New Measurements of the Capacity and the Resistance of the Myelin Sheath and the Nodal Membrane of the Isolated Frog Nerve Fiber

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IT HAS BEEN SHOWN that the myelin sheath and the surface membrane at the node of Ranvier of the myelinated nerve fiber behave, to a weak penetrating current, like a condenser with an ohmic resistance connected in parallel (1–5). The absolute values of these capacities and resistances have been estimated already with some accuracy. In order to analyze the process of action potential production at the nodal membrane, it is desirable to obtain more accurate measurements on the physical constants of the membranes at rest.

In the present investigation, the physical constants of the myelin sheath were measured by recording the current that passes through this membrane when two nerve impulses, each coming from an end of a single fiber preparation, collide at the internode under investigation. This collision of impulses (6) makes the spatial distribution of the potential inside the myelin sheath uniform and consequently increases the accuracy of the measurement. Measurements of the capacity and the resistance of the resting nodal membrane were made, using the collision technique, on a node treated with a sodium-free Ringer or with a diluted cocaine-Ringer solution. The effects of several chemicals and of temperature changes upon the physical properties of the nerve membrane were investigated by the same technique.

METHODS

Material and Instruments. Large myelinated nerve fibers (12–15 μ) innervating the sartorius or the semitendinosus muscle of the bull frog were used. The dissection (5, p. 141) was done at a point on the nerve approximately halfway between the sartorius muscle and the proximal end of the sciatic nerve. The connective tissue sheath of the nerve was removed for a length of about 2 mm, and a fiber without a node of Ranvier exposed in the operated region was selected for the myelin sheath experiments. For the measurements on the nodal membrane, a fiber with a node in the middle of the operated region was chosen.

The preparation was stretched across three pools of Ringer solution separated by two bridge-insulators (fig. 1). The width of the middle pool in most experiments was 1 mm, and the two lateral pools were larger (approximately 20 mm square). The insulating air-gaps were 0.1–0.15 mm wide. The lateral pools were grounded by means of Ag-AgCl (agar) electrodes. An electrode of the same type in the middle pool was connected to an amplifier.

Stimuli were brief rectangular pulses from two Grass stimulators (model S4A with stimulus isolation units) applied to the nerve trunk near its cut ends through pairs of platinum electrodes. The amplifier used was a Tektronix preamplifier (Type 122), giving a voltage amplification of almost exactly 1000. This preamplifier has an input capacity of 50–100 $\mu\mu$f. When a resistance of 0.3–0.5 megohms (r in fig. 1) is connected between the input and the ground, therefore, the time constant of the recording system is not small enough compared with the internodal conduction time (approximately 100 μsec.). In most of the present investigation, a cathode-follower with an input capacity of approximately 5 $\mu\mu$f (RCA 1620 in triode connection operated at a low plate voltage) was inserted between the electrode in the middle pool and the Tektronix preamplifier, as shown in figure 1.

The output of the preamplifier was led directly to channel A of a dual-beam oscilloscope (Du Mont type 322) and simultaneously, through a simple network of a condenser and resistor (k and w in fig. 1), to channel B. Channel A recorded the time course of the current flowing through the nerve membrane and channel B recorded the total amount of electricity that passed through the membrane. The resistance w in most of the experiments was 50 kilohms. The capacity k was 0.4 or 0.8 μf for the myelin experiments and 0.8 or 1.1 μf for the node experiments.

Under the conditions of our experiments the resistance r in the arrangement of figure 1 is far smaller than the resistance of the nerve fiber and the product of the resistance w and the capacity k is far longer than the duration of the action potential of the nerve fiber. The current $I(t)$ passing through the membrane at time t and the quantity of electricity $Q(t)$ carried through the membrane up until time t are therefore given by

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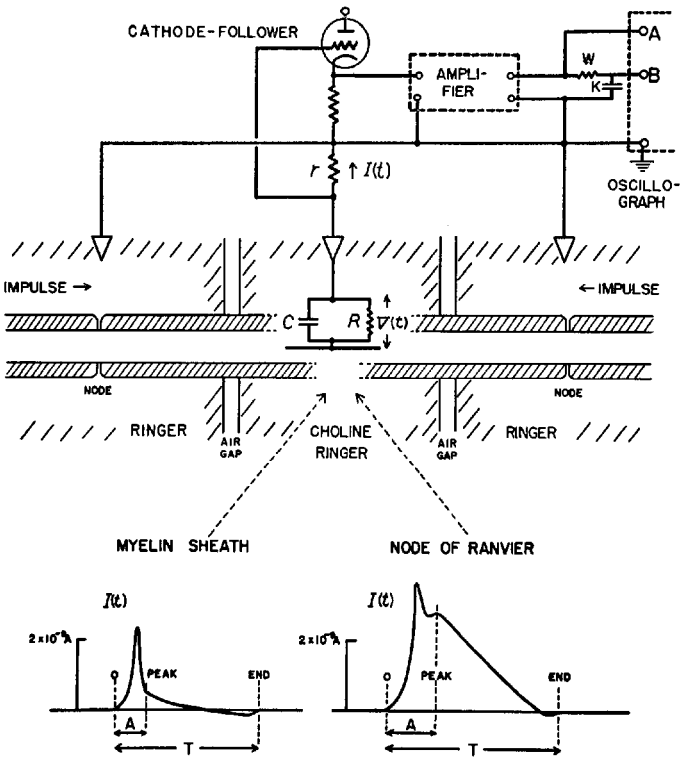

FIG. 1. *Top:* experimental arrangement used for measuring the capacity and the resistance of the nodal membrane and of the myelin sheath; the nerve fiber is sketched disproportionately thick and short. *Bottom right:* time course of the membrane current induced by two colliding impulses when a node of Ranvier was introduced into the middle pool of the arrangement shown above (node ✻4 in table 2). *Bottom left:* membrane current induced by two colliding impulses when there was no node of Ranvier in the middle pool (myelin sheath ✻14 in table 1). The symbols 'o', 'peak' and 'end' in the diagram refer to the start, the time of maximum and the end, respectively, of the potential of the axis-cylinder. The total duration T was approximately 0.9 msec. in both cases. Further detail in text.

$$I(t) = \frac{1}{\mu r} V_a(t), \quad \text{and}$$

$$Q(t) = \frac{kw}{\mu r} V_b(t),$$

respectively, where μ is the over-all amplification by the cathode-follower and the preamplifier and $V_a(t)$ and $V_b(t)$ are the voltages observed on channel A and B at time t, respectively.

Principle of Measuring the Capacity and the Resistance of the Nerve Fiber. The principle of measuring the capacity and the resistance of the myelin sheath by the collision technique is as follows: when the two nodes of Ranvier in the lateral pools in figure 1 are brought into an active state by nerve impulses arriving simultaneously at these nodes, the potential of the axoplasm in the middle pool referred to the surrounding fluid medium goes up and then down with a relatively simple monophasic time course. Let this time course of potential variation be denoted by $V(t)$. The current, $I(t)$, which flows through the myelin sheath during the period of potential variation will be given by the sum of its capacitative and ohmic components, namely, by

$$I(t) = C\frac{dV(t)}{dt} + \frac{1}{R} V(t), \qquad (1a)$$

where C and R are the capacity and the resistance of the myelin sheath immersed in the middle pool of figure 1. The problem is to separate the observed current $I(t)$ into its two components by taking advantage of the fact that $V(t)$ is a simple triangular voltage pulse with a maximum value of approximately 100 mv (7).

The solution of this differential equation for the present problem is

$$V(t) = \frac{1}{C}\int_0^t e^{-(t-\lambda)/RC} I(\lambda)\, d\lambda, \qquad (1b)$$

where λ is a parameter. This equation can be used for checking the result of our determination of R and C. Knowing $I(t)$, R and C, the numerical calculation of this integral should give the actual size and the shape of the single fiber's action potential.

Records were taken of the quantity of electricity, $Q(t)$, defined by

$$Q(t) \equiv \int_0^t I(t)\, dt, \qquad (2)$$

where the limit zero signifies the time immediately before the start of an action potential at which $V(t)$ is still zero. Integrating each member of *equation 1a* with respect to time, we have

$$Q(t) = CV(t) + \frac{1}{R}\int_0^t V(t)\, dt \qquad (1c)$$

It follows from this that the total quantity of electricity, Q(end), which crosses the myelin sheath during one whole period of activity is given by

$$Q(\text{end}) = \frac{1}{R}\int_0^{\text{end}} V(t)\,dt. \qquad (3a)$$

The capacitative term in *equation 1c* drops out from *equation 3a*, because the potential of the axis-cylinder after the end of a spike is equal to that before the start. Since the action potential of a nerve fiber is approximately triangular (7), the integral in *equation 3a* is approximately equal to a half of the product of the peak voltage, V(peak), and the spike-duration, T. Therefore, an approximate relation is:

$$Q(\text{end}) = \frac{T}{2R}V(\text{peak}). \qquad (3b)$$

We can determine Q(end) from the records; and, knowing the peak voltage V(peak) of the action potential and the spike-duration T, this equation (*3b*) serves for determination of the membrane resistance R.

The quantity of electricity at the moment when the potential inside the axis-cylinder reaches the maximum, Q(peak), is given by:

$$Q(\text{peak}) = CV(\text{peak}) + \frac{1}{R}\int_0^{\text{peak}} V(t)\,dt. \qquad (4a)$$

For the myelin sheath, of which the resistance R is very large, the last term in this equation is far smaller than the middle, capacitative term. We can adopt without reducing the accuracy of the measurement the approximation:

$$\int_0^{\text{peak}} V(t)\,dt = \frac{A}{2}V(\text{peak}), \qquad (5)$$

A being the time-interval from the start to the peak of the action potential. *Equation 4a* combined with *5* was used for the determination of the capacity of the myelin sheath.

The method of evaluating the capacity and the resistance of a non-responding nodal membrane is similar to that applied to the internode. When two nerve impulses collide at a node of Ranvier treated with a dilute cocaine-Ringer solution or with a sodium-free Ringer solution, the situation is similar to the collision of impulses at an internode. All the equations from *1* to *4* for evaluation of R and C of the internode can be used. The peak value of the potential V(peak) inside a non-responding nodal membrane has not been directly measured; it should be smaller than 100 mv for the internode because of a stronger leakage at the node.

It is known that along a non-res onding stretch of a nerve fiber the spread of potential is governed by a simple exponential law:

$$V_n = V_0 e^{-n\alpha},$$

where V_n is the potential drop across the nth node and the factor $e^{-\alpha}$ represents the attenuation per internode. For the frog motor nerve fiber, this factor is approximately 0.4 (5, p. 31 and p. 39). Then, from the consideration of symmetry, it can be shown that the potential drop across a non-responding node between the two normally responding nodes is equal to $(\cosh \alpha)^{-1}$ times the normal action potential. Using the above mentioned value of the attenuation factor, we find that the peak value of the potential inside a non-responding nodal membrane is 70% of the normal action potential. {Note that $\cosh \alpha = \frac{1}{2}(e^{\alpha} + e^{-\alpha} = \frac{1}{2}(0.4 + 2.5) = 1.45 = 1/0.7$}. In the following assessment we assume the peak voltage V(peak) for the non-responding node of Ranvier to be 70 mv. The maximum error arising from this assumption does not exceed 15% because previous measurements indicate that the attenuation factor is between 0.3 and 0.5.

At a non-responding node of Ranvier, the current caused by collision of two nerve impulses shows a time course illustrated by the lower right diagram in figure 1. It has an initial peak, which undoubtedly represents a capacitative flow of current, followed by a plateau or frequently by a small hump. The peak of this small hump represents the time at which the potential inside the node has reached its maximum, since the potential rises and falls along a simple monophasic curve with a single maximum. The capacitative flow of current being zero at the maximum of potential, it follows from *equation 1a* that the resistance R of the nodal membrane can be determined simply by the relation:

$$R = \frac{V(\text{peak})}{I(\text{peak})}, \qquad (6)$$

where I(peak) is the observed current intensity at the peak of the hump. The value of R determined by (6) agrees very well with that obtained by using equation (*3b*) which can be rewritten as:

$$R = \frac{TV(\text{peak})}{2Q(\text{end})}, \qquad (7)$$

For convenience in later description, the combination of *equations 3b*, *4a* and *5* for determination of the capacity is rewritten as follows:

$$C = \frac{Q(\text{peak}) - (A/T)Q(\text{end})}{V(\text{peak})}, \qquad (8)$$

The last three equations serve for the determination of the resistance R and the capacity C, since the spike-duration T, the peak potential inside the membrane V(peak), the quantity of electricity at the end of a spike Q(end), the quantity observed at the peak of potential Q(peak), and the duration of the ascending phase of the inside potential A are all either known or measurable on each nerve fiber. The practical problems in the procedure of the determinations will be described under RESULTS.

RESULTS

Capacity and Resistance of the Myelin Sheath. When a short stretch of the myelin-covered portion of a nerve fiber is introduced

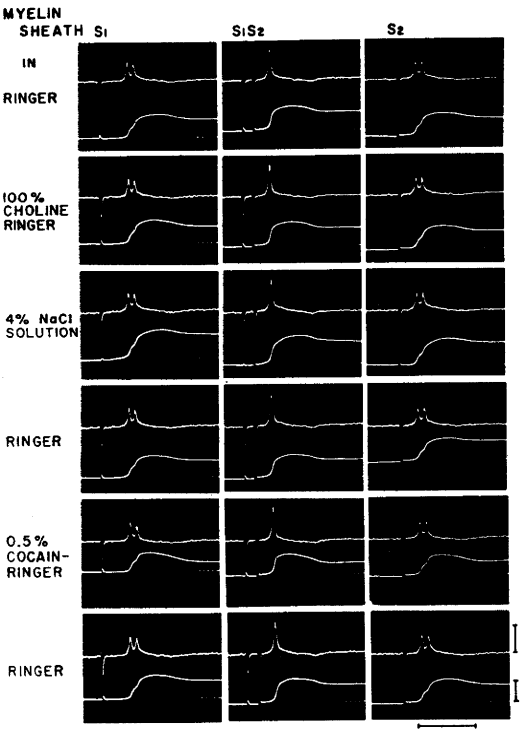

FIG. 2. Action current recorded through the myelin sheath when the nerve fiber was excited at one end or at the two ends. Shock S_1 was applied near the proximal end of the fiber and S_2 was delivered at the distal end. The time interval between the shocks was adjusted to give a collision of the two impulses at the site of recording. The fluid media in which the portion of the myelin sheath under observation was immersed are given. The calibration of the upper beam is 2×10^{-9} amp., that for the lower beam (the integral of the membrane current with respect time) 2×10^{-13} coulomb and the time marker 1 msec. *Dotted lines* indicate the extension of the zero level for the lower beam. Myelin sheath # 13 in table 1.

into the middle pool of Ringer in the experimental setup of figure 1, top, a nerve impulse arriving here from the proximal end of the fiber elicits an action current with two well-defined peaks at an interval of approximately 0.1 msec. (fig. 2, *left*). The first peak is caused by the start of activity at the node of Ranvier on the proximal end of the internode under observation and the second peak by the activity at the distal node (2; 5, p. 67). The potential of the axis-cylinder in the middle of the internode rises one step when the activity starts at the proximal node and another step when the distal node comes into action (8). A nerve impulse starting at the opposite end of the fiber induces, through the myelin sheath, an action current of similar double-peaked configuration (fig. 2, *right*).

Two nerve impulses, one arising at the proximal end and the other at the distal end of the fiber, induce an action current through the myelin sheath which has only one sharp peak if those impulses arrive at the internode simultaneously (6). To be more accurate, this phase of strong outward-directed current is followed by the second phase of weak (first outward- and then inward-directed) current. The end of the second phase corresponds to the end of the spike at the two adjacent nodes (see fig. 1, *left bottom*).

In the present investigations, simultaneous records were taken of the current $I(t)$ flowing through the myelin sheath and the quantity of electricity $Q(t)$ crossing the sheath (see fig. 2). The shock labeled S_1 in the figure was applied to the proximal nerve trunk of the single fiber preparation and S_2 to the distal side of the operated region of the preparation. The delays of these two shocks from the start of the oscillograph beam were adjusted so that an action current with the highest single peak was obtained when the two shocks were applied in succession. To illustrate the effect of the collision of two impulses, the records showing the responses to each of the two shocks alone are also presented in figure 2.

The time at which the potential of the axis-cylinder reached the maximum ('peak' in fig. 1, *bottom left*) was determined by considering that the action potential of a nerve fiber is nearly triangular with a short rising phase and long falling phase; the time 'peak' was taken simply as the beginning of the second, slow falling phase of the current $I(t)$ recorded through the myelin sheath. There is a slight arbitrariness in this procedure, but this causes only a minor error in determining the quantity Q(peak) at that moment since the current intensity $I(t)$ is then very small. As can be seen from the records in the middle column of figure 2, the quantity of electricity at the end of an action potential, Q(end), is approximately equal to Q(peak). In determining the capacity C by *equation 8* described under METHODS, the second term in the numerator containing Q(end) is a correction term of the order of 10%.

(Note that the ascending phase of the action potential A is about 10% of the total duration T.) The resistance R of the myelin sheath was determined by *equation 7*. As mentioned under METHODS, the value of V(peak) was taken to be 0.1 v. in the myelin sheath experiments.

In table 1 are collected the results of the measurements on 14 nerve fibers at room temperature. In the early stage of these measurements neither the collision technique (for reducing the complexity of the inside potential spatially and temporally) nor the cathode-follower stage (for reducing the input capacity of the amplifier) was used. The measurements were made simply on the records as shown in the left column of figure 2. The results obtained by this crude method agree with the results obtained later. The peaks of the action currents recorded in the early experiments are undoubtedly less sharp than those of the records furnished in figure 2. As seen in *equations 7* and *8*, a slight distortion of the records by the input capacity of the amplifier does not affect the results appreciably.

Including the data taken from two afferent fibers, the average values of the capacity and the resistance of the myelin sheath of the bull frog large myelinated fiber (12–15 μ outside diameter) of 1 mm length are 1.6 $\mu\mu$f and 290 megohms, respectively. The maximum rate of voltage rise in the middle of the internode, measured from the maximum current intensity in the collision experiment, was 1.3–1.7 kv/sec.

The distance between the nodes of Ranvier is in general between 2 and 2.5 mm in the large motor nerve fiber of the bull frog (cf. e.g. 5, p. 124). There is reason to believe that the physical properties of the myelin sheath are uniform from one node of Ranvier to the next. The total capacity of the myelin sheath of one whole internodal segment should be between 3 and 4 $\mu\mu$f.

The ohmic resistance of the myelin sheath can be changed by altering the physico-chemical environment of the sheath. The capacity stays unchanged when the ionic environment of the nerve fiber is modified.

Capacity and Resistance of the Nodal Membrane. The electric current that flows through a normal node of Ranvier when a nerve impulse travels along the fiber is triphasic (see fig. 3, *top right*). The first phase consists of a strong outward-directed current of approximately 0.1 msec. duration; the second phase is also short but the direction of the current is inward. In the third phase, a weak, irregular, mainly outward-directed current flows through the nodal membrane for approximately 1 msec. (at 24°C). This triphasic membrane current has been interpreted in terms of successive excitation of the nodes by local currents (cf. 5, p. 58).

TABLE 1. CAPACITY AND RESISTANCE OF MYELIN SHEATH OF 1 MM LENGTH

Exper. No.	Capac., $\mu\mu$f	Resist., megohms	Remarks
1	1.7	220	Single pulse, Tektronix
2	1.7	280	Single pulse, Tektronix
3	1.5	330	Single pulse, Tektronix
4	1.8	350	Collision, Tektronix
5	1.9	250	Collision, Tektronix
6	1.3	300	Collision, Tektronix
7	1.5	330	Collision, cathode-follower
8	1.6	290	Collision, cathode-follower
9	1.2	310	Collision, cathode follower (afferent fiber)
10	1.7	260	Collision, cathode-follower
11	1.9	340	Collision, cathode-follower
12	1.7	260	Collision, cathode-follower
13	1.6	290	Collision, cathode-follower (afferent fiber)
14	1.7	260	Collision, cathode-follower
Av.	1.63	290	
	σ = 0.20	σ = 38	

12–15 μ diameter frog nerve fibers at 24–25°C.

It is unavoidable that membrane action currents recorded by the bridge-insulator technique of figure 1 contain the current flowing through the short myelinated portions of the fiber on both sides of the node under observation. Since the current that flows through the myelin sheath is mainly outward-directed, the observed current intensity in the second phase of the nodal action current is greatly reduced if a long myelinated portion is included in the pool of Ringer in which the node under observation is immersed.

When the colliding impulses reach the node under observation simultaneously, the action current through the node becomes almost purely monophasic, consisting only of a phase of strong outward-directed current (6). The explanation for this short monophasic current is that the node under observation is brought into excitation by a very strong outward-directed current generated by the two adjacent

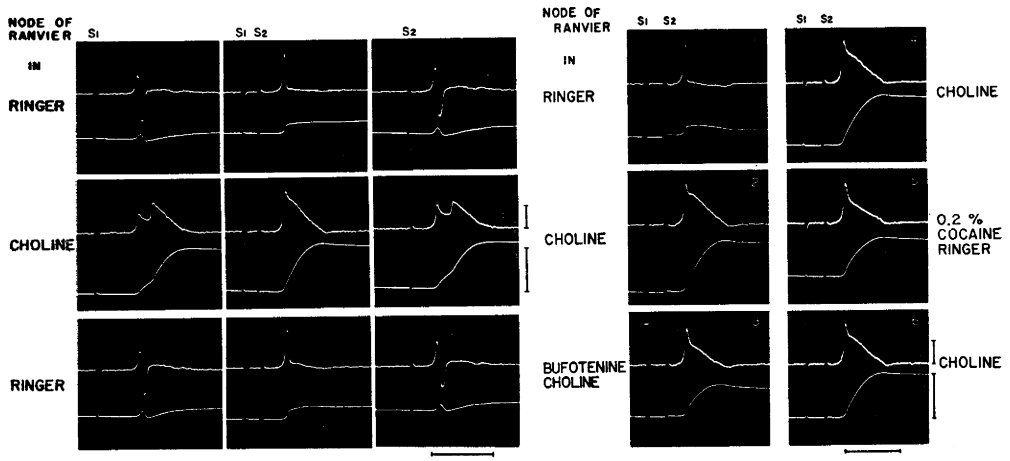

FIG. 3 (*left*). Same as in fig. 2, but there was a node of Ranvier in the region from which the action current were led off. Calibration for the upper beam 2×10^{-9} amp., that for the lower beam 10^{-12} coulomb, and time marker 1 msec. Node # 5 in table 2.

FIG. 4 (*right*). Effect of various chemicals upon the membrane action current recorded from a 1-mm long portion of a nerve fiber with a node of Ranvier in the middle. The action currents were elicited by two impulses colliding at the site of recording. Calibration for the upper beam 2×10^{-9} amp., that for the lower beam 10^{-12} coulomb and time marker 1 msec. Node # 4 in table 2.

nodes. The difference in the time of start of activity at these three successive nodes at the site of collision is small, i.e., the potentials at these nodes rise and fall almost synchronously. This synchronous activity means that there is practically no potential gradient along the axis-cylinder in the active region during the whole period of activity. In a region where there is no potential gradient, there is no membrane current, and the measured membrane current is practically zero except at the very beginning of activity.

A portion of a nerve fiber including a node of Ranvier treated with a sodium-free (choline) Ringer solution or with a dilute cocaine-Ringer solution becomes immediately inexcitable. Since a nerve impulse can jump one or two completely inexcitable nodes of Ranvier (1; 5, p. 44), the action current through this inexcitable node shows two distinct pulses of outward-directed current at the interval required for the impulse to jump across the non-responding node of Ranvier (see fig. 3, 2nd row, *right* and *left*).

The collision of two impulses at the non-responding node of Ranvier produces a membrane current with an initial peak followed by a plateau or small hump (fig. 3, *middle* and fig. 4, *2*). The capacity and the resistance of the nodal membrane were determined by applying *equations 6–8* to the records of the membrane current caused by colliding impulses through a non-responding node of Ranvier.

Comparison of the two tracings at the bottom of figure 1 shows that the major portion of the current flowing through a non-responding nodal membrane is ohmic. *Equation 3b* shows that the quantity of electricity at the end of the spike, Q(end), divided by a half of the spike-duration should be the membrane current at the peak of the inside potential, I(peak). The value of I(peak) determined in this manner was found to agree perfectly with the current intensity observed at the peak of the hump or during the plateau in the $I(t)$ recording. The following example will illustrate this better:

In the second record of figure 4, the lower trace shows that the quantity of electricity at the end of the spike is 1.2×10^{-12} coulomb. Since the upper trace of the same record indicates that the spike-duration of this fiber is approximately 0.9×10^{-3} seconds, *equation 3b* indicates that the maximum ohmic current is $2 \times 1.2 \times 10^{-12} \div (0.9 \times 10^{-3}) = 2.7 \times 10^{-9}$ amp. The current intensity at the plateau of the upper trace is 2.6×10^{-9} amp. and agrees with the calculated value. Dividing our estimate of the peak value of the inside potential, 70 mv, by the current intensity just men-

tioned, the resistance of the nodal membrane is found to be 38 megohms in this preparation.

The capacity of the nodal membrane was determined by *equation 4a*, not by the simplified *equation 8*. Since the start, the point of steepest ascent (at which the current shows the sharp maximum), and the peak of the inside potential were directly discernible on the records, estimation of the time course of the voltage rise was not very difficult. From the estimated time course of $V(t)$, the integral in *equation 4a* was calculated. The quantity Q(peak) in this equation was determined directly from the lower oscillograph beam at the end of the plateau. Introducing these values and V(peak) and R mentioned above, the capacity C of the nodal membrane was evaluated.

Table 2 shows the results of measurement on nine different single fiber preparations at room temperature. In all these fibers the nerve impulse was shown to jump across the node of Ranvier treated with a sodium-free Ringer solution. There were three preparations in which propagation of impulses across the inexcitable node was not constant or was only unidirectional.[1] Date from those fibers with a low safety factor were excluded from the table.

The average value of the capacity of a 1-mm long portion of a large nerve fiber including a node of Ranvier is 3.1 $\mu\mu$f, and the average value of the resistance of this portion is 36 megohms. Subtracting the contribution from the myelin sheath, the best estimate of the capacity of the nodal membrane is 1.5 $\mu\mu$f and that of the resistance 41 megohms.

Effect of Some Chemicals Upon the Physical Properties of the Myelin Sheath and the Nodal Membrane. After the records had been taken for determination of the resistance and the capacity of the myelin sheath in normal Ringer, the fluid in the middle pool was replaced by a solution of some chemical. The replacement was accomplished by sucking out the fluid at one corner of the long, narrow middle pool and pouring in the new fluid at the other corner. A complete replacement of the fluid took 1–2 minutes. An equilibrium between the myelin sheath and its fluid medium was reached immediately after replacement of the fluid with all the chemicals examined (except saponine). Recovery from the effect of a chemical on the myelin sheath after washing with normal Ringer was also prompt.

TABLE 2. CAPACITY AND RESISTANCE OF A NODE OF RANVIER INCLUDING 1 MM LONG MYELIN SHEATH

Exper. No.	Capac., $\mu\mu$f	Resist., megohms	Remarks
1	3.2	33	Collision, Tektronix
2	2.3	48	Collision, Tektronix
3	3.5	32	Collision, Tektronix
4	3.4	38	Collision, cathode follower
5	3.0	33	Collision, cathode follower
6	3.6	32	Collision, cathode follower
7	2.8	35	Collision, cathode follower (afferent fiber)
8	3.1	40	Collision, cathode follower
9	3.0	36	Collision, cathode follower
Av.	3.1	36.3	
	$\sigma = 0.4$	$\sigma = 5.2$	

Large nerve fibers of the frog at 24–25°C.

Examples of records showing the effects upon the myelin sheath of a choline (Na-free) Ringer, a hypertonic sodium chloride solution and a strong cocaine-Ringer solution are presented in figure 2. All these records show that the initial sharp peaks of outward-directed currents are practically unaffected by the chemicals. The final value of the quantity of electricity (the final level of the lower trace in each record), however, is markedly increased by hypertonic (4%) NaCl solution and lowered by 0.5% cocaine-Ringer solution. In other words, these chemicals change the ohmic resistance of the myelin sheath but not the capacity. Sodium-free (choline) Ringer and cocaine-Ringer solution of below 0.1% did not change the physical property of the myelin sheath. The results of these measurements agree with the previous results obtained by the shock test method (3; 5, p. 74).

The results of the present observations on the myelin sheath are summarized in the upper half of table 3. The results of treatment with 'sinomenine' are included in the table. It is an alkaloid[2] which lengthens the falling phase of the action current of a single nerve fiber (10).

The effects of some chemicals upon the physical properties of the nodal membrane were also investigated (fig. 4). The resistance

[1] Wolfgram and van Harreveld (9) reported that they failed to demonstrate propagation of nerve impulses across an inexcitable node of Ranvier. Apparently their preparations had a low safety factor as in these three preparations.

[2] A hydrochloride of a synthetic sample, supplied kindly by Dr. K. Takeda, Shionogi & Co., Amagasaki, Hyogo-ken, Japan.

TABLE 3. CHANGES IN THE RESISTANCE OF THE MYELIN SHEATH AND OF THE NODAL MEMBRANE CAUSED BY VARIOUS CHEMICALS

Myelin sheath (resistance in normal Ringer taken as unity)	
Cocaine Ringer solution, 0.1%	0 ~ +0.1
Cocaine Ringer solution, 0.2%	+0.2 ~ +0.5
Cocaine Ringer solution, 0.5%	+0.7 ~ +1.0
NaCl solution, 3 ~ 4%	−0.2 ~ −0.35
Na-free choline Ringer	No change
Na-free TEA Ringer	0 ~ +0.1
Sinomenine Ringer solution	+0.5
Non-responding nodal membrane (resistance in Na-free choline Ringer taken as unity)	
Bufotenine choline-Ringer solution, 0.05%	+0.4 ~ +0.7
Cocaine Ringer solution, 0.01%	No change
Cocaine Ringer solution, 0.05%	No change
Cocaine Ringer solution, 0.2%	+0.3 ~ +0.5
Cocaine Ringer solution, 0.5%	+0.4 ~ +0.6
Dextrose (isotonic) solution 4 parts plus choline Ringer 1 part	0 ~ +0.1
TEA (Na-free) Ringer	+0.1 ~ +0.2
Urea (isotonic) solution 1 part plus choline Ringer 1 part	0 ~ +0.15

'+' signifies an increase and '−' a decrease in the resistance.

in sodium-free (choline) Ringer was taken as the standard for comparison. A node of Ranvier which was made inexcitable by a dilute solution of cocaine (dissolved in normal Ringer) showed the same resistance as the node in a sodium-free choline-Ringer. As shown in figure 4, the strong cocaine-Ringer solution increased the resistance of the nodal membrane as it raised the resistance of the myelin sheath.

Bufotenine[3] was found to increase the resistance of the resting nodal membrane without changing the action current developed by the node; this is an interesting contrast to the effect of a weak cocaine-Ringer solution which reduces or completely abolishes the action current from a node without changing its resting resistance.

Tetra-ethyl-ammonium (TEA) ions are known to lengthen the duration of the action potential of the whole nerve (12). This chemical, supplied kindly by Doctor R. Lorente de Nó, causes a similar change in the action current of a single nerve fiber at a proper concentration. Single nerve fibers, large or small, immersed in Ringer's solution in which the Na-ions were completely replaced by TEA-ions cannot carry impulses. The nodal membrane kept in 100% TEA Ringer shows a resistance slightly higher than that in 100% choline Ringer.

As shown in the lower half of table 3, a mixture of an isotonic solution of non-electrolytes (dextrose and urea) with choline Ringer did not change the resistance of the nodal membrane appreciably.

Effect of Saponine Upon the Resistance and Capacity of the Myelin Sheath. Saponine and other detergents are known to dissolve the myelin sheath and to increase the leakage of current through the myelin sheath when applied locally (13; 5, p. 78). An interesting question with these chemicals is whether the increase in the leakage of current is due solely to a decrease in the ohmic resistance of the myelin sheath or to an increased flow of currents through both the ohmic and capacitative channels.

Figure 5 shows the results of one of a number of experiments performed to answer this question. The current passing through a 1-mm long portion of the myelin sheath was recorded as in the previous experiments. Then, the fluid in the middle pool, in which only a myelinated portion of the fiber is immersed, was replaced with a 2% saponine-Ringer solution. Within about 5 minutes after application, a marked increase in the flow of current through the myelin sheath was observed. As time elapsed, the current leakage through the myelin sheath became gradually stronger, until propagation of nerve impulses across the internode started to fail. This block of propagation occurred in general between 20 and 40 minutes after administration of the drug. Until this block occurred, there was no sign of depolarization of the membrane of this internode. The action current always had a sharp rising phase and there was no direct current flowing through the myelin sheath. The results are consistent with previous observations (5).

Measurements were made of the quantity of electricity at the peak of the potential inside the membrane, Q(peak), and of the quantity

[3] A crystalline organic base isolated by Stromberg (11) of the National Heart Institute from the leguminous shrub *Piptadenia peregrina* and kindly supplied to the author by Dr. E. Evarts of the National Institutes of Mental Health. The curarizing action of this drug upon the synaptic transmission at the geniculate body is being studied in this laboratory.

at the end of the spike, Q(end). As with the normal myelins sheath, the time at which the initial capacitative flow of current (in the collision records) came to an end was taken as the time of the highest inside potential. This time could be clearly defined on the records until the value of Q(end) became greater than three to four times the normal value (until 15 min. in the example of fig. 5). All the measurements showed that both Q(end) and Q(peak) are increased by the action of saponine. In the early stage of saponification, the ratio of Q(end) to Q(peak) remained approximately constant, indicating that, according to *equations 7* and *8* mentioned under METHODS, the capacity C increased proportionately with the increase in conductance $1/R$. This result is expected if the action of saponine is to dissolve the outer layer of the myelin sheath without changing the properties of the inner layer.

In a later stage of saponification (after 25 min. in the example of fig. 5), the quantity Q(end) was found to increase more rapidly than the quantity Q(peak), indicating that the conductance $1/R$ had increased more rapidly than the capacity C.

Effect of Temperature Changes Upon the Capacity and the Resistance. A single fiber preparation, mounted across a set of bridge-insulators as shown in figure 1, was placed in a lucite box ($14 \times 20 \times 12$ cm^3). After action current records had been taken at room temperature (24–25°C), the single fiber was cooled by filling the space around the preparation with ice. As the air surrounding the preparation was slowly cooled, records were taken of the action currents at every temperature. In this type of temperature regulation, there was an error of about 1° or slightly more in the temperature reading.

An example of the records of the temperature effect is presented in figure 6. The preparation had a node of Ranvier immersed in the middle pool of sodium-free choline-Ringer. The temperature around the preparation was gradually lowered down to 15°C. It can be seen in the figure that as the spike became longer with lower temperature the final value of the quantity, Q(end), gradually became greater. The current at the plateau following the initial capacitative flow of current, I(peak), showed a distinct decrease with the lowering of the

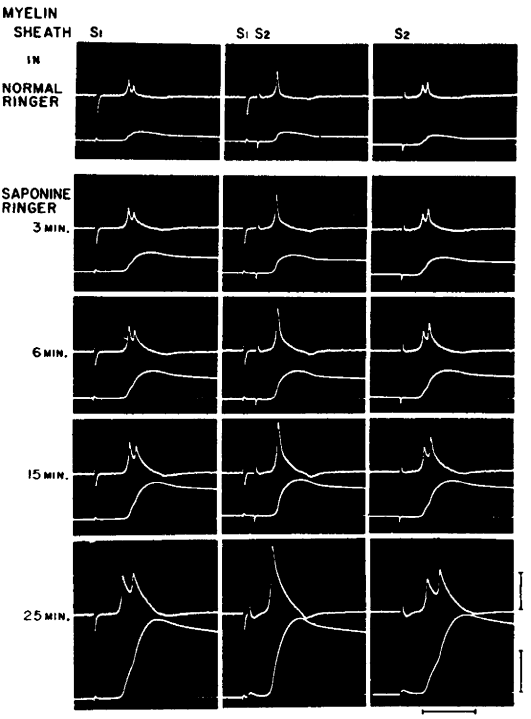

FIG. 5. Effect of saponine upon the membrane action current recorded from a 1-mm long myelinated portion of a nerve fiber. The collision technique was used. Calibration of the upper beam 2×10^{-9} amp., that for the lower beam 10^{-12} coulomb and time marker 1 msec. Myelin sheath # 11 in table 1.

temperature. The change in the membrane resistance and the duration of the action current were reversible.

The ratio of the current I(peak) at 15°C to that at 25°C was found to be between 1.5 and 1.7 (data from three preparations). It has been shown in a recent paper (7) that the maximum value of the action potential was almost independent of temperature. The attenuation factor (see METHODS) also seems to be almost temperature independent. It seems certain that the temperature dependence of membrane resistance is much greater than that of Ringer solution. The capacity of the nodal membrane showed no noticeable temperature dependence in the range from 15–25°C.

The capacity of the myelin sheath was found to be independent of temperature. The resistance of the myelin sheath depended appreciably on temperature, the resistance being higher at lower temperature. Because of tech-

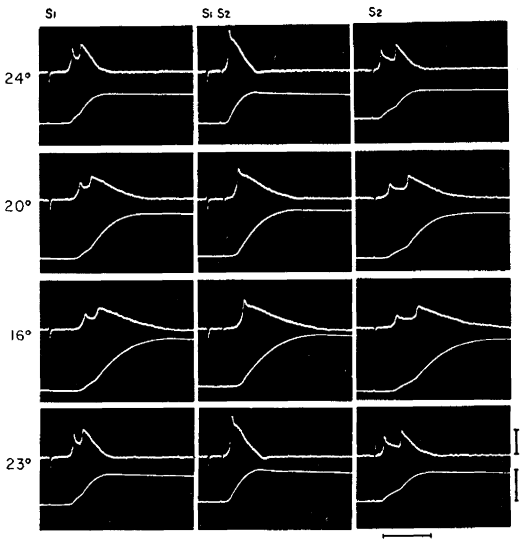

FIG. 6. Effect of temperature changes upon the membrane current recorded through a non-responding node of Ranvier. As in the previous figures, shock S_1 was delivered to the nerve fiber near its proximal end and shock S_2 near its distal end. The node was made inexcitable with a Na-free choline Ringer solution. Calibration for the upper beam is 2×10^{-9} amp. and that for the lower beam 10^{-12} coulomb. Time marker, 1 msec. Node ✱10 in table 2.

nical difficulties involved, no attempt was made to determine the temperature coefficient of the resistance of the myelin sheath.

DISCUSSION

It is of some interest to compare the results of the present measurements with the previous data. From an analysis of the spread of electricity along a cocainized region of a nerve fiber, Hodler, Stämpfli and Tasaki (8) estimated the capacity of the nodal membrane to be approximately 2 $\mu\mu$f. The new figure for the capacity is 1.5 $\mu\mu$f. (S.D. ca. 0.45 $\mu\mu$f.). The previous estimate of the resistance of the nodal membrane is approximately 30 (1), 80 (8) and 50 megohms (5); the present estimate is 41 ± 5 megohms. The capacity of the myelin sheath was estimated previously to be 1.3 (4) and 1.0 $\mu\mu$f/mm. (5); and the new result is 1.6 ± 0.2 $\mu\mu$f/mm. The previous estimate of the resistance of the myelin sheath is approximately 1000 megohm·mm (5), and the new figure is 290 ± 38 megohms·mm. Except for the resistance of the myelin sheath, which is very difficult to measure without using an integrating circuit, the difference between the old and the new data is rather small.

The largest source of error in the present measurements on the myelin sheath is probably produced by difficulty in placing the nerve fiber precisely perpendicularly across the middle pool of Ringer (fig. 1, *top*), but the error arising from this difficulty does not seem to exceed 10%. The large standard deviation in the measurement of the capacity of the nodal membrane is due to the uncertainty in calculation of the integral in *equation 4a*.

A rough estimate of the ratio of the inside (axis-cylinder) diameter to the outside diameter of the frog motor nerve fiber gives a figure between 0.7 and 0.8. The equation for a cylindrical condenser is $C = \frac{1}{2} D/\{\log (r_2/r_1)\}$, where C is the capacity per unit length, D the dielectric constant of the material between the two cylindrical conductors and the denominator is the natural logarithm of the ratio of the diameters of the two cylinders. The capacity of the myelin sheath obtained in the present investigation is 14.5 electrostatic units/cm. This figure and the ratio of the diameters introduced into the equation above yields a dielectric constant for the myelin sheath of 6–10, a very common value for liquid and solid organic compounds. The previous estimate of this constant by Huxley and Stämpfli is 5.6 (4).

Assuming that the exposed part of the axis-cylinder has a cylindrical surface of 0.5–1 μ length and approximately 8 μ diameter, the area of the nodal membrane is between 2 and 5 times 10^{-7} cm^2. It follows from this estimate that the nodal membrane has a capacity of 3–7 μf/cm^2 and a resistance of 8–20 ohm·cm^2. The corresponding figures for the myelin sheath are approximately 0.005 μf/cm^2 and 10^5 ohm·cm^2.[4] Calculated from the equation for a parallel plate condenser, the thickness of the nodal membrane expressed in Ångstrom units is 1.2–3 times the dielectric constant of the substance of the membrane. Assuming that

[4] This enormous difference in the ohmic resistance per unit area between the nodal membrane and the myelin sheath will answer the argument by Rosenblueth, Garcia and Mileđi (14) against the theory of saltatory transmission. It is true, as they stress, that the myelin sheath is not a perfect insulator. But, the density of the ohmic, or ionic, current through the myelin sheath is only of the order of one ten-thousandth of that through the nodal membrane.

the dielectric constant is 6–10, the thickness of the nodal membrane is estimated to be between 8 and 30 Å. There is no direct evidence showing that the dielectric constant of the nodal membrane is the same as that of the myelin, but the similarity between the nodal membrane and the myelin sheath in their behavior to modified chemical environments (table 3) suggests that the materials for these two membranes are chemically similar.

In the squid giant axon Cole and Curtis (15) demonstrated a pronounced reduction of the ohmic resistance of the membrane during activity which is not associated with any measurable change in the membrane capacity. A similar drastic reduction of the membrane resistance during activity has been observed in the nodal membrane (10; 5, p. 55). Various chemicals applied from outside of the myelin sheath and the nodal membrane do not affect the capacity of the membrane, while the ohmic resistance is often modified by more than 50%. In this respect there is a striking similarity between the impedance change during activity and that caused by application of various chemicals to the nodal membrane.

The process of action potential production is undoubtedly associated with chemical changes on the inner side of the plasma membrane. It is natural to assume that these chemical changes are responsible for the reduction of membrane resistance during activity. The constancy of the membrane capacity during activity may be interpreted as indicating that the thickness of the hydrophobic layer of the membrane is not altered by the active process, as is the myelin sheath treated with a hypertonic sodium chloride solution.

The strong temperature dependence of the membrane resistance of the myelin sheath and of the nodal membrane is interesting in relation to the temperature dependence of the velocity of the nerve impulse. In careful experiments, Tamashige (16) demonstrated that the membrane resistance of an isolated single muscle fiber shows a strong temperature dependence, i.e., the resistance of the muscle membrane at 15°C is nearly twice as great as that at 25°C. In addition, Tamashige found that the sarcoplasm resistance shows an equally strong temperature dependence. These findings present a satisfactory interpretation for the lowering of the conduction velocity at low temperatures. Since the membrane capacity is not affected by temperature changes, the wave of potential which spreads along the myelinated portion of the nerve fiber (8) should travel slower at lower tenperatures. This probably is the major, if not exclusive, factor which determines the temperature coefficient of the conduction velocity which is 1.8 for a change of 10 degrees in the temperature of the frog nerve fiber (cf. 5).

Another interesting implication of the data presented in this paper is that the capacitative component of the membrane current at an active node of Ranvier occupies only a negligibly small portion of the total action current. It is known that the peak value of the action current of a single node of Ranvier, recorded longitudinally between the active and the adjacent inactive nodes (15–25°C), amounts to $(2 \sim 3) \times 10^{-9}$ amp. (cf. 5, p. 19). Since there is a current of the same intensity between the active node and the inactive node on the opposite side, the peak membrane action current is $(4 \sim 6) \times 10^{-9}$ amp. This inward-directed current through the active node decays approximately linearly with time. The capacitative current in the falling phase of the action potential, calculated by the formula $C \cdot dV/dt$, C being the capacity of the nodal membrane (1.6 $\mu\mu$f) and dV/dt being the rate of potential decay (100 mv in 1 msec. at 24°C), gives a figure as low as 1.6×10^{10} amp. This figure drops at 15°C to 5×10^{-11} ampere which is only 1% of the total membrane current in the early falling phase of the action potential. This fact is of utmost importance in consideration of the mechanism of action potential production, which will be discussed in subsequent papers.

SUMMARY

The leakage of action current through the myelin sheath was recorded at the site of collision of two nerve impulses. By separating this leakage current into the capacitative and the ohmic components, the capacity and the parallel resistance of the myelin sheath were determined. For large myelinated nerve fibers of the bull frog, the capacity was found to be 1.6 $\mu\mu$f/mm and the resistance to be 290 megohm·mm. The capacity and the resistance of a node of Ranvier treated with a Na-free Ringer were determined by using a similar

collision technique. The capacity of the nodal membrane was found to be 1.5 $\mu\mu$f and its resistance to be approximately 40 megohms. Various chemicals change the resistance of the nodal membrane, just as they do the resistance of the myelin sheath. The capacity of the nodal membrane, just as that of the myelin sheath, is not altered by chemicals. A hypertonic NaCl solution reduces the membrane resistance. A strong cocaine-Ringer solution increases it; but a weak cocaine-Ringer solution, which is still strong enough to abolish the response of the nerve fiber completely, does not change the membrane resistance. Gradual removal of the myelin substance by saponine increases the capacity and decreases the resistance of the myelin sheath. In the early stage of saponine action, the product of the capacity and the resistance remains constant. Lowering of the temperature increases the membrane resistance; this temperature dependence is greater than that of the specific resistance of a Ringer solution.

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