gap was invariably longer than for stimuli given close to it. The difference in the latency at the shock strength of 105 per cent threshold was in general between 70 and 90 μsec. As the length of the myelinated portion of the fiber which gave rise to this difference in latency was approximately 1 mm., this might indicate that the process responsible for release of an action current spreads along the myelinated portion of the fiber at a rate of about 10 to 15 m/sec. It should, however, be emphasized that this 'rate' becomes unmistakably shorter when the shock strength used for comparison becomes

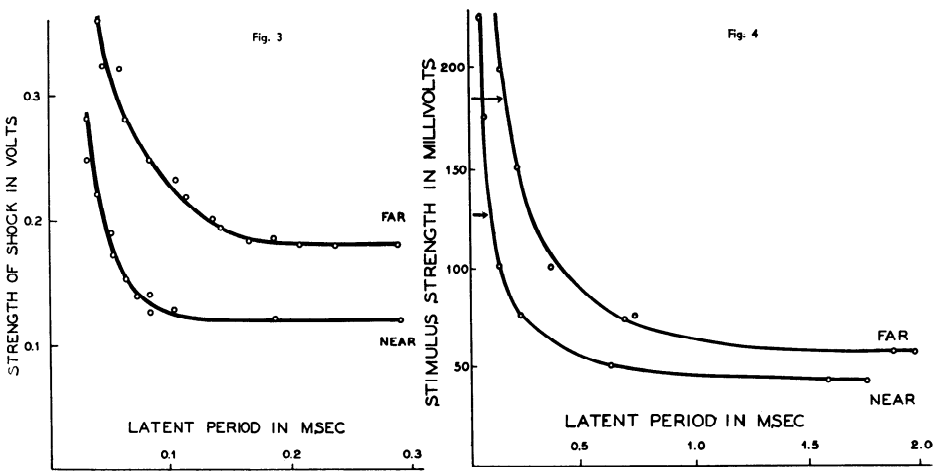

Fig. 3. RELATION BETWEEN the strength of brief shock (7-μsec. duration) and the latency of action current obtained with the arrangements of fig. 1*A*. Temperature, 20°C.

Fig. 4. RELATION BETWEEN the strength of a long rectangular voltage pulse and the latency obtained with the arrangements of fig. 1*A*. Temperature, 20°C.

higher. This excludes the existence of a 'process' which propagates along the internode at a well-defined rate.

When, with the experimental arrangement of figure 1*A*, *long rectangular voltage pulses* were employed instead of brief shocks, it was found that the configuration of the action current changes to some extent with the strength of the pulses. For very strong rectangular pulses, therefore, it was not possible to determine the latency accurately and we could measure the latency only up to about 2.5 times the rheobase.

In figure 4 is given an example of the strength-latency relations obtained by this method. The curves in general have a form similar to those obtained by Blair and Erlanger (8). For long rectangular pulses of rheobasic strength the latency showed a pronounced variation, the longest latency observed being about 3 msec. at 20°C. Here again, we found that there is a marked difference in latencies for the two modes of stimulation, the remote site of stimulation giving longer latencies than the one situated near the excited node. At the strength of twice the rheobase and for a difference of distance of about 1 mm., the difference in the latency was of the order of 100 μsec. and was much shorter for stronger stimuli.

It may be expected that there should be a definite relation between the strength-latency curves obtained by two different modes of stimulation, namely, by brief shocks and by long rectangular pulses. In figure 4 the latency for a brief shock of 105 per cent threshold strength is indicated by *arrows*. This corresponds to the latency for rectangular pulses of about three times the rheobasic voltage.

One might take the results of the experiments just mentioned as indicating that in the internodal stretch of a myelinated nerve fiber the process of excitation and conduction takes place in an entirely continuous manner, just as in nonmyelinated fibers, the latency being determined solely by the distance from the site of stimulation to the recording locus. But that this is not the case is readily shown by the following experiment in which the action currents were recorded with the stimulating electrodes themselves.

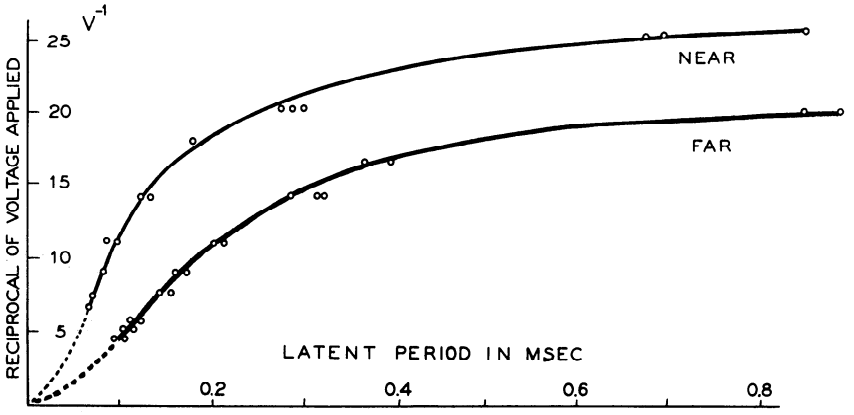

Fig. 5. RELATION BETWEEN the reciprocal of the strength of a long rectangular voltage pulse and the latency, obtained with the arrangement of fig. 1*B*. Temperature, 18°C.

Strength-Latency Relation Determined by the Oil-Gap Method. Figure 2*B* shows several examples of the records obtained with the experimental arrangements of figure 1*B*. With the Wheatstone bridge well balanced, the start of a long rectangular pulse gave rise to a transient artefact which decayed approximately exponentially with a time constant of about 100 μsec. We could trace the strength-latency relation by this method in the range of strength up to about three times the rheobase. Figure 5 gives the results of latency measurements obtained with the oil-gap method. In this figure, unlike the preceding one, the reciprocal of the strength of rectangular pulse is plotted as ordinate against the observed latency as abscissa.[2] It is seen that the observed points lie on curves showing sigmoid ascent.

With this arrangement it was possible to bring a node of Ranvier of the fiber first very close to the partition and then to shift the fiber so that the partition lay at the opposite end of the same internode. Again, we found a distinct difference in the latencies measured at the two opposite ends of one and the same internode of the

[2] This way of plotting the data was adopted in this case for the following reason: If we assume that the action current is released at the moment when the voltage across the nodal membrane attains a certain critical level and also that the nerve fiber behaves in linear manner, then a graph of the reciprocal of the strength should be a curve representing the time course of voltage rise at the node. The observed strength-latency relation, plotted this way and extrapolated as shown in the figure, actually gives curves showing sigmoid ascent, typical of the voltage rise in a network with resistances and capacities.

fiber. When a node was located near the distal wall of the partition, stimulation by descending currents, i.e. with cathode on the distal side, invariably gave a shorter latency than excitation by a current of the same strength but flowing in the opposite direction. If however the fiber was shifted so that a node lay close to the proximal side of the partition, ascending-current stimuli gave always shorter latencies than descending-current stimuli of the same strength.

It is therefore clear that *the latency for a rectangular pulse is determined both by the strength of the pulse and by the distance from the partition to the nearest node of Ranvier on the cathodal side.* This is what is expected from the structure of the nerve fiber: The effect of stimulation has to spread along the capacitative myelin-covered portion of the fiber before it reaches the node where a nerve impulse is initiated. It has been well known since the time of Lord Kelvin that a signal travels along a cable with distributed capacity at only a finite rate.

From these considerations it immediately follows that the difference in the latencies for close and remote stimulations should be greater in the experiments with the oil-gap partition than in those with bridge-insulators. With the arrangement of figure 1*B*, the effect of the stimulating current first spreads as far as the node and then the action current developed by the node spreads back towards the oil gap, thus finally causing an observable potential change in the input of the amplifier.

Actually the latency observed by the oil-gap method was not as long as would be expected from the cursory consideration just mentioned. It was even difficult to prove experimentally that the oil-gap method gives a longer latency than the bridge-insulator method. For a full understanding of these results, therefore, a more analytical consideration is necessary.

Spread of Longitudinal Current Along the Nerve Fiber. For the analysis of the mode of spread of longitudinal currents along the nerve fiber a propagated impulse can be used to advantage. With a source of current applied to a nerve fiber from outside, a considerable portion of the current from the source flows through the fluid medium surrounding the fiber and it is difficult to determine the time course of the current flowing through the axis-cylinder of the fiber. In an analysis of the action current associated with a propagated impulse we are not troubled by this difficulty, because the input resistance of our amplifier is always far smaller than the resistance of the preparation, including the leakage resistance of the partition.

Development of an EMF at a normal node of Ranvier seems to progress, under the condition of our experiments, with great rapidity. When a node of Ranvier situated close to an oil-gap partition is brought into action by a propagated impulse, it is always observed that a current with an extremely sharp rising phase is started through the low input resistance of the amplifier (see fig. 6 *A*-1, *B*-1 and *C*-1). The speed of excursion of the electron beam of the oscillograph at this moment is not much different from that caused by a rectangular voltage pulse applied directly to the input of the amplifier (fig. 6*D*).

Under ordinary experimental conditions this abrupt rising phase of the action current is preceded by a *gradually rising 'foot' which mainly derives from the EMF* of the preceding nodes. The action current record labeled 1 in figure 6*A*, was taken from a nerve fiber immersed in normal Ringer's fluid divided into two pools by means of an oil-gap partition. One of the nodes of Ranvier of the fiber was brought close to the partition on the proximal side, and the response of the fiber to a brief shock sent into the proximal nerve trunk of the single fiber preparation was recorded with an amplifier with a low input resistance (166 kilohms).

Action currents led from the fiber at a point some distance away from a node

show unmistakably a slow ascending phase. This tendency increases with the distance from the node to the recording locus. Action currents taken from the distal end of an internode (*record 2* in fig. 6*A*) reach their maximum in 20 to 50 μsec. after the action current recorded from the proximal end (*record 1*) has attained its maximum. At the distal end, however, the downward stroke of the oscillograph line becomes very sharp, owing to the short distance of the distal node from the partition. Similar records have been obtained by Huxley and Stämpfli (3) with the same experimental arrangement.

Although the records of action currents associated with propagated nerve impulses show an increasing delay in the rising phase of the longitudinal current with increasing distance from a node, this delay is relatively small as compared to the

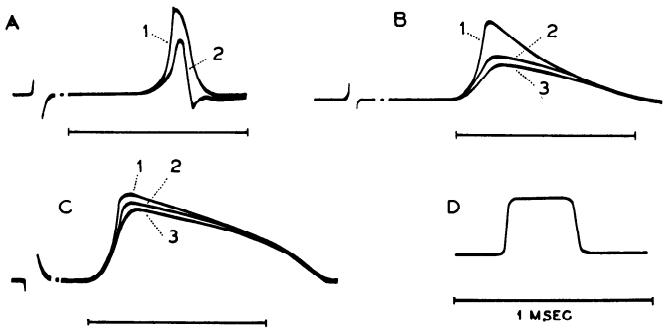

Fig. 6. *A:* ACTION CURRENTS RECORDED at two opposite ends of the same internode; observation with the oil partition, stimuli on the proximal nerve trunk. Temperature, 18°C. *B:* Records of action currents obtained when a 0.2 per cent cocaine-Ringer solution was applied to the region of the fiber on the distal side of the oil partition; *record 1*, taken with the partition located near the proximal node; *2*, the partition in the middle of the internode; *3*, the partition close to the distal node. Temperature, 19.5°C. *C:* Action currents recorded from the fiber of which the first node on the distal side of the oil partition had been crushed and the injury potential was compensated; *record 1*, taken with the partition located close to the proximal node; *2*, taken at the middle of the internode; *3*, from the distal end of the internode; at the points beyond the crushed region, there was no spread of current observable. Temperature, 19.5°C. *D:* Calibration of the amplifier with a rectangular pulse of 0.3 mv. Temperature, 19.5°C. Time marks, 1 msec.

difference in latency obtained by excitation with rectangular pulses at the two ends of an internode. Now, without going into the mathematical treatment of the problem, one may be convinced that, in an electrical network with condensers, the rise of potential at any point in the network can lag behind the rising phase of the current at that position. We assume that it is the potential difference across the nodal membrane of the nerve fiber, and not the longitudinal current, that is directly responsible for initiation of an impulse. It is therefore quite understandable that the latency varies more markedly than the longitudinal action current as the distance from the node.

Before going into the description of the next experiment, showing the time relation between the longitudinal current and the potential across the membrane, it seems worthwhile to consider the effect of the capacity of the myelin sheath and the nodal plasma membrane upon the configuration of the action current. Let us take *records B* and *C* in figure 6, which were obtained after making the lead 'monophasic' either by narcosis (*B*) or by mechanical injury (*C*). It is seen in these records that the decrease in the strength of action current with the distance from the active

node occurs mainly in the early phase of the current. An increase in the distance of the partition from the node does not appreciably affect the later (descending) phase of the action current. This fact suggests that this change in the configuration of action current is mainly due to leakage of current through the capacity of the myelin sheath.

It is also of some interest to point out the difference in the form of action current in the narcotized (*B*) and crushed (*C*) nerve fibers. The crushed region of the fiber shows practically no resistance to the current leaving the axis-cylinder; and, if the injury potential is kept compensated by a constant voltage applied from outside, the adjacent node of Ranvier retains its ability to develop action currents of almost normal strength. The quicker subsidence of the current strength in the narcotized nerve fiber (*B*) than in the crushed fiber (*C*) is considered to be due to the capacity of the cocainized nodal membrane.

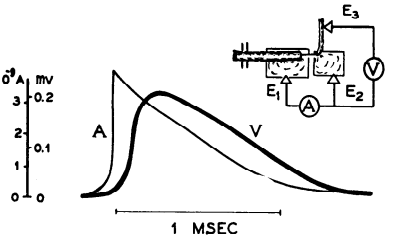

Fig. 7. COMPARISON OF THE TIME-COURSES of the longitudinal action current and the outside action potential led off from the same internode. Temperature, 19°C.

Time Course of Potential Change Caused by the Action Current. It is well known that the action potential of a single nerve fiber led from the surface of the nerve trunk has a much slower rising phase than the action currents shown in figure 6. In an electrical network consisting of only ohmic resistances, such a difference in the time-course of current and potential cannot exist. The distributed capacity of the surface membrane of the nerve fiber is without any doubt responsible for this difference. In the diagram of figure 7 is illustrated the arrangement of our simple experiment designed to show how remarkable is the difference between the records of potential and of current.

From the fine nerve trunk entering the frog sartorium muscle, a large motor nerve fiber was isolated for a length of about 0.5 mm. No node of Ranvier of the fiber was exposed in the operated region. The fiber was mounted on a bridge-insulator about 0.1 mm. wide. Both the proximal and distal nerve trunk regions of the preparation were immersed in the lateral pools of Ringer's fluid. The fluid on the distal side of the bridge-insulator was then replaced with a 0.3 per cent cocaine-Ringer solution. When the narcosis became deep enough, the distal end of the nerve trunk was gradually lifted above the surface of the fluid by elevating it with a metal forceps and by keeping its proximal end in the distal pool of Ringer's solution by another forceps. Thus, the entire length of the distal nerve trunk was exposed to air. The nonpolarizable electrodes E_1 and E_2 dipping into the pools of Ringer were grounded and the metal forceps (E_3) holding the distal end of the nerve trunk was connected to the grid of an amplifier of the cathode-follower type. The time course of potential difference between E_2 and E_3, induced by a nerve impulse arriving at the gap, was recorded with a cathode ray oscillograph. The nerve impulse was initiated by a brief rectangular pulse of three times the threshold strength applied to the proximal nerve trunk.

Next E_3 was disconnected from the amplifier, and then, with an amplifier hav-

ing an input resistance of 166 kilohms, and with the same stimulus strength, the action current of the fiber was recorded through the electrodes E_1 and E_2. Then the potential recording and also the current recording were repeated alternately once more.

With this experimental arrangement, the current (*curve A* in fig. 7) flowing into the distal pool through the axis-cylinder on the bridge-insulator is the cause of the potential difference developed between E_2 and E_3 (*record V* in fig. 7). As no node of Ranvier was exposed in the operated region of the fiber, the action potential record is considered to show the time-course of potential change at the surface of the nerve trunk outside the very internode from which the action current record was obtained. The remarkable difference between the form of the potential record and that of the current record reveals the importance of the capacity of the nerve fiber when considering the spread of potential wave along an inactive (narcotized in this case) region of the nerve fiber.

Spread of Potential Wave in Normal Conduction of Impulse. In normal nervous conduction, nodes of Ranvier of the fiber are successively thrown into action at intervals of about 0.1 msec. The time-course of potential change at the surface of the nerve trunk should, therefore, be different from that given in figure 7*V*, which shows the time-course of potential change spreading into a narcotized region of a fiber.

In a nerve fiber introduced into a long tube of Ringer's fluid of uniform diameter, the time-course of potential change in the fluid medium outside the fiber is considered to approximately reproduce the potential change inside the fiber (see DISCUSSION). The absolute value of potential is then determined simply by the ratio of the resistance per unit length of the fluid medium to that of the axis-cylinder. As the potential change in the axis-cylinder can readily be correlated with the longitudinal action current by virtue of Ohm's law (see below), the analysis of the configuration of the action potential of a fiber kept in a uniform cylindrical conducting medium should give us direct information about the processes taking place in normal nervous conduction.

The experimental set-up we used for this purpose was substantially the same as one of those used by Huxley and Stämpfli. A long nerve fiber, to the distal end of which was tied a nylon fiber, was introduced into a glass capillary of 103 microns diameter and 18 mm. length, filled with Ringer's fluid. The capillary has its ends in two troughs of Ringer's solution (34 x 16 x 16 mm.) which were connected, by means of Ag-AgCl-Agar electrodes, to a cathode-follower. The time-course of the potential difference between the troughs caused by a nerve impulse arriving there from the proximal nerve trunk and travelling further along the fiber in the capillary was thus recorded. The distal, injured end of the fiber remained as a rule in the capillary. Then, by means of two pairs of forceps, one holding the distal end of the nylon fiber and the other catching the damaged fibers at the distal end of the nerve trunk, the fiber in the capillary was shifted in the fluid, and at intervals of about 0.2 mm. along the fiber, action potentials of the fiber were recorded.

Under these experimental conditions, the portion of the action current flowing in the two large lateral troughs makes no contribution to the recorded action potential. The effective load-off electrode is, therefore, situated at the proximal opening of the glass capillary, the indifferent electrode being located at the cut end of the fiber.

A set of action potential records obtained by this method is furnished in figure 8. As the falling phase of the observed action potential was slow and almost linear,

regardless of the position of the effective lead-off electrode, this part of the action potential record was omitted in the diagram. We noticed at once that the maximum height of the action potential does not vary appreciably as a function of distance along the fiber. The most conspicuous change in the time-course of the action potential along the fiber is in its steepness of the rising phase. When one of the nodes of Ranvier of the fiber was brought in the vicinity of the proximal opening of the capillary, the observed action potential curve showed a remarkably sharp rising phase. But, even these sharp rising phases have to be considered as being considerably rounded off by the spread of the potential field in the space around the opening of the capillary and also by the sluggishness of our recording device in responding to a sudden change of potential. In our records, the maximum steepness of the action potential at the node is approximately three times as great as that at the middle of the internode. The real variation in the steepness must therefore be greater than 3.

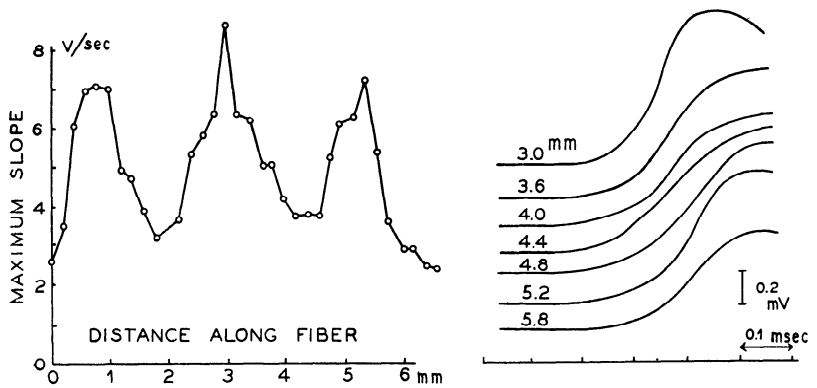

Fig. 8. *Right:* TIME-COURSE OF THE ACTION POTENTIAL recorded from outside the fiber at various positions along the fiber; the effective position of the electrode is given. *Left:* Maximum steepness of the action potential along the fiber. For the experimental arrangement, see text. Temperature, 19°C.

As can be seen in the diagram, the start of the rising phase of these action potential curves is so gradual that it was practically impossible to make an accurate measurement of the shock-response interval as a function of distance along the fiber by this method. As in Laporte's recent observation (11) on the nerve fiber in the lateral line of the carp, the shock-response interval appeared to vary continuously along the fiber.

DISCUSSION

In a previous paper (4), it was shown by the shock-test method that the effect of a brief shock applied at an internode of a fiber reaches the node after an appreciable lapse of time. When a conditioning subthreshold shock was applied to a fiber at a point far from a node, the maximum threshold change, revealed by another (testing) shock applied now directly to the node, was observed not at the time of delivery of the conditioning shock but at least 20 to 30 μsec. after the end of the conditioning shock. This fact, together with other evidence showing capacitative leakage of current through the myelin sheath, indicates that the potential wave spreads along the myelin sheath at a finite (but not well-defined) rate. Now we have shown that the latent period of the action current for direct electrical stimulation

varies considerably as a function of distance along the fiber. This fact is not surprising, since the action current is considered to be released at the node only after the effect of the stimulus has reached the node. It is well known that both the longitudinal current (in the axis-cylinder) and the potential difference across the distributed capacity (at the surface of the fiber) spread along such a network at a finite velocity.

Another finding, mentioned in this paper, namely the fact that the steepness of the rising phase of the action potential varies regularly as a function of distance from the node of Ranvier, calls for a comment in connection with a misunderstanding concerning the mechanism of nervous conduction. Laporte (11) saw that the time interval between the brief shock and the start of the action potential varied continuously as the distance along the nerve fiber, and believed that this was evidence against the saltatory theory of nervous conduction. Although we could confirm the experimental result on which Laporte's argument was based, we believe that his conclusion is incorrect. We shall first show, by virtue of simple Ohm's law, how the action current recording is correlated to the action potential recording, and then discuss the process of nervous conduction in the myelinated fiber.

Let $I(x, t)$ = time-course of the current flowing through the axis-cylinder at distance x and at time t;
$V(x, t)$ = potential of the axis-cylinder at distance x and at time t, the potential of the resting region ahead of the active region taken as zero;
w = resistance per unit length of the axis-cylinder.

The zero points for the distance x and the time t are arbitrary. If we assume that the axis-cylinder obeys Ohm's law, then we have

$$V(x, t) = \int_{\infty}^{x} I(x, t)w \, dx.$$

Since the nerve fiber which we used for our experiments has a uniform diameter in the operated region, w should be approximately constant in the range of x under investigation. Therefore, the time-course of the potential $V(x, t)$ should be given by an integration of the spatial distribution of the current $I(x, t)$ at that moment. We carried out this integration by means of a planimeter on a typical set of action current records.

The curves $x = a$ and b in figure 9*A*, show the time-courses of the action currents recorded with an oil-gap partition of 0.6-mm. width placed close to the proximal and distal ends, respectively, of an internode of a nerve fiber. The internodal conduction time for this fiber was approximately 0.1 msec. The curves c, d and e are those drawn in such a manner that they lag behind the curves a or b by 0.1 or 0.2 msec. The actual records taken from the adjacent internodes were not very different from these curves.

From these five curves, a graph showing the spatial distribution of the longitudinal current at several different moments was constructed (fig. 9*B*). As our oil gap was 0.6 mm. wide, the curves $a, b, \cdots e$ are considered to show the time-courses of currents at a distance of about 0.4 mm. away from the nodes. These loci ($a, b, \cdots e$) and the positions of the nodes ($N_1 \cdots N_3$) are marked on the abscissa in *diagram B*. The curve marked 1, for instance, was obtained by plotting the strengths of currents $I(x, t)$ at the moment $t = 1$ in *diagram A* as ordinate against the distance x

along the fiber and connecting the four plotted points with a continuous curve. Similarly, the curves labeled *2*, *2.3* and *3* were constructed. Judging from the time-course of the action currents, the times $t = 1$, 2 and 3 correspond to the moments shortly before the start of the (action) EMF at the nodes N_1, N_2 and N_3, respectively. The time $t = 2.3$ is the moment at which the longitudinal current recorded at $x = c$ reaches its maximum. The discontinuities on these curves at the positions of the nodes correspond to the currents flowing outwards through these nodes.

We measured the area under the curves in figure 10*B*, from positive infinity to x, and plotted the values as ordinate against the distance x. *Diagram C* in figure 10 shows the spatial distribution of potential $V(x, t)$ thus obtained.

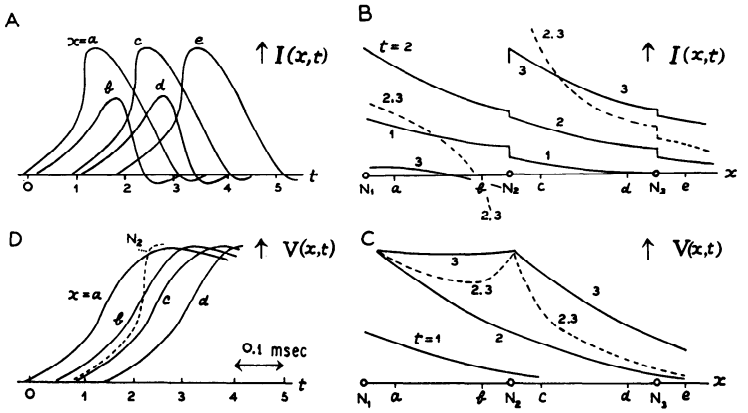

Fig. 9. DIAGRAMS showing the process of constructing the time-course of the potential of the axis-cylinder $V(x, t)$ from the records of the longitudinal action currents $I(x, t)$. Details in the text

Attention should be called to the fact that there is no discontinuity in the curves representing the spatial distribution of potential. At the moment $t = 3$, both the nodes N_1 and N_2 are in 'active' state, the potential difference across the surface membrane being determined by the state of the node. Hence, the potential at N_1 is approximately equal to that at N_2. At $t = 2.3$, it is difficult to determine the potential at the node N_2 from the observed action current record, because the exact time-course of the longitudinal current in the immediate neighborhood of the node can not be determined by this method. The dotted line in *diagram C* is drawn on the assumption that the EMF at N_2 is full-sized at this moment.

From the diagram of figure 9*C*, the time-course of the potential of the axis-cylinder shown in *D* of this figure was constructed. It is seen here that the start of the potential change (in the axis-cylinder) shifts continuously as the distance x. It is also clear from the diagram that the point of inflection of the curve changes with x in a somewhat complicated manner and that the maximum steepness varies periodically as the distance x. At the position of the node (see the curve marked N_2), the potential of the axis-cylinder is seen to rise more rapidly than anywhere else along the fiber. The results of all these analytical considerations agree, at least qualitatively, with the result of our actual observation on the action potential of a single nerve fiber.

In the experiment of figure 8, the fiber was kept in a cylindrical fluid medium of which the resistance per unit length was far lower than that of the axis-cylinder.

Since at any moment the current flowing through the axis-cylinder is equal (but opposite in direction) to the current flowing in the medium at that position, the action potentials we recorded are exactly what we have mapped out above. From fibers placed in a glass tubing of 103-micron diameter, we obtained action potentials of 0.6 to 1 mV. If we assume, therefore, the resistance of the axis-cylinder to be 170 megohm/cm. and the specific resistance of Ringer's fluid to be 90 ohms-cm. at 20°C., the absolute value of the action potential of the axis-cylinder is found to be about 120 mV. It should be borne in mind, however, that this method of determining the absolute value of the action potential is subject to a large source of error arising from the estimation of the resistance of the axis-cylinder (12).

The fact that both the longitudinal action current and the action potential of the fiber in a uniform conducting medium show the greatest steepness at the node can very readily be interpreted in terms of the saltatory nature of nervous conduction, *saltatory with respect to space* in the sense mentioned in the introduction of the present paper. The 'active' change in the EMF across the surface membrane of the fiber takes place only at the nodes, and the potential wave spreads along the myelin sheath on both the proximal and the distal side of the node.

Our experimental results agree with those of Huxley and Stämpfli (3) in showing that the shock-response interval measured to the peak of the action current record rises continuously within each internode and also increases discontinuously as each node of Ranvier is passed. In our results, however, the gradual rise in the shock-response interval within each internode accounted for a larger proportion of the total internodal conduction time than in the previous results. Thus the shift in the maximum of the action current observed with an oil-partition of 0.6 mm. wide in an internode of 2 mm. long was found to be 20 to 50 μsec. at 20°C. while figure 7 in Huxley and Stämpfli's paper shows only about 10 to 30 μsec. (The difference may be due to poorer high frequency response in the amplifier used by those authors which may have retarded a sharp rise of current more strongly than a slow rise.) Since the total internodal conduction time at this temperature is 70 to 100 μsec., we conclude that the discontinuity in shock-response interval at the node itself is smaller than the gradual increase within the internode.

Nervous conduction in the myelinated fiber differs considerably from conduction of the activation wave in Lillie's model of the myelinated fiber, because the model is so designed that there is no leakage of current through the myelin sheath. *Diagram B* in figure 9 shows how significant the leakage of current through the myelin sheath actually is, at least for the rapidly rising phase of the action current. The plasma membrane at the node is short (less than 1 micron) as compared with the myelinated portion of the fiber, and its thickness is far less than that of the myelin sheath. Therefore, the current density and the intensity of electric field through the nodal membrane is certainly much greater than those through the myelin sheath. We therefore feel that saltation of the nerve impulse with respect to space is not at all surprising.

We may conclude from what has been mentioned above that, under normal conditions of experimentation, *conduction in the myelinated nerve fiber is saltatory with respect to space, but not quite so with respect to time.* At relatively high room temperature (*ca.* 20°C.), the velocity of conduction is determined to a major extent by the rate of spread of the potential wave along the myelin sheath with its distributed capacity. At low temperatures, however, it seems probable that the rate of the processes taking place at the node, namely the movement of Na and K ions (10), becomes the main factor which determines the velocity of conduction.

Summary

Using two different types of partition, stimulating voltages were applied at several points along an internode of a frog nerve fiber, and the latent period of the action current was measured at the site of stimulation. For both brief and long stimulating pulses, longer latencies were observed when the partition for stimulation was located farther from the node. This variation in the latency along the internodal stretch is attributed to the spread of the potential wave along the myelinated portion of the nerve fiber at a finite rate.

The action current of a conducted nerve impulse changes its shape when the recording partition is shifted along the nerve fiber. Within an internodal segment, the rising phase of the action current is shortest and the maximum is attained earliest when the recording partition is located at the proximal end of the internode. The rising phase is retarded, within an internodal stretch, continuously with increasing distance of the recording partition from the proximal node. This fact is also ascribed to the distributed capacity of the myelin-covered portion of the nerve fiber.

The difference between the time-course of the action *current* (flowing through the axis-cylinder) and that of the action *potential* (recorded from the surface of the nerve trunk) is shown to be due to the capacity distributed along the surface of the nerve fiber.

The latent period of the action *potential* of a conducted nerve impulse varies continuously as the distance along the fiber. The steepness of the rising phase shows a sharp maximum at each node of Ranvier regularly. This result was correlated with the measurements of action currents recorded by the partition method.

Conduction of the nerve impulse in myelinated fibers is saltatory with respect to space but not, in general, with respect to time.

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