ON SALTATORY CONDUCTION IN PERIPHERAL MYELINATED NERVE

Thesis by

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ABSTRACT

Anatomically speaking, there are two types of nerve fibers in vertebrates: myelinated and unmyelinated fibers. In the former the axon is covered with a protein-lipid structure (myelin) whish is interrupted at more or less regular intervals (Ranvier nodes). In unmyelinated fibers this myelin structure is absent. Myelinated fibers conduct impulses much faster than unmyelinated fibers. In order to account for the high conduction velocities of medullated nerves, Lillie has proposed that excitation takes place only at the nodes of Ranvier with the circuit between adjacent nodes being made through the axoplasm of the fiber and the medium external to the fiber. This is the theory of saltatory conduction.

The literature on saltatory conduction is critically reviewed and it is concluded that only two experiments that have been done seem to indicate that saltatory conduction occurs. These are the narcosis experiments of Taskai and the air gap experiments of Huxley and Frankenhaeuser. These experiments have been carefully repeated and improved. From the results of these experiments it is concluded that medullated nerve can conduct in a situation where the conditions necessary for saltation do not exist. The possibility that saltation is the normal physiological process of conduction cannot be discounted.

INTRODUCTION

The theory of saltatory conduction in medullated nerve was first proposed by Lillie in 1925. The theory implies that impulses are conducted along medullated nerves in a discontinuous manner from one node to the next. Lillie felt that the theory adequately explained the high conduction velocities typical for medullated nerves. The experiments from which he formulated the theory were not actually performed on myelinated nerve, but on an "iron-wire model" of nerve. Following the publication of Lillie's experiments the theory was generally accepted by physiologists despite the fact that there was no evidence based on experiments with living nerve to substantiate it. Eleven years after the publication of Lillie's thoery, Blair and Erlanger performed the first experiments on nerve that were offered as evidence for saltatory conduction. Since then the problem has been investigated by a number of authors, most of whom have concluded that saltatory conduction is, indeed, the mechanism of conduction in medullated nerve fibers.

PART I. A REVIEW OF THE LITERATURE

A. Some Histological and Physiological Properties of Myelinated Nerves

It is customary to divide nerve fibers histologically into two classes: myelinated and non-myelinated fibers. Myelinated fibers are almost exclusively confined to the vertebrates, while the unmyelinated axons appear in both vertebrates and invertebrates. Actually, the distinction between myelinated and non-myelinated nerve fibers is not a sharp one. for all nerves seem to possess myelin to some degree. The myelinated fibers consist of a central core, the axis cylinder of axon, surrounded by a layer of protein-lipid material (myelin) that can be stained black by treatment with osmic acid. In the frog the diameter of the axon represents about seventy percent of the total fiber diameter (1), the rest being myelin. The non-myelinated fiber is enveloped by only a very thin layer of myelin, the axis cylinder constituting between 95 to 99% of the total fiber diameter (2,3). Both types of fibers are covered by a layer of flat cells, the Schwann cells of neruilemma.

On longitudinal section the unmyelinated fibers appear uniform, while the myelin sheaths of medullated axons are interrupted by two types of structures. The most obvious interruptions of the myelin sheath are the nodes of Ranvier. The segment of axon between two consecutive nodes of Ranvier is known as an internode. The internodes contain the "Clefts

of Lantermann" which penetrate the myelin obliquely all the way to the axis cylinder. It was long believed that the Lantermann clefts were artifacts, but their existence seems to be beyond question now that von Muralt (4) has observed them in living nerve fibers under polarized light. The physiological significance of the Lantermann clefts is unknown. The controversy on the role of the Ranvier nodes in nerve conduction is the basis of this thesis.

There are many conflicting opinions in the literature about how nodes are formed. The following three paragraphs are quoted from the work of J.Z. Young (5) because they more or less summarize the current views.

> "The nodes of Ranvier are interruptions of the coating of myelin without any break or membrane across the axon. The protoplasm of one Schwann cell covers each internode and the neruilemmal tube wall turns in at each node to make a ring, the so-called cementing disc, which does not extend into the axoplams. Nodes occur on fibres of all sizes in vertebrate peripheral nerves but not on fibres of the central nervous system. They are present in the medullated nerves of prawns but only on the larger fibres. They are also absent from regenerating nerve fibres in the early stages of myelination.
>
> "In a normal mammalian nerve the internode length increases with fibre diameter but at a rate which varies in different nerves. In man the smallest fibres (2-3 micra) have internode lengths up to 1.3 mm in the anterior tibial and ulnar nerves, 0.7 mm in the mandibular nerve. The slope of the line relating internode length to diameter is therefore steeper in the faster growing nerves. This suggests that when myelin is first laid down on the fibres of 1-2 micra diameter the internode length is about 200 micra and the extent to which it increases depends on the amount by which the nerve is subsequently stretched during growth.
>
> "Observations on developing and regenerating nerves confirm this view. The increase of internode

> lengths during development is proportional to the growth of the part. Regenerating nerves are at first covered with a continuous sheet of myelin which then breaks into segments about 200 micra long, varying considerably. Although the fibres subsequently increase in diameter the internode length remains short. Sanders and Whitteridge have shown that these large fibres with short internodes conduct as rapidly as normal fibres of the same diameter. Nodes and internodes are therefore probably not of critical importance for conduction as is suggested by their absence from many medullated fibres."

Young (6,7) has since reversed his stand that fibers of the central nervous system are not segmented by nodes. Other investigators, too, have found nodes in the central nervous system (8,9,10) and they regard the myelin as a highly labile substance which quickly "closes" the nodes after death or during the usual straining procedures. Lorente de No (11) agrees with the concept of the lability of the myelin sheath. He states that:

> "- - - with freshly excised nerves the myelin sheath is discontinuous at all, or to be less positive, at practically all the nodes of Ranvier. If a nerve has been excised some time, however, the myelin is found to be continuous at a number of nodes of Ranvier -- nerves fixed 8 hours or more after excision have been examined in which nodes lacking a myelin sheath were a rarity."

The most striking physiological characteristic of myelinated nerve is the very high conduction velocity. For example, a 20 fiber of the cat may propogate impulses at a velocity exceeding 120 meters per second. It was formerly thought (12) that the conduction velocity was proportional to the square of the fiber diameter, but more recent investigations agree with Gasser's finding (13) that a linear relationship exists between the conduction velocity and fiber diameter

in mammalian medullated nerve. Zotterman (14) has found a linear relationship between axon diameter and internodal length. Hursh (15) claims that the conduction velocity is directly proportional to the internodal length. Tasaki (16) came to a similar conclusion and gives the following relations between conduction velocity, fiber diameter, and internodal length for the bullfrog sciatic at 24°C:

$V = 0.146D$ V = conduction velocity (M/sec)
D = fiber diameter ( )
$L = 2.05D$ L = internodal length (mm)

The conclusion can be drawn from this that, according to Tasaki, the conduction velocity is directly related to internodal length and this in turn would indicate that the time needed for an impulse to jump from one node to the next is a constant one for all myelinated nerves of the same species. Thus, according to this view, a 20μ fiber of the frog conducts at a velocity of 30 M/sec while a 10μ fiber conducts at 15 M/sec simply because the Ranvier nodes in the 20μ fiber are twice as far apart as those in the 10μ fiber, and not because of any difference in the time needed to jump from one node to the next.

Sanders and Whitteridge (17) have investigated the relation between velocity and internodal length in another way. They crushed the peroneal nerves of rabbits and when they had been allowed to regenerate between 456 to 486 days, the internodal distances were about half normal. There was, however, no significant differences in the conduction velocity between these regenerated nerves and normal nerves. Further-

more, they found that the myelin sheaths of the nerves proximal to the lesion thickened and their conduction velocities increased by 11% while the thickness of the axis cylinder remained constant. They concluded that the conduction velocity is related to the thickness of the myelin sheath and is not related to the internodal length.

Van Muralt (4) similarly concludes that the conduction velocity of a fiber is related solely to the amount of myelin surrounding it. He has chosen to compare two fibers that conduct at 25 M/sec. One was a 10μ fiber from the frog sciatic in which the ratio of axis cylinder to fiber is 0.73. The other was a 4μ fiber from the saphenous nerve of the cat and its axis cylinder/fiber ratio is 0.58. He quite arbitrarily assumes that the reason a 4μ fiber of the cat conducts at the same rate as a 10μ fiber of the frog is that it has proportionately more myelin. This is obviously doubtful, because, among other things, he fails to consider that the metabolic rate of a cat nerve (as measured by oxygen consumption) is about seven times that of a frog nerve (18). Indeed, it has been demonstrated that fibers of the same diameter and myelin thickness of different species of mammals can conduct at entirely different rates. Fibers of 20μ diameter of the cat and rabbit peroneal nerves have the same myelin thickness (2.5) yet the cat nerve conducts at 108-111 M/sec while the rabbit nerve does not exceed 69 M/sec (17).

In any given species of mammals there is a striking difference in the conduction velocities of myelinated and

unmyelinated fibers of the same diameter (19). A medullated A fiber of one micron in diameter of the cat will conduct at about 6 - 7 M/sec., while the unmyelinated C fibers which are as large as 2.5μ do not conduct faster than 2 M/sec. It is clear that myelinated nerves have some special property which enables them to conduct impulses at velocities greatly in excess of those that unmyelinated fibers can attain.

B. The Nature of the Nerve Impulse and the Problem of Saltatory Conduction

In an isolated nerve fiber the only tangible evidence of the propagation of an impulse is the action potential. This is essentially a wave of negativity moving with a finite velocity over the surface of the nerve fiber. At rest the nerve membrane is polarized so that the outside is positive with respect to the inside. In terms of equivalent circuits (fig. 1) the nerve membrane is usually pictured as a series of units each consisting of a resistance (R_2) and a capacitance (C_1) in parallel and a battery (E) which supplies the E.M.F. for the membrane. The inside (core) of the fiber is represented by a series of resistances (R_3) and the resistance of the external medium surrounding the fiber by (R_1). The arrows in fig. 1 give the flow of current through the membrane model when a current is passed through electrodes A (anode) and C (cathode).

The classical concept of a nerve impulse (Bernstein Theory) postulated that at rest the nerve membrane was polar-

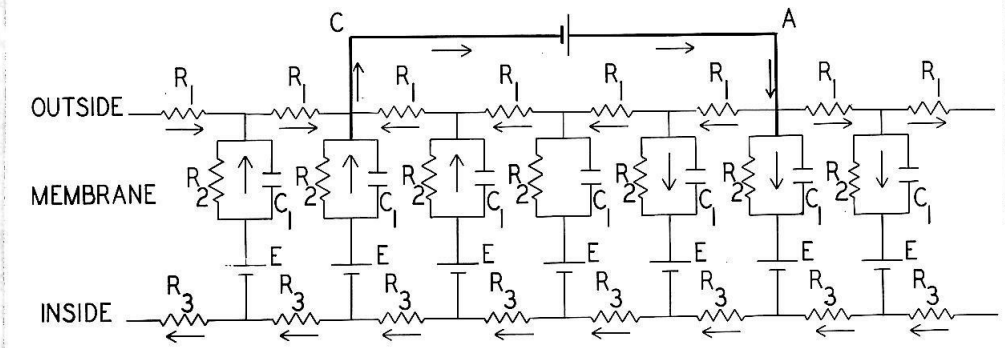

Figure 1. The Equivalent Electrical Circuit of the Nerve Membrane. Modified after Fulton (19).

ized but during activity the charge across the membrane was reduced to zero. Then during the absolute and relative refractory periods the membrane was repolarized. The Bernstein theory was based on measurements made with externally placed electrodes. Curtis and Cole (20), and later Hodgkin and Huxley (21) and Marmont (22) reinvestigated the electrical events during the passage of an impulse and their work has led to a modification of the Bernstein theory. In their experiments they used the giant axon of the squid (550 diameter) and made measurements with one external electrode and one microelectrode (100μ diameter) inserted into the axoplasm of the nerve. At rest the potential across the membrane was of the order of 40 to 100 mV. When an impulse passed over the fiber the potential was first abolished, then reversed in sign (outside negative with respect to the inside), after which it returned to normal. This reversal of the membrane potential occurs at the height of the action potential and is significant because it shows that more than a simple depolarization and repolarization takes place.

As an impulse passes along an ummyelinated nerve fiber current* flows inward through the excited part of the membrane, through the core of the axon, then outward in front and in back of the excited region and then through the external

* The term "current" is used here in the conventional sense as flowing from positive to negative.

medium to complete the circuit. This "local circuit" mechanism for impulse propogation in unmyelinated nerve is universally agreed upon.

For medullated nerve two theories of conduction are being proposed at the present time. The theory of continuous conduction maintains that impulses in medullated nerve are propagated by local circuits beneath the myelin exactly as in unmyelinated nerve. On the other hand, the theory of saltatory conduction maintains that the action potential of one node excites the next node and that the circuit between two successive nodes is made through the axis cylinder in the internode and through the medium external to the fiber. According to the saltatory conduction hypothesis the current flowing through an internode is a passive current (in the sense that it does not depolarize a membrane in the internode).

C. A Review of the Literature on Saltatory Conduction

The Ostwald-Lillie Iron Wire Model

If a piece of iron wire (23) is put in concentrated nitric acid (60 - 70 vol % HNO_3) a film of oxide forms over the surface of the wire. This oxide is impermeable to the nitric acid and prevents it from dissolving the wire. When the surface of this "passive" wire is scratched or stimulated electrically the film is broken down, and then the nitric acid attacks the wire and forms another oxide film on it. This breaking down and reforming of the oxide coating is

propagated along the entire surface of the wire. Lillie found that it took 2.7 seconds for an impulse to pass over an iron wire 75 cm long in a bath of 70% nitric acid at 4° C. This inorganic system exhibits certain properties of nerve. For example, there is a definite threshold for electrical stimulation, and absolute refractory period follows excitation, and under certain conditions there may be repetitive discharges. The system also shows accommodation to slowly increasing currents, summation of inadequate stimuli, and after potentials. Furthermore, the "impulses" are propagated by local circuits because the velocity is a function of the conductivity of the external medium.

In Lillie's opinion the bare iron wire in nitric acid is a model of an unmyelinated nerve fiber. He was able to construct a model of a myelinated nerve by threading the iron wire through segments of glass capillary each 10 cm long and with a gap of 2 mm between the segments. In this model the segments of glass capillary represent the myelin and the gaps represent the nodes of Ranvier. When one end of the iron wire was stimulated the conduction time for 75 cm of wire was too brief to be measured by the methods available to Lillie. During the passage of an impulse each node became active first, and then the impulse spread in both directions through the internodal regions. Lillie therefore postulated that in myelinated nerve excitation occurs only at the nodes of Ranvier. He believed that the high conduction velocities of myelinated nerves are due

solely to their being segmented by myelin so that excitation occurs in a discontinuous manner.

The Polarization Experiments of Blair and Erlanger

Figure 2 diagrams the first published experiments (24) on single nerve fibers that were considered to show evidence of saltatory conduction. A single nerve fiber of the frog extends from D to N with nodes at a,b,c,d, etc. It is stimulated to the left of D and a cathode is placed at D, an anode at node d. Action potentials are recorded at d and N. Two shocks 3 msec. apart were delivered to the nerve. "A" shows a recording without anodal polarization of node d. S_2 is the stimulus artifact of the second shock and both spikes are diphasic; the second spike travels in the refractory period of the first and is therefore smaller than normal. In "B" anodal polarization has been applied at d and the recording shows that the first spike has been blocked there because it is monophasic. The second spike is normal and traveled over the entire length of the fiber in spite of the anodal polarization. This second spike was supposed to have skipped the anodally polarized node d.

Their second experiment is really a corollary of the first. Figure 3 shows a single fiber extending from G to N with nodes at a, d, c, etc., and with an anode placed approximately at node c and a cathode at the next node, d. The recording oscilloscope is placed between nodes d and N. When anodal polarization was applied at node c an impulse

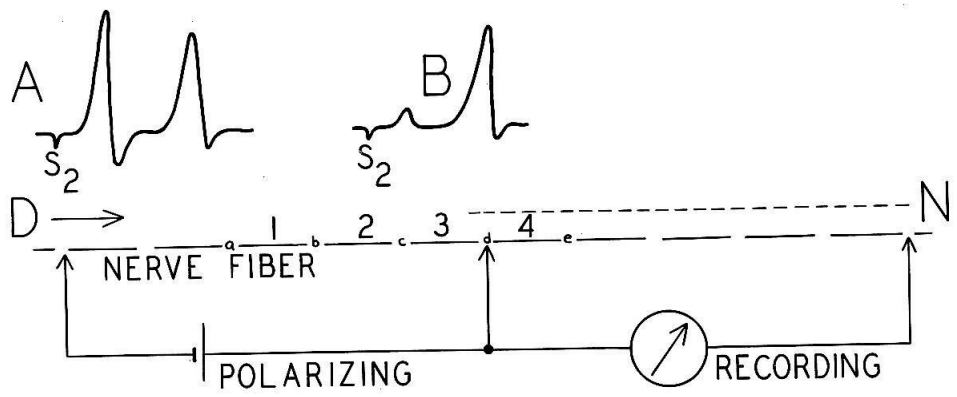

Figure 2. Anodic Polarization of a Ranvier Node. Modified after Blair & Erlanger (24).

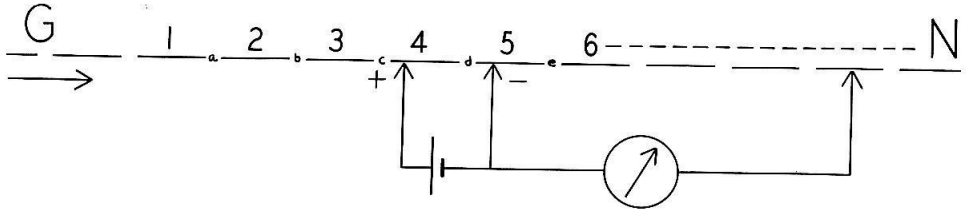

Figure 3. Anodic and Cathodic Polarization of Adjacent Ranvier Nodes. Modified after Blair & Erlanger (24).

passing over the fiber was blocked at this node and apparently reinitiated at the next node. It then passed over the rest of the fiber.

These experiments may be interpreted in the light of data obtained later by Hodgkin (25) on the potential changes occurring at an anodic block in unmyelinated nerve. Hodgkin found that with various types of nerve blocks he could record an exponentially decrementing potential beyond the block even though no impulse could pass through the block. This "extrinsic potential" was found to exist as far as 5.4 mm beyond a block. It was repeatedly found that one impulse may die out at a block but in doing so it lowers the threshold at the block to such an extent that a second impulse arriving at the proper time can pass unimpeded through the block. It is sometimes said that the second impulse "jumps over the block." This is not true because the block has been temporarily removed by the first impulse. This may explain why in Blair and Erlanger's first experiment the second impulse was able to pass over the entire nerve.

The second experiment of Blair and Erlanger also is no argument for saltatory conduction. To prevent an impulse from passing completely through the block the anodically polarized node had to be polarized enough to raise its threshold several times above normal. The cathodically polarized node consequently had its threshold lowered. When the impulse arrived at the anode it was

not completely extinguished. The electrotonic potential extending beyond the block would presumably be enough to bring the cathodically polarized node (which would be hyperexcitable) to threshold and initiate a propagated response.

Strength-Duration Curves and Action Potentials of Single Medullated Nerve Fibers.

Unmyelinated nerves have a uniform threshold of excitation over their entire length. According to Kato (26) the situation is quite different for myelinated nerve. By placing an anode on the central nerve trunk and a cathode at a node of Ranvier he determined the strength of current needed to excite as a function of the time for which the current is applied. Curve A (fig. 4) was obtained with the cathode at a node, curve B with the cathode half-way along an internode. When a shock of 1 msec. duration was delivered at a node 10 volts were required to bring the fiber to threshold, but when a shock of the same duration was delivered halfway along an internode roughly 70 volts were required. (It should be noted that such high potentials were required because high resistance capillary electrodes were used for stimulating).

Most investigators agree (26-29) that the action potential measured at a node with externally applied electrodes is greater than that measured at an internode. Many have interpreted this as meaning that potentials are set up only at the nodes, hence saltatory conduction exists.

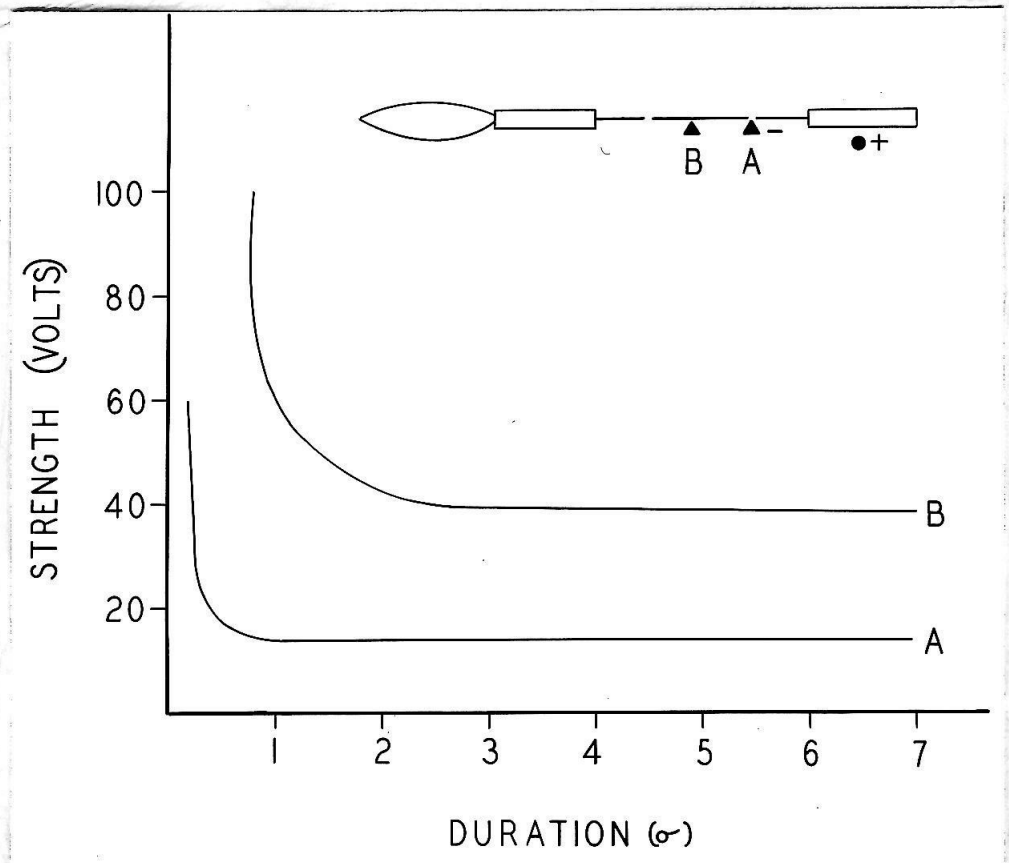

Figure 4. Strength-Duration Curves for a Single Medullated Nerve Fiber. After Kato (26).

On the other hand, these measurements of the action potential with external electrodes may be interpreted as meaning that the transverse resistance at a node is less than that of an internode, hence it is possible to measure a greater potential at a node. This is to be expected on the basis of Kato's experiments.

Recently, Laporte (30,31) has challenged the idea that the action potential measured at a node with external electrodes is greater than that measured at an internode. He recorded potentials from the lateral line nerve of the carp in which only one fiber was conducting. He found a continuous action potential with no manifestation of segmentation. It is difficult to reconcile his results with those obtained on dissecting single fibers, but one might possibly attribute his results to the shunting of current by the tissue surrounding the fiber.

Tasaki's Narcosis Experiments

It is very difficult to assess accurately the significance of Tasaki's many experiments (32-35). Some of the results are completely contradictory, and many of the experiments are so complicated that it is difficult to understand their meaning.

In 1936 Kato and Tasaki (36) reported that when a single Ranvier node was exposed to a narcotic solution, conduction was immediately blocked. The narcotics used were urethane in Ringer's solution (1.0% to 4.0%) and cocaine in Ringer's

solution (0.005% to 0.1%). Three years later, Tasaki (32) reported that narcosis of a single node does not stop conduction. He found that only very concentrated solutions of narcotics (over 5% cocaine and over 10% urethane) would stop conduction at a single node, but this conduction block was usually irreversible. However, when two nodes were exposed to a dilute narcotic solution conduction was usually reversibly blocked.

Obviously these experiments are completely contradictory. If narcosis does block conduction at one node this does not mean that saltatory conduction does not occur. It may be that saltation takes place but that the safety factor in frog nerve is too small for an impulse to pass over an inexcitable node. If narcosis of one node does not block conduction then this would seem to indicate a discontinuous system.

The Air Gap Experiments

These experiments stem from an observation by Kato and Tasaki (36) that part of an internode may be suspended in the air across two small pieces of glass capillary without the fiber being damaged. The myelin sheath protects the axon against drying, but if a node is suspended in the air gap then the nerve dries and dies immediately. Kato is quite explicit in stating that a nerve with a desiccated internode can conduct for hours.

In 1949 Huxley & Stampfli (27) claimed that if an

internode was desiccated over an air gap and the lateral pools were not connected, then conduction ceased. If, however, the pools were connected by a thread moistened in Ringer's solution conduction was restored. They interpreted this as evidence for saltatory conduction, the implication being that the external resistance surrounding the nerve was raised to such a value that current could not flow between the nodes of Ranvier. Unfortunately, they made no quantitative measurements on the resistance between the experimental nodes. It should be pointed out that the results of this experiment, of correct, constitute conclusive evidence for saltatory conduction.

More recently, Frankenhaeuser and Schneider (37) have published some quantitative data on air gap experiments and, like Huxley and Stampfli, they came to the conclusion that saltatory conduction exists. They assume that a reasonable approximation of the longitudinal resistance of the axoplasm of an internode can be obtained by assuming the axoplasm to be a cylinder of Ringer's solution. Using Hodgkin's value (27) of 90 ohms/cubic centimeter as the specific resistance of Ringer's solution they calculate, from the following equation, a longitudinal internal resistance of 17 megohms for a 10μ fiber with a 1.5 mm internodal length.

$$R = \frac{l}{A} = (90)\frac{(0.15)}{(3.1)(25 \times 10^{-8})} = 17 \text{ megohms}$$

l = internodal length (cm)
A = cross section of axoplasm (cm^2)
σ = specific resistance of Ringer's solution

Actually the resistance is higher than 17 megohms because they made a false assumption in calculating the axis cylinder diameter. In their experiments they used fibers between 10μ and 12μ diameter. About thirty percent of the diameter of frog nerve is myelin (1), so that a 12μ fiber would have an axis cylinder of 8.4μ, and a 10μ fiber would have an axis cylinder of 7.0μ. Assuming an internodal length of 1.5 mm, the 12μ fiber would have an internodal axoplasmic resistance of 24.7 megohms, and a 10μ fiber would have a resistance of 35.7 megohms.

They placed a 10 or 12μ fiber over an air gap and recorded action potentials from the nodes on each side of the gap. They "estimate" the resistance of their gap to be somewhere between 10 to 30 megohms. This 10 to 30 megohms of the air gap was in parallel with a 15 megohm resistance of the grid leak of the oscilloscope. The effective external resistance in their experiment was 10 megohms, if we assume the external resistance of their air gap to be 30 megohms. They found that this 10 megohm resistance blocked conduction in about two or three minutes after the fibers had been put over the air gap.

A clue to what happened in their experiments is contained in their admission that their nerves died within five to ten minutes after they put them in the air gap. Furthermore, they had to wait a few minutes after they put the fibers in the gap before conduction stopped. This was to allow the fluid to evaporate from the surface of the gap. As previously

mentioned, Kato and Tasaki have conclusively demonstrated that internodes of uninjured fibers can be desiccated for hours without any resulting injury. One can only conclude that Frankenhaeuser and Schneider performed their experiments on damaged fibers that were about to die.

Cold Block Experiments

Various attempts have been made to get a reversible conduction block by cooling the internodal region of a single nerve without cooling the adjacent nodes. The results from different investigators are completely conflicting. Those that favor saltatory conduction (38) maintain that a conduction block only supervenes when the nodes have been cooled, others (39) claim that conduction can be reversibly blocked without cooling the adjacent nodes.

Pressure Block Experiments

Schneider (39) has shown that it is possible to block conduction reversibly by applying pressure to an internode. He felt that this was evidence against saltation. In later experiments (37) he found that pressure applied to an internode blocked conduction at the node next to the block. He concludes that since a pressure block is not confined to an internode his previous results do not constitute evidence against saltation.

PART II. METHODS AND RESULTS

A. A Method for Dissecting a Single Motor Fiber

Tasaki (40) and Stampfli (41) have briefly described their methods for preparing a single motor fiber - muscle preparation. In their preparations they used the gastrocnemius muscle, but it was found in the present investigation that it is better to use the sartorius muscle. The gastrocnemius is a bulky muscle and it is difficult to see the contraction of a single motor unit when it is not located on the surface of the muscle. The motor fibers innervating the gastrocnemius are about the same diameter as many of the sensory fibers and, at best, one has about a 50-50 chance of selecting a motor fiber. In the sartorius nerve there are about a dozen fibers that are 20μ or larger. Because they are so much larger than the rest of the fibers they are easy to recognize, and it was found that they are almost invariably motor. A sartorius nerve-muscle preparation is more difficult to prepare than a gastrocnemius preparation, and a single fiber is more difficult to dissect from the sartorius nerve because of the greater amount of connective tissue. But the greatly increased chances of dissecting a motor fiber, and the ease with which a single motor unit can be recognized more than compensate for these disadvantages.

The dissecting instruments are made from ordinary sewing needles and glass capillary tubing. A piece of

capillary 6 inches long is used as a handle and the blunt end of the needle is inserted a short distance into the capillary. This end of the capillary is then heated and crushed with pliers so as to hold the needle firmly. The tip of the needle is ground on an oilstone and polished with jeweler's rouge. The needle tip should come sharply to a point because a long tapering point bends or breaks. It is best to have some coarse-tipped needles for removing the sheaths and some fine-tipped needles for dissecting the nerve fibers.

The best preparations can be obtained from five and one-half inch frogs (measured from snout to anus). A sartorius nerve-muscle preparation is dissected out together with about two centimeters of sciatic nerve proximal and distal to the sartorius branch. The dissection is done in a 25 x 140 mm petri dish whose inner surface has been coated 1 cm deep with paraffin except for a circle 3 cm in diameter in the center of the dish. The nerve is illuminated from beneath this hole in the paraffin. The two ends of the muscle and the distal end of the sciatic are pinned to the paraffin. There must be no slack in the nerve yet great care must be taken not to stretch it.

The nerve is now examined under a dissecting microscope (15x ocular, 1x objective). With blunt dissecting needles all of the blood vessels and debris are cleaned from the nerve. During this phase of the operation there should be enough Ringer's solution in the dish to barely cover the

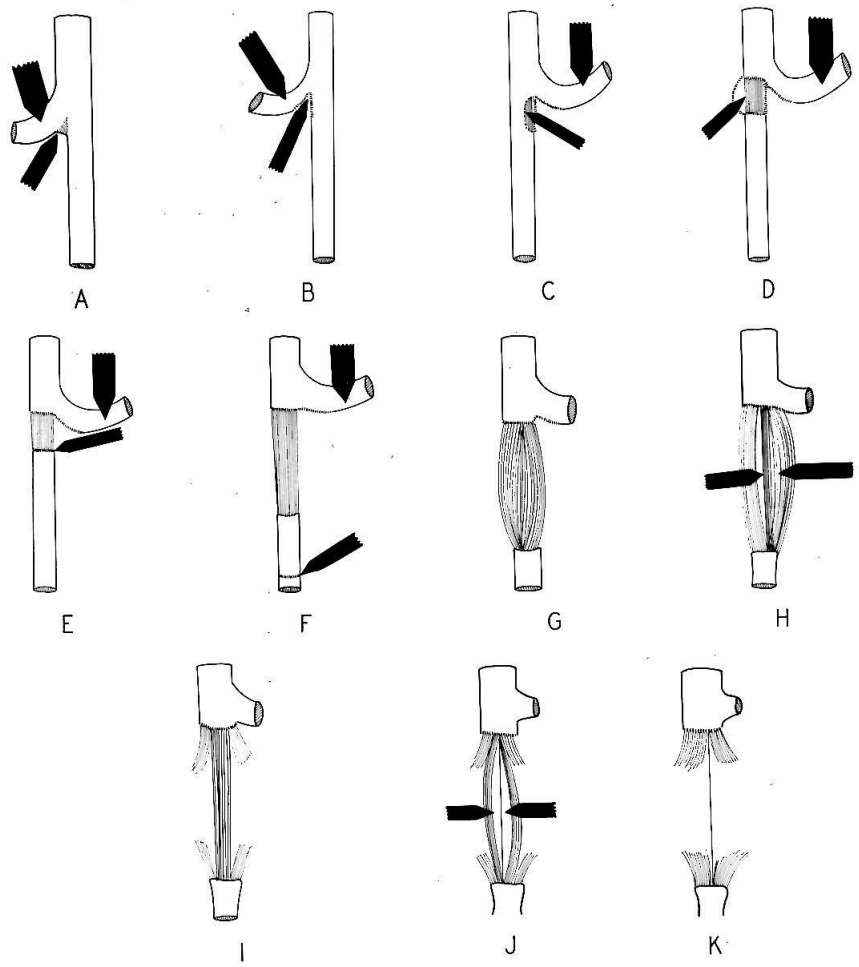

Figure 5. The Dissection of a Single Motor Fiber - Muscle Preparation.

nerve. The dissection is begun at the point where the last nerve branch leaves the sartorius nerve (fig 5a), this is about 2 cm from the sartorius muscle. High power (15x ocular, 6x objective) is now used. The branch is pulled back with a dull-pointed needle and the two connective tissue sheaths (epineurium and perineurium) between the branch and the sartorius nerve are cut through. This cut is extended for about 1 cm (fig 5b). In making the cut great care must be taken not to touch any of the nerve fibers, and yet the thin, transparent perineurium which closely invests the nerve bundle must be cut through. A fine needle is now inserted (fig 5c) between the two sheaths and they are gently pulled to one side and cut through all around the nerve bundle (fig 5d). The cut ends of the sheaths are then gently pulled back over the intact nerve in the direction of the muscle for a distance of about 1.5 cm (fig 5e,f). The branch is then cut off and the nerve is allowed to become slack by removing the pins from the muscle and the sciatic nerve. If the dissection has been properly done, the 1.5 cm of undamaged nerve fibers will flatten out on the surface of the dish when the tension is removed (fig 5g). If the perineurium has not been removed the fibers will remain together in a compact bundle. It is useless to attempt to dissect a single fiber from a bundle that is still ocvered by the perineurium.

Now the dissection of the single fiber begins. In order to flatten out the nerve bundle as much as possible most of the Ringer's solution is removed from the dish. For

this reason the dissection must be done quickly. Two small needles (fig 5h) are laid on each side of the nerve bundle and are drawn apart so that the nerve is separated into three bundles with the center bundle containing about ten fibers. The lateral bundles are cut through so as to leave only the center bundle intact (fig 5i). This center bundle is now carefully examined for a large medullated fiber (about 20μ diameter). The fiber should preferably be in the center of the bundle, not only because it is easier to dissect there but also because the fibers in the center have been less subject to damage. When the fiber has been selected the bundle is again separated and the lateral bundles cut through so that only the single fiber remains (fig 5j, k).

In the experiments described in this thesis the cut fibers were only dissected back for a short distance. In the narcotic experiments about a 3 mm stretch of single fiber with a node in the middle was dissected free. In the air gap experiments about 1.5 to 2.0 mm of internode was dissected free. It was necessary to use such short lengths of fiber because it is very difficult to transport a long length of single fiber from the dissecting dish to the site of the experiment.

B. The Narcosis Experiments

Since Tasaki's narcosis experiments are completely conflicting it seemed desirable to repeat them. These experiments are significant only in that if narcosis of a

single node does not block conduction then it would seem that saltatory conduction exists. If narcosis of a node does not block conduction, then it may be argued that saltation may occur but the safety factor is not great enough for an impulse to pass over one inactive node.

A narcotizing chamber was constructed in a 15 x 90 mm petri dish. A small piece of glass capillary (about 1/6 mm in diameter) was bent in the form of a loop and fused to the inner surface of the dish. The small pool that was formed by the loop and the inner surface of the dish was about 1/6 x 1 x 2 mm. The pool was shown to be watertight by placing a drop of water in it with a micropipette and observing under a microscope that the water did not leak out of the pool. The entire inner surface of the petri dish except for a circle 2.5 cm in diameter around the inner pool was coated with paraffin 0.5 cm deep. It was possible to narcotize a single Ranvier node in the small center pool while the nodes on either side of the narcotized node lay in a common pool of Ringer's solution. This provided a path of low electrical resistance between the latter nodes.

The procedure used for narcotizing a single node is as follows. The narcotic was dissolved in Ringer's solution and a small amount of fastusol* (1 drop of a 5% solution in 25 ml of narcotic solution) was added. A fastusol- Ringer's solution without a narcotic had no effect on a single fiber

* Fastusol is a red aniline dye that is commonly used as an anticoagulant.

that had remained in it for several hours. Because the narcotic solution was dyed red with the fastusol it was possible to determine if any of the narcotic leaked over the edges of the small center pool. In these experiments one can therefore be certain that none of the narcotic reached the surrounding nodes. The contraction of a single motor unit in the sartorius muscle was used as an index of nerve conduction.

In all the experiments urethane concentrations from 7.0% to 1.25% stopped conduction immediately when applied to a single Ranvier node (table 1). The conduction block was fully reversible with these concentrations of urethane because conduction returned immediately when the narcotic was removed and the nerve was washed with Ringer's solution. A saturated solution of phenylurethane (about 0.2%) also stopped conduction immediately and reversibly when applied to a single node.

A 0.04% solution of eserine salicylicate stopped conduction reversibly in about 40 seconds. Lower concentrations of eserine seemed to have no effect. The conduction block produced by eserine was somewhat unusual in that when the nerve was stimulated at about ten shocks per minute it seemed that every second or third impulse was blocked before the complete block supervened. A 0.35% solution of prostigmine bromide had no effect when applied to a node for as long as ten minutes. Nachmansohn (42) claims that even though prostigmine is a powerful anticholinesterase it has

Table 1

Narcotic Solution	Concentration of Narcotic	Results
Urethane-Ringer's	7%	Immediate Conduction Block
"	5%	" " "
"	2.5%	" " "
"	2.0%	" " "
"	1.25%	" " "
"	1.0%	No Effect in One Minute
Phenylurethane-Ringer's	Saturated (About 0.2%)	Immediate Conduction Block
Eserine-Ringer's	0.04%	Conduction Block in 40 Sec.
Eserine-Ringer's	0.02%	No Effect in One Minute
Prostigmine-Ringer's	0.35%	No Effect in Ten Minutes

no effect on peripheral nerve because it cannot penetrate the nerve membrane.

These experiments failed completely to substantiate Tasaki's recent claim that narcosis of a single node does not stop conduction. Tasaki (43) and Curtis (44) have objected to these experiments by claiming that if a long length of single fiber is used the current produced at the last active node will be dissipated in the low resistance path surrounding the nerve and will return through several of the more distal nodes. Under these conditions the current strength in the individual nodes might not be sufficient for stimulation. The objections are not valid because in these experiments only one node of the single fiber was dissected free and the rest of the fiber lay in the nerve bundle. If nerve has a safety factor it should not have been changed by this small amount of dissection. The concept of the safety factor was introduced by Hodgkin in 1937 (25). He definied it as the ratio of the magnitude of the action potential (recorded externally) to the amount of depolarization produced by a just subthreshold stimulus of long duration. For unmyelinated fibers he found safety factors of 3 to 7. Tasaki (35) claims that there is a safety factor of about 5 for saltatory transmission between nodes in medullated fibers. (Tasaki uses the term "safety factor" to mean the ratio of the maximum potential developed at a Ranvier node to the amount of potential, applied for a long duration, that is necessary to stimulate a node). The experiments reported here do not

necessarily mean that saltatory conduction does not take place, but they do indicate that the safety factor in nodal transmission is less than Tasaki claimed.

C. The Air Gap Experiments

In these experiments it was assumed that a good approximation of the longitudinal internodal resistance of the axoplasm can be obtained by assuming that the axoplasm is a cylinder of Ringer's solution. The myelin sheath has a very high electrical resistance (27) compared to the axoplasm. To obtain the diameter of the axon twice the thickness of the myelin is subtracted from the external diameter of the fiber. By assuming that the myelin represents roughly thirty percent of the external diameter of the fiber (1), and that the specific resistance of Ringer's solution is 90 ohms/cc at 20° C (27), the axoplasmic resistance of a 20μ fiber with a 2.5 mm internodal length may be calculated from the equation that Frankenhaeuser and Schneider used. This gives a value of 14.8 megohms. In order to test for saltatory conduction the external resistance must be raised to at least several times this calculated internodal resistance.

It immediately became clear that a high external resistance is not easily attained. If, as was done by Tasaki, two pieces of glass capillary 0.16 mm in diameter are cemented to a glass plate with a gap 0.2 mm between them, the electrical resistance between the two pieces of capillary is of the order of 500 to 1000 megohms. If the pieces of capillary are covered

with Ringer's solution for a few minutes and then the liquid is withdrawn from the center pool (between the pieces of capillary), and the gap thoroughly dried with strips of filter paper, the resistance between the two external pools is only about 0.3 megohm. This low resistance is due to electrolytes from the Ringer's solution which have adhered to the surface of the glass plate. Since in all of the previously reported air gap experiments the nerves were floated across the gaps in Ringer's solution, it is extremely doubtful that in any of these experiments the external resistance was made high enough to actually test the saltatory conduction hypothesis. Furthermore, data will be presented later to show that electrolytes from the Ringer's solution adhere not only to the surface of the gap, but also to the outside of the myelin of the internode which is suspended in the gap. Even if it were possible to make the resistance of the gap extremely large, the electrolytes from the Ringer's solution which adhere to the surface of the myelin would lower the external resistance so much that it would not be possible to test for saltatory conduction.

These difficulties can be overcome in part by floating the nerve across the gap in a solution of a nonelectrolyte such as an isotonic glucose solution. The glucose solution is then withdrawn from the middle pool and the gap thoroughly dried with strips of filter paper. Then the glucose solution in the lateral pools is replaced with Ringer's solution. It was found that if single nerve fibers are

repeatedly stimulated in isotonic glucose they rapidly deteriorate. If they are not stimulated they can safely remain in the glucose for fifteen minutes. In the experiments reported here the fibers were never in glucose for more than two or three minutes and they were not stimulated during this time.

In the first experiments, when the fiber was floated across the capillary ridges and most of the fluid withdrawn from the middle pool the muscle contracted violently. After this contraction the preparation would not respond to stimulation even if it were refloated in Ringer's solution. Because the contraction occurred when the fiber first touched the glass ridges it was believed that it was being damaged by being stretched across the gap. Many months were spent constructing gaps of various shapes and sizes but the nerves always died when placed across them. It was then found, as Tasaki (45) has previously noticed, if the two lateral pools were connected with nonpolarizable zinc-zinc sulfate-agar electrodes that all of the fluid could be safely withdrawn from the middle pool. This is not a solution to the problem because when these electrodes were withdrawn the same violent muscular contraction resulted. But if the experiment is done in a grounded iron cage then there is no muscular contraction when the electrodes are withdrawn.

It seemed, therefore, that in an unshielded room a potential developed between the glass plates, and when the nerve fiber was placed across the gap enough current passed

through the fiber to stimulate it and perhaps even kill it. (Although it must be admitted that the failure of the nerve-muscle preparation to respond to stimulation after the violent tetanus may have been due to fatigue of the neuro-muscular junctions).

In order to determine the potential between the plates in an unshielded room, a twenty-four megohm resistance (R_1) (representing a rough average of the measured internodal resistances) was placed in series with the two megohm impedance of an oscilloscope (R_2) and was connected to a pool of Ringer's on one of the glass plates. The other plate was connected to the oscilloscope. The potential recorded on the oscilloscope was then $\frac{R_2}{R_1 + R_2}$, or 2/26 of the total potential. It was found that potentials of 30 to 100 mV existed between the plates, depending on the electrical appliance put in the immediate vicinity.

Because the resistance of the gap was extremely large practically all of the current that flowed between the plates passed, in the actual experiment, through the fiber. From Ohm's law it may be calculated that this current amounts to about 4×10^{-12} amperes. This is a very small amount of current, but since the structure through which the current passed is also very small a sufficient current density seems to have been produced to cause stimulation.

Two different types of air gaps were used. The first type of air gap experiment (fig 6) was done with a gap 0.2 mm wide between two pieces of glass capillary cemented

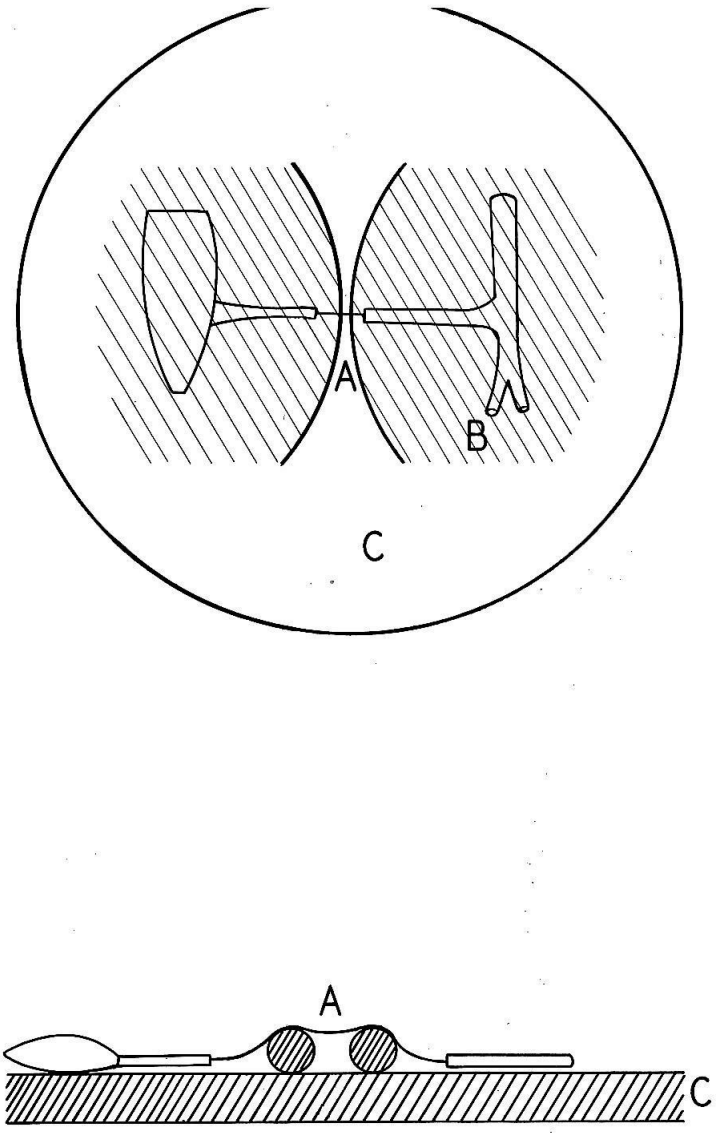

Figure 6. Air Gap Constructed on a Glass Plate.
(A) Air Gap; (B) Pool of Ringer's Solution; (C) Petri Dish

to a glass plate. Table 2 shows the results of six experiments in which the nerve was floated across the gap in glucose solution. All of the nerves conducted for an hour with part of the experimental internode suspended in the air gap. At the end of the hour the experiment was terminated by measuring the resistance of the gap with the fiber in place. Then the desiccated internode was cut through and drawn back from the gap. The resistance of the gap itself was then measured. In all of the experiments the nerves were stimulated by induction shocks applied to the central end of the sciatic and the contraction of the sartorius motor unit was used as an index of nerve conduction. To make sure that the nerve was being stimulated only at the stimulating electrodes the threshold of the fiber was determined before floating it across the gap. There was no appreciable change of threshold in any of the experiments after arrangement of the fiber over the gap. As an added precaution, at the end of the hour the nerve was stimulated by pinching the central end of the sciatic with a forceps. In doing this the central end of the sciatic was touched with the forceps for a moment before the nerve was pinched. When the forceps touched the nerve the muscle usually twitched, indicating that the nerve had been stimulated. This might be due to the fact that the shielding was inadequate and the investigator's body acting as an antenna, picked up enough current to stimulate the nerve. Or the stimulation may have been due to the forceps acting as a galvanic couple. After this twitch the nerve was

TABLE 2

Fiber Diameter ( )	Resistance with Fiber in Gap (M)	Resistance of Gap (M)	Time
20	15	65	1 hr.
20	15	100	"
16	13	70	"
16	20	70	"
18	23.6	130	"
18	22	500	"

TABLE 3

Fiber Diameter ( )	Resistance with Fiber in Gap (M)	Resistance of Gap (M)	Time
18	26	1000	20 min.
19	28	"	30 "
19	30	"	"
18	26	"	"
20	23	"	"
18	27	"	"

TABLE 4

Fiber Diameter ( )	Resistance with Fiber in Gap (M)	Resistance of Gap (M)	Time
18	5	1000	1 hr.
18	4.5	"	30 min.
18	5	"	30 min.

pinched and the motor unit in the muscle always twitched in response to this mechanical stimulation of its motor nerve.

In all of these experiments the nerve conducted even though the resistance of the gap was several times the calculated internal resistance of the fiber. The exact external resistance cannot be determined simply because it is not known how far the calculated internal resistance deviates from the actual internal resistance. Even though these two factors cannot be precisely determined, it can be safely said that the external resistance achieved in these experiments is high, and probably higher than that which other investigators have attained.

Because saltatory conduction has become such an integral part of the concept of nerve conduction, it was decided to improve these experiments. To do this an air gap 0.2 mm wide was constructed between two unconnected glass plates (fig. 7). These two plates were mounted on wooden supports (D) that were embedded in paraffin (E). The resistance of this gap was always greater than 1000 megohms -- the highest resistance that the available electronic ohmmeter could measure. The resistance was high because the supports of the plates were set far apart in paraffin which is a good insulator. Table 3 shows the results of six experiments in which the nerves were floated across the gap in isotonic glucose solution. The nerves were stimulated as previously described. They were left in the gap for only thirty minutes but they could have survived a much longer period if

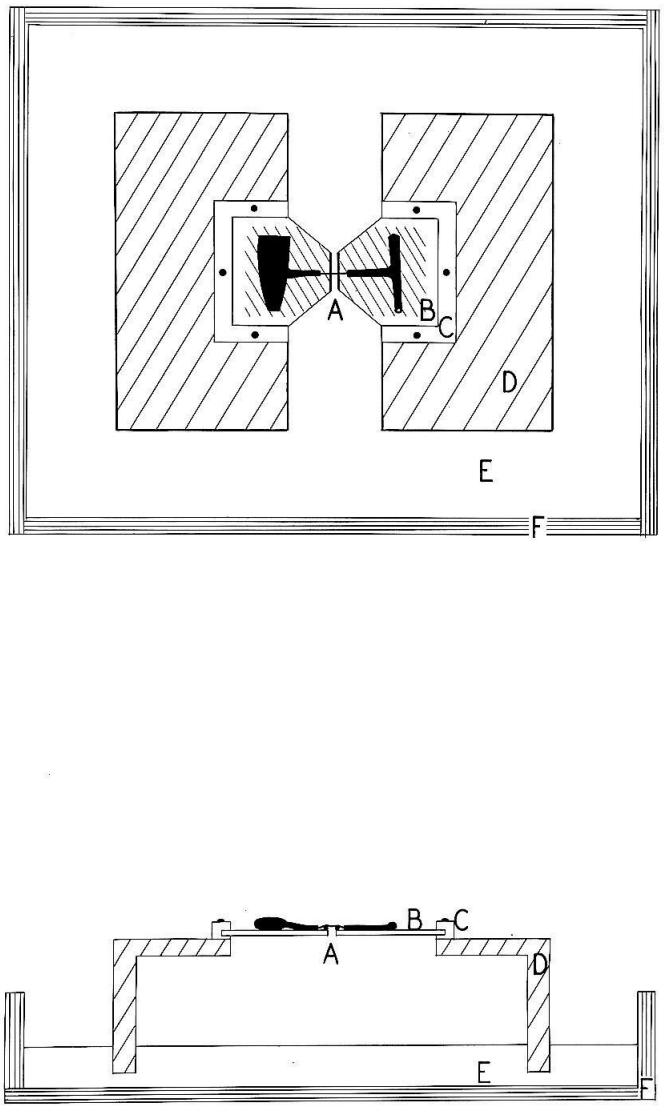

Figure 7. Air Gap Between Two Unconnected Plates.
(A) Air Gap; (B) Glass Plate; (C) Metal Holder for Glass Plate; (D) Wooden Support; (E) Paraffin; (F) Wooden Box.

necessary.

It will be noted in table 3 that the measured internodal resistances are significantly greater than the calculated resistance. One can think of several reasons for this. It was only possible to measure the diameter of the fibers when they were lying in a very shallow pool of fluid. In this condition the fibers may have been somewhat flattened out, and the measured diameters are probably greater than the true diameters. Furthermore, the resistance that was calculated was the longitudinal resistance of the axoplasm, and the measured resistances were this plus the transverse membrane resistances at the nodes. Since the amount of membrane which is exposed at a node is very small, its transverse resistance may be very large.

The resistance of the outside path is formed by the resistance of the gap which is very high (more than 1000 megohms) and coverings of the axoplasm (myelin and neurilemma). The fact that the best resistance measurements are higher than that computed for the axoplasm would tend to indicate that the resistance of the coverings of the axon is very high. Thus the total resistance of the outside path of the circuit postulated as necessary for saltatory conduction is very high when compared to the inside resistance through the internode. Since the gap used was of very high resistance, and the outside of the fiber was freed, as far as possible from electrolytes by treatment with glucose solution, the resistances given in table 3 probably come quite near to the

real internal resistance of the current path postulated in saltatory conduction.

The effect of electrolytes adhering to the nerve fiber is demonstrated in table 4, where the more than 1000 megohm air gap was used, but the fiber was floated over the gap in Ringer's solution. When Ringer's solution is used the outside resistance is low compared to the internal resistance. In all previous investigations Ringer's solution has been used to float the fibers over the gaps. Furthermore, the gaps that were previously used had lower resistances than those used in the present investigation. Both of these factors tend to lower the resistance of the outside current path. It is therefore doubtful that any of the air gap experiments reported in the literature can be seriously regarded as evidence for saltatory conduction. In none of the experiments were precise measurements made of the resistance of the gap and of the resistance of the fiber in the gap, but it seems doubtful that in any of them the external resistance was made high enough to test for saltatory conduction.

On the basis of the air gap experiments reported here it seems justifiable to conclude that medullated nerve can conduct under circumstances where one of the conditions for saltatory conduction is not fulfilled, namely the presence of an outside current path of sufficiently low resistance. In these experiments it has to be assumed that the impulse was transmitted by local circuits beneath the myelin of the desiccated internode. It is conceivable that a medullated

nerve can conduct either by continuous conduction or by saltatory conduction, but it should be pointed out that there is no reasonable evidence to demonstrate this.

CONCLUSION

Many different types of experiments have been advanced as evidence for saltatory conduction. Cold and pressure blocks have been applied to internodes, Ranvier nodes have been narcotized, and internodes have been suspended in air to increase the external resistance. After having critically reviewed these experiments it was concluded that the cold block experiments are of doubtful value because it has not been demonstrated that cooling an internode does not change the temperature of the adjacent nodes. On the other hand, pressure blocks may raise the internal resistance of an internode. Tasaki has made the claim that narcotizing a single node does not stop conduction. This has been a strong piece of evidence in favor of saltation. When his experiments were carefully repeated it was found that narcosis of a single node blocked conduction immediately and reversibly. According to the saltatory conduction hypothesis current flows between nodes in the medium external to the fiber. If this is true then it should be possible to block conduction if the resistance external to an internode is made high enough. Several attempts have been made to do this and all of the investigators concluded that conduction is blocked when the external resistance is made high enough. However, none of these

experiments yielded quantitative measurements as to the external resistance necessary to block conduction. When these experiments were repeated and improved upon, it was found that due to technical difficulties none of the experiments were able to achieve an external resistance high enough to test for saltatory conduction. In the experiments reported in this thesis it was possible to achieve external resistances which probably were large with respect to the internal resistance without blocking conduction. On the basis of these results it has been concluded that medullated nerve can conduct in a situation where only continuous conduction is possible. It may be that medullated nerve can conduct by saltation but it is felt that no one has yet demonstrated this.

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