INITIATION AND ABOLITION OF THE ACTION POTENTIAL
OF A SINGLE NODE OF RANVIER

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In recent years the process of initiation of the action potential has been in-
vestigated extensively on the squid axon. Internal recording of action po-
tentials with glass pipette electrodes (Curtis and Cole (4); Hodgkin and Huxley
(7)) and elimination of the cable properties of the axon with a long internal
metal wire electrode (Marmont (11); Cole (2); Hodgkin et al. (9)) are the two
main techniques which have yielded important results. These techniques, how-
ever, are not applicable to vertebrate myelinated nerve fibers, at least in their
original forms, because of the small size of the vertebrate fibers.

Despite their small size, frog nerve fibers have certain advantages over squid
giant axons as materials for analyzing the process of action potential produc-
tion. The plasma membrane at the node of Ranvier, at which the process of
action potential production takes place (14), is a small cylindrical section of
membrane which has a length of about half a micron. This structure is so small
compared to the diameter and the internodal length of the fiber that the po-
tential difference across the nodal membrane can be treated as the membrane
potential at a point. In other words, the use of a single node preparation (Tasaki
and Takeuchi (19)), in which all except one of the nodes of a single nerve fiber
were made inexcitable, automatically eliminates all the complications arising
from the cable properties of the excitable membrane. In the squid axon the
situation is not simple; even in a voltage-clamped axon, a difference in the ex-
citability in different parts of the large axonal membrane with a large number
of severed branches could cause a serious difficulty in the treatment of the re-
sults obtained.

There is another, important advantage of using frog myelinated fibers for
the analysis of the excitatory processes. The time constant of the resting nodal
membrane, which is given by the product of the resistance and the capacity
of the membrane, is between 0.06 and 0.1 msec. under the ordinary experi-
mental conditions. The corresponding value for the squid axon is approxi-
mately 1 msec. (Cole and Hodgkin (3)) and the value for the muscle membrane
of the frog or toad is 20 to 60 msec. (Katz (10); Hagiwara and Watanabe (5)).

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The duration of the action potential is not much different in these three different excitable tissues. This indicates that the time sequence of the excitatory processes can be followed in the frog fiber more accurately than in other tissues.

In the present investigation, the process of initiation of the action potential has been analyzed taking advantage of the structural simplicity of the elementary section of excitable membrane at the node. The results obtained, which are presented in this paper, indicate that there are two stable potential levels at the node and that the process of excitation is a transition from the lower potential level to the higher. The converse process, namely a transition from the higher potential level to the lower, or an abolition of an action potential, can be induced by passing a relatively strong pulse of inward current through an active node. The nature of the recovery process following an action potential has been investigated by the same technique.

Method

Large motor nerve fibers 10 to 14 microns in outside diameter of the toad (*Bufo marinus*) or the bull frog (*Rana catesbiana*) were used. The node of Ranvier to be examined, which is labelled N_1 in all the diagrams in this paper, was situated in the middle of the operated region of the single fiber preparation. The internodal segment on each side of this node was carefully cleaned under a high power dissecting microscope. The node in the middle was always left covered by pieces of damaged nerve fibers which served to locate the node under a low power microscope as well as to protect the node from mechanical injuries.

The nerve fiber was mounted across a set of bridge insulators between three independent pools of Ringer (Fig. 1, top). The node in the middle, N_1, was introduced in the small middle pool of Ringer (1 mm. wide and 10 mm. long), taking all the precautions mentioned in an earlier paper (Tasaki and Frank (16)) against damage to the preparation. One of the two unoperated portions (containing node N_0 in the diagram) of the preparation was kept in the large pool of Ringer separated from the small pool by an air gap of about 0.2 mm. The other portion (containing N_2) was mounted on a small electrode of the Ag–AgCl–Ringer agar type separated from the small pool of N_1 by an air gap of about 0.5 mm. The major portion of the electrode was covered by a metal shield driven by a positive feed-back voltage from a MacNichol-Wagner preamplifier (12). The purpose of this driven shield is to correct for the loss in high frequency response produced by the resistance of the preparation and the capacity of the electrode to ground. The small middle pool was generally grounded by means of two large Ag-AgCl electrodes placed at the two ends of the pool. Stimulating pulses were applied between the small and the large pools, and the action potential evoked was recorded between the ground and the shielded grid electrode.

In the experiment of Fig. 1, the small middle pool was not directly grounded; it was connected to a source of rectangular stimulating pulses (Tektronix pulse generator number 1) with a 500 ohm output resistance. A 0.3 megohm resistor was inserted between ground and the electrode (Ag–AgCl) in the large pool, and the potential drop across this resistor caused by the action current of the fiber was amplified with

a Tektronix preamplifier (number 122). A DuMont 322 dual beam oscilloscope and a Grass camera were used for recording.

Most of the experiments were done in a refrigerated room at 10–12°C.

RESULTS

1. Relationship between the Membrane Action Current and the Action Potential of a Node.—The first observation made with the arrangement of Fig. 1, top, was to compare the action potential and current of a node of Ranvier (N_1) during the course of narcosis of the adjacent node (N_0). The large and small pools were first filled with normal Ringer, and node N_2 was made inexcitable by insulation combined with application of isotonic KCl or cocaine solution (16). Node N_1 in the middle was excited by rectangular voltage pulses of approximately 0.5 msec. duration applied through the electrode in the middle pool.

Under these experimental conditions, the current recorded with amplifier labelled A in the figure is the membrane current that flows through node N_1 since there is, theoretically, no current between N_1 and N_2. The potential variation recorded by means of amplifier V with a high input impedance is the change of the voltage across the membrane of N_1.

The action current observed when the pool containing N_0 was filled with normal Ringer (Fig. 1, record 1) consisted of a short period of inward current at N_1 followed by a longer phase of weak outward current; this is a typical "binodal" action current (14, p. 38) associated with propagation of an impulse from N_1 to N_0. The action potential recorded simultaneously showed a time course similar to those evoked by propagated nerve impulses (16).

When the fluid in the large pool carrying N_0 was replaced with a 0.1 per cent cocaine–Ringer solution, the action current soon became purely monophasic (record 2 in Fig. 1) followed by a gradual prolongation of the phase of inward current (record 3). A certain period of time after the start of narcosis, which varied from 5 to 20 minutes depending on the amount of connective tissue removed, propagation of impulses from N_1 toward N_0 was suspended, resulting in a simple triangular deflection in channel A for action current recording (record 4). During the whole course of narcosis, there was very little change in the time course of the observed action potential. When the propagation of the impulse from N_1 toward N_0 was delayed by narcosis, there was a slight prolongation in the observed action potential (records 2 and 3). When the propagation was completely suspended (record 4), the spike height, the duration, and the shape of the action potential were indistinguishable from those of a normal nerve fiber (record 1).

The finding that the action potential of the node is practically independent of the membrane current through the node is not new. This conclusion was drawn first from an experiment made by the shock test method (13) and later
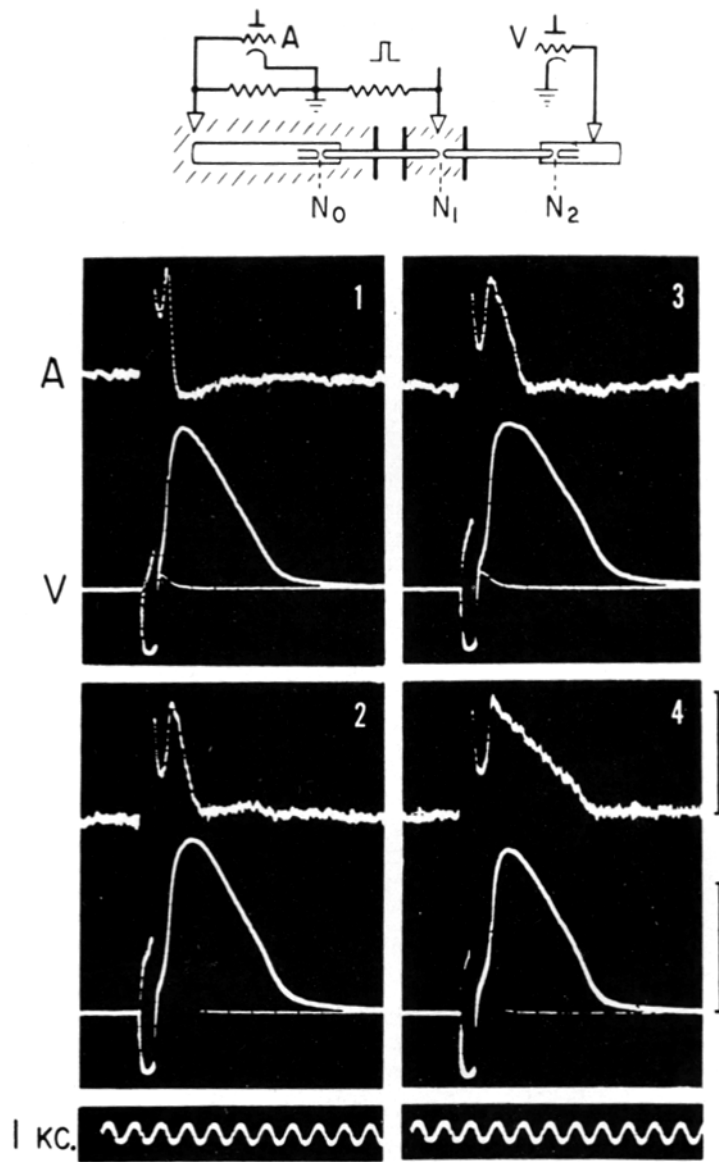
FIG. 1. Membrane action current (A) and action potential (V) of a single node of Ranvier recorded during the course of narcosis of the adjacent node. In record 1, the pool carrying node N_0 was filled with normal Ringer. In record 4 response of N_0 was completely abolished by 0.1 per cent cocaine–Ringer solution. Note that there is practically no difference in the action potential recorded before and after narcosis. In these and other records in this paper, upward deflections in the potential channel (V) represent positivity at the grid electrode. In the current channel (A), upward deflections represent inward currents at N_1. Calibrations: 50 mv. for V and 10^{-9} ampere for A. A bull frog nerve fiber at $6°C$.

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from measurements of the longitudinal action current in the internode (19; 14, p. 53; 15).

Other features of the relationship between the action potential and the membrane current are presented in a later section.

2. *Threshold Membrane Voltage.*—The next observation made with the experimental arrangement of Fig. 2 was on the variation in the potential difference across the nodal membrane caused by subthreshold stimulating pulses. Node N_0 in the large pool was treated with a 0.1 per cent cocaine–Ringer solution and stimulating voltages (S in the figure) were applied between the small and large pools as in the preceding observation. The potential inside the node N_1 in normal Ringer was recorded through the grid electrode carrying the KCl-treated node N_2.

Examples of such observations are reproduced in Figs. 2 and 3. In response to relatively long rectangular voltage pulses of subthreshold intensities (right column in Fig. 2), the potential inside N_1 referred to ground was found to rise approximately exponentially with a time constant in agreement with earlier measurements (15). The myelin sheath 1 mm. long containing one node of Ranvier has a capacity of approximately 3 $\mu\mu$f. and a resistance of the order of 60 megohms under the conditions of the present observation. Since there is a resistance of the same order of magnitude in the axis cylinder between N_0 and N_1, the time constant of voltage rise in the arrangement of Fig. 2 should be of the order of 100 μsec. The voltage rise in this network is not precisely exponential and the frequency response of the amplifier does not permit one to measure it accurately, but the observed time constant is certainly not far from the predicted value.

When the stimulating voltage was increased, it was found that the change in the membrane voltage increased *proportionately* with the applied voltage. The proportionality between the applied and the recorded membrane voltage was, in many "good" preparations, almost perfect up to the threshold (Fig. 2, right bottom). With several preparations which had accommodation time constants (Hill's λ) smaller than 10 to 15 msec.,[1] it was observed that barely subrheobasic voltage pulses generated a disproportional rise in the membrane voltage followed by a rapid "accommodative" fall. The details of the accommodation in potential inside the node will be discussed in a subsequent paper.

With rectangular voltage pulses of relatively short duration (shorter than about 0.3 msec.), the proportionality relation between the applied voltage and the variation in the membrane voltage was seen only up to about 80 per cent of the threshold stimulus strength. The deviation from proportionality manifests itself, at stimulus strengths of about 80 to 90 per cent of the threshold, as a disproportional prolongation in the rate of potential fall after the end of

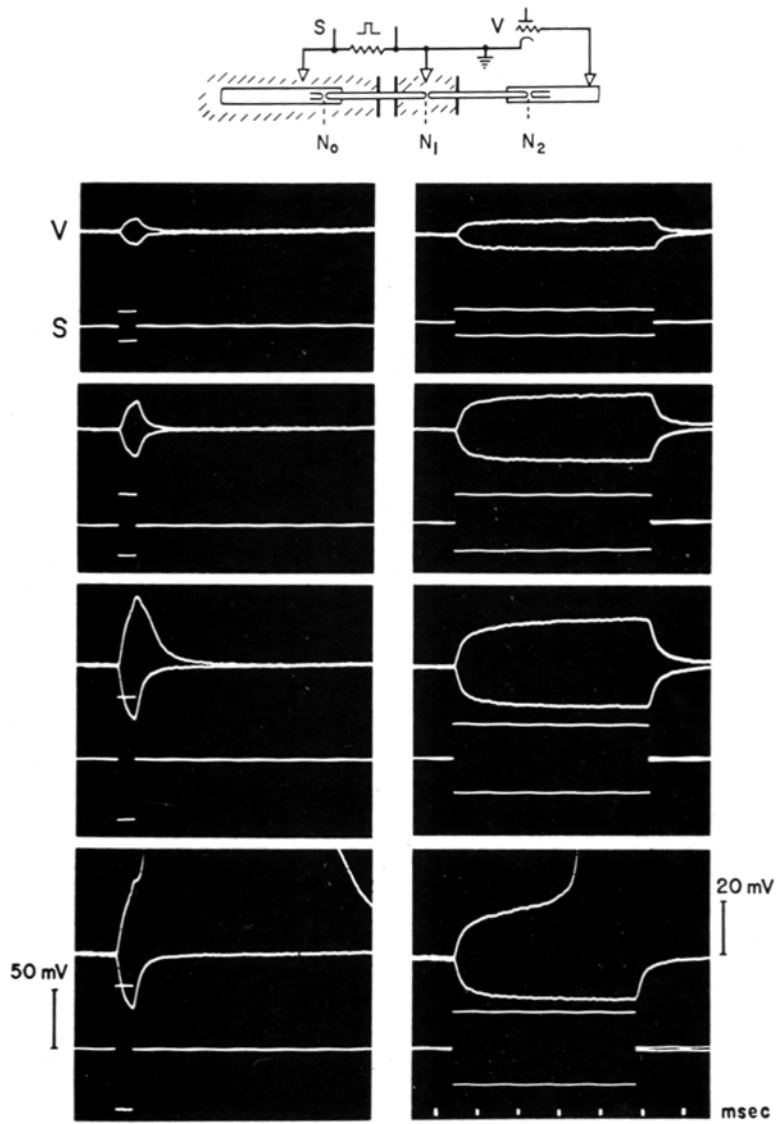[1] For the time constant of accommodation in the isolated nerve fiber, *cf.* Tasaki and Sakaguchi (18).

FIG. 2. Relationship between subthreshold rectangular pulses (S) applied between N_0 and N_1 and the variation in the potential drop (V) across the nodal membrane of N_1. Note the short time constant of potential rise in channel V and the proportionality between intensity of S and voltage V in the right-hand records. A toad nerve fiber at 10°C.

the applied pulse. At barely subthreshold strengths, a pronounced variability in the rate of potential fall was observed. The phase of potential rise was stable and was proportional to the applied voltage.
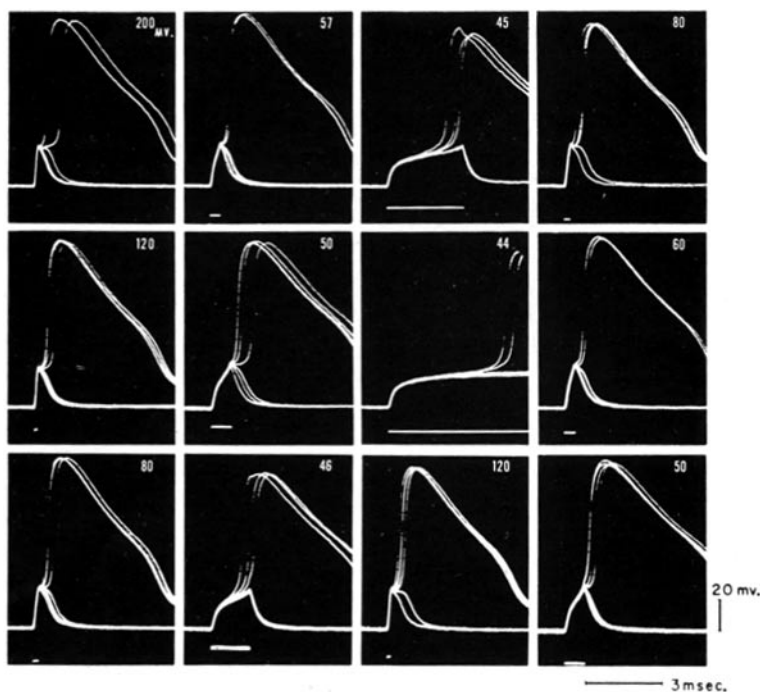

FIG. 3. Threshold membrane potentials at varying stimulus durations. Stimulus intensities (threshold) in millivolts are indicated. The bars in the records indicate the stimulus duration that varied between 0.05 and 6.4 msec. in logarithmic scale. In each record 5 to 6 sweeps were superposed at constant stimulus strength and duration. The same nerve fiber as in Fig. 2. The strength-duration relation for this fiber fits the empirical formula $S = 40(1 + 0.2/t)$, in which S is the threshold voltage in millivolts and t the duration in milliseconds.

In the range between about 0.3 and 3 msec. in stimulus duration, the potential variation caused by a barely subthreshold pulse was slightly greater than what was expected from the proportionality relation. This type of deviation, or non-linearity, was more marked with longer pulse durations in this range; but the maximum deviation did not exceed about 25 per cent under the conditions of the present observations (cf. records for stimulus strengths 45 and 50 mv. in Fig. 3). The potential variation caused by voltage pulses of the reversed polarity (generating inward currents through N_1) was consistently propor-

tional to the strength of the applied pulse so long as the increase in the membrane voltage did not exceed 20 to 30 mv.

Another interesting feature that has become clear from observations of this type is the constancy of the *threshold membrane voltage* in stimulation of the node by rectangular pulses of varying durations. If the threshold membrane voltage is defined as the highest potential level that decays after the end of a stimulating pulse without producing an action potential, it is practically independent of the duration of the stimulating pulse (Fig. 3). Under the conditions of the present observations, the threshold membrane voltage, measured as a rise in the inside potential from its resting level, was between 10 and 20 mv., in general around 15 mv., in a series of about a dozen preparations. A similar constancy of the membrane voltage in threshold excitation has been observed in the squid axon (Cole and Curtis, personal communication).

When the potential inside a node was raised up to this critical level at the end of an applied pulse, there appeared a great variation in the following course of the membrane voltage. As can be seen in all the records presented in Fig. 3, there was a variable delay in the start of the steep rising phase of the action potential; sometimes the action potential failed to appear at the same stimulus intensity. This is the variability in response at threshold stimulation, or threshold play, which has been known since the classical work of Blair and Erlanger (1). When the membrane voltage was raised quickly 5 to 10 per cent above this threshold level, the variability disappeared and the quick rising phase of the action potential started immediately at the peak of the passively raised membrane voltage.

The threshold membrane voltage was practically independent of whether it was reached quickly by a short stimulating pulse (as in the experiment of Fig. 3) or stepwise by a relatively long subthreshold pulse followed by a short pulse (as in the following experiment of Fig. 4), provided that the voltage caused by the long threshold pulse did not suffer an accommodative, or nonlinear, reduction. During the relatively refractory period or in a partially narcotized node, the threshold membrane voltage was larger than in a normal node at rest. Since relative refractoriness and light narcosis do not change the resistance of the resting nodal membrane (14, 15), the rise in the threshold membrane voltage under these conditions is expected to parallel the rise in the threshold value of the applied stimulus. The rise in threshold by refractoriness and by light narcosis has been described earlier (14, p. 82 and 92).

3. Effect of Applied Currents upon the Action Potential of a Node.—It is seen in the records of Fig. 3 that the action potentials which started a long period after the beginning of the applied pulse are smaller in their spike height than those with short latencies. This decrease in the spike height is associated with the slow, disproportional rise in the inside potential that precedes the start of the quick rising phase of the action potential. Under those conditions

for which this slow rise in potential is absent, a sustained subthreshold voltage does not affect the spike height of the action potential evoked later. This is shown by the records furnished in Fig. 4.

The experimental arrangements shown by the upper diagram of this figure are essentially the same as those for the preceding experiments. Two rectangular voltage pulses were applied between the inexcitable node, N_0, and the normal node to be studied, N_1. The technique of applying two stimulating
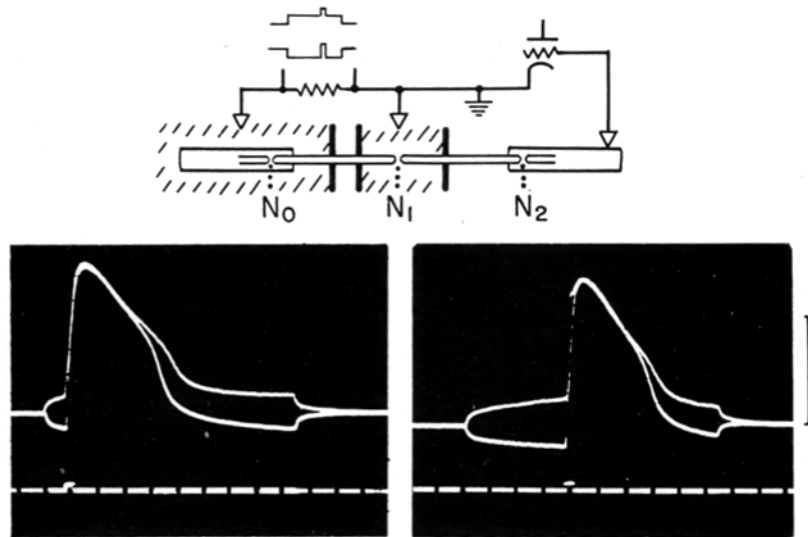

FIG. 4. Effect of changing the resting membrane voltage by a direct current upon the action potential of a single node. Long voltage pulses, applied between N_0 and N_1, were started at the moment when the two traces in the photograph begin to separate. The action potential of N_1 was initiated by a short stimulating pulse approximately 1 (left) and 4 msec. (right) after the start of the long pulse. Voltage calibration: 50 mv.; time marks, 1 msec. A toad nerve fiber at 11°C.

pulses has been described earlier (17). The potential inside N_1 was recorded with a high input–impedance amplifier connected between N_1 and N_2. The variation in the membrane voltage induced by the applied voltage consisted of two components, a maintained (subthreshold) change in the membrane voltage and an additional quick rise in it which initiated an action potential.

In each photograph in the figure, two traces of the oscillograph line were superposed, one showing the response of the node evoked during passage of an outward current of subthreshold intensity through the node and the other the response initiated while the node was traversed by a current of the opposite sign but of the same intensity. As can be seen in the figure, the variation in the

membrane voltage caused by the long subthreshold pulse was still proportional to the applied voltage, as indicated by the equal deflections to opposite polarity currents. The maximum positive potential of the responses evoked was not affected by the passage of these currents through the nodal membrane[2] (see also Fig. 6, left).

There is one strange feature in the record of Fig. 4 that deserves further investigation. It is the fact that not only the peak of the action potential but also *the major portion of its falling phase remains unchanged by passage of a direct current through the nodal membrane*. It has been shown in a preceding paper (17) that the impedance of the nodal membrane, measured by short pulses or by A.C., falls at the peak of activity to a value below 5 per cent of the resting impedance and that it returns to normal in proportion to the fall in the membrane voltage. The *IR* drop across the membrane, which tends to separate the two traces in the experiment of Fig. 4, is expected to increase as the potential falls from the peak of the response. The almost perfect coincidence of the two traces in the figure during the first half of the active period definitely conflicts with the simple picture that the membrane voltage and resistance vary proportionally to each other (17; 14, p. 55).

A comparison of the records of Fig. 4 with those in Figs. 4 and 5 of the preceding paper (Tasaki and Freygang (17)) clearly indicates that the "resistance" of the active nodal membrane, unlike that of the resting membrane, depends markedly upon the duration of the measuring current pulse (or upon the frequency of the measuring A.C.). At least in the first half of the falling phase of the action potential, the voltage across the membrane is practically independent, within a range of about 10^{-9} ampere, of the direct current traversing the membrane, provided that the current is started before or at the peak of the action potential.

Fig. 5 further illustrates the strange property of the active nodal membrane just mentioned. Rectangular voltage pulses of ± 45 mv. were applied between nodes N_0 and N_1, the pulse being started near the peak of the action potential and withdrawn in the middle of the falling phase. This voltage is expected to cause a difference in the membrane current of the order of 1.5×10^{-9} ampere at the peak of the action potential, since the internal resistance of the fiber internode is of the order of 60 megohms (15). This large difference in the membrane current, however, did not bring about any measurable difference in the membrane voltage in the first half of the falling phase (see also Fig. 6, left). When the applied voltage pulse was suddenly withdrawn in the middle of the falling phase, there appeared a quick change in the membrane voltage, indi-

[2] On the squid giant axon, Cole and Curtis (personal communication) examined the effect of a direct current polarization upon the internally recorded action potential. They found that the peak value of the action potential referred to the external fluid medium was unaffected by the direct current.

cating that the membrane resistance, measured by short (or suddenly started) current pulses, was reasonably large at this moment.

An easy and practical way of describing this puzzling phenomenon is to assume the active nodal membrane to be represented by a variable resistance and a variable electromotive force in series (14; p. 52). The membrane resistance can then be measured in terms of the variation of the membrane voltage caused by a short current pulse, and the effect of a long current pulse can always be accounted for by assuming a slow change in the electromotive force of the membrane. (Note that the small capacity of the membrane cannot play any role in this phenomenon.) From this point of view, the distinct di-
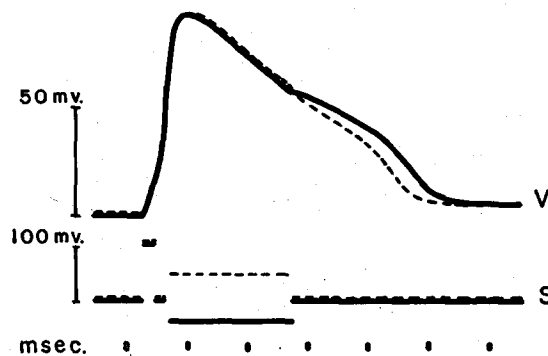

FIG. 5. Modification of the action potential of a single node by a strong inward (solid line) or outward (broken line) current through the node. The current was generated by a voltage pulse started at the peak of the action potential and withdrawn in the middle of its falling phase. A toad nerve fiber at 12°C.

vergence of the two traces in Fig. 5 upon withdrawal of the applied voltage can be taken as a sign of a modification of the membrane electromotive force by the strong current. The change in the membrane electromotive force caused by a direct current applied during activity is in such a direction that the change in the action potential (*i.e.* the membrane electromotive force minus the IR drop) is thereby reduced.

The property of the active nodal membrane just mentioned explains also the absence of interdependence between the action potential and the membrane action current of a single node (Results, section 1). The records of Fig. 1 show the same phenomenon when the membrane current is altered by the activity of the adjacent node, N_0. For a delayed activation of N_0, the current generated by the activity of N_0 caused a detectable IR drop across the membrane of N_1 (records 2 and 3). But, when the current from N_0 started at the peak of activity at N_1, there was no detectable modification in the action potential of N_1 by this linearly decreasing current.

4. Abolition of Action Potentials of a Single Node by Inward Current Pulses.—
In the experiment of Fig. 5 discussed in the preceding section, it is seen that a
pulse of inward current applied through an active nodal membrane lengthens
the total duration of the action potential and a current pulse of the opposite
polarity shortens the spike duration. If, however, the pulse is made longer in
duration or stronger in intensity, it is found that an inward current pulse also
shortens the spike duration.

The records of Fig. 6 show the effect of a long pulse of inward current ap-
plied during the action potenial of a single node. The arrangement for this
experiment is the same as for the previous one. A rectangular voltage pulse for
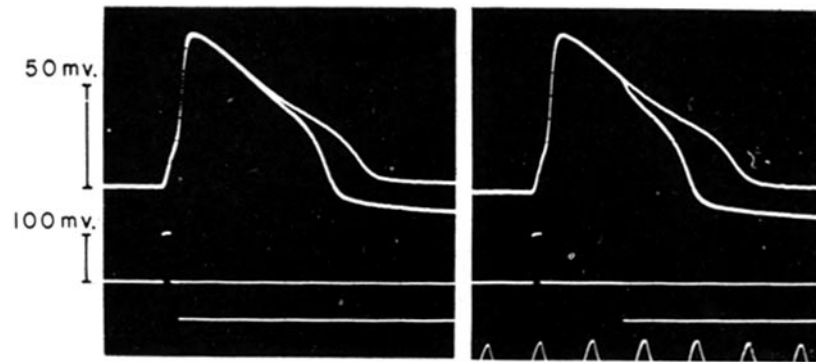generating inward currents through the active node was started either near

FIG. 6. Shortening of the action potential of a single node by inward current through
the active membrane. A toad nerve fiber at 11°C.

the peak (left hand record in the figure) or in the middle of the falling phase
(right hand record) of the action potential. Two sweeps of the oscillograph
beam were superposed, one displaying the action potential modified by the
applied inward current and the other a control.

In agreement with the results of the preceding section, the voltage pulse
started at the peak of the action potential did not change the following course
of the action potential except near its end. When the voltage pulse was started
later in the falling phase, the membrane voltage was lowered by the pulse to
a greater extent and reached the steady final level sooner.

When the pulse of inward current through the active nodal membrane was
made short and strong, a new phenomenon was observed: *the portion of the
action potential following the applied anodal pulse was "abolished" at a certain
strength of the pulse in all-or-none manner.*

The records in Fig. 7 show that a pulse applied to the node in the falling
phase of the action potential lowered the membrane voltage temporarily in

proportion to its intensity. After the end of the pulse, the potential inside the node went up approximately to the level which would have been reached if the pulse had not been delivered. When the applied pulse was made strong enough so that the membrane voltage at the end of the pulse was close to the threshold voltage of the node, the potential inside the node did not go up after
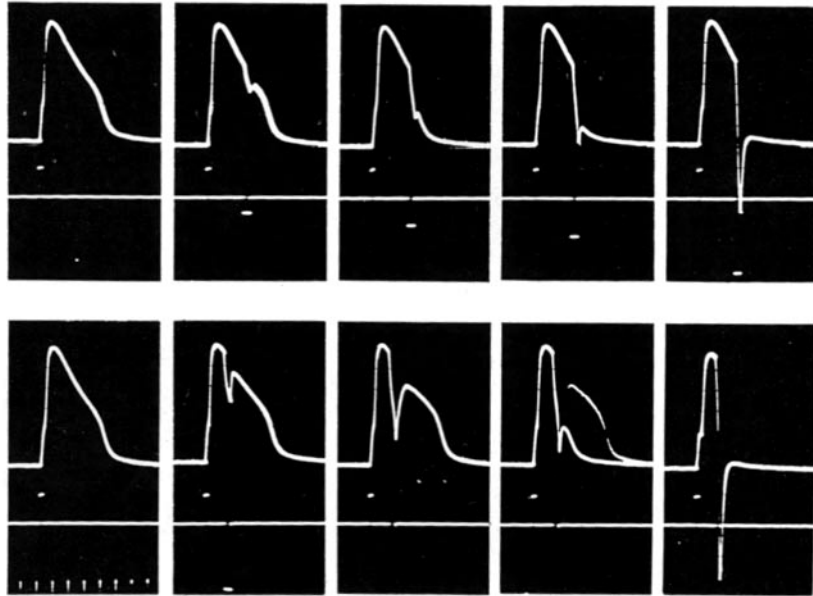

FIG. 7. Abolition of the action potential of a single node by anodal pulses. The lower trace in each record indicates the time course of the voltage applied between N_0 and N_1 in the previous diagram; the first voltage pulse was 100 mv. in amplitude. In the fourth record, lower column, two successive sweeps of the oscillograph lines were superposed at the same stimulating and abolishing voltages. The spike height of the recorded action potential was approximately 100 mv. Time marks in milliseconds. A toad nerve fiber at 10°C.

the end of the pulse but instead it decayed rapidly until the potential level in the resting state was reached. A further increase in the strength of the applied pulse lowered the potential level further, and consequently the resting potential level was reached sooner.

The minimum intensity of the pulse needed for abolishing an action potential depended markedly upon the relative position of the pulse on the action potential. Much stronger pulses were needed in the early stage of the falling phase of the action potential than in its later stage (see Fig. 7). When the pulse strength was raised well above the minimum value required for abolition, it

was seen in some preparations that the portion of the action potential follow-
ing the pulse reappeared after some delay. Since this phenomenon of failure
of abolition at high pulse intensity was observed only on several preparations,
the details of the phenomenon have not been worked out. A plausible explana-
tion of this phenomenon is to attribute it to stimulation of N_1 by the counter
electromotive force at N_0 caused by the strong pulse.

RECOVERY AFTER NORMAL RESPONSE
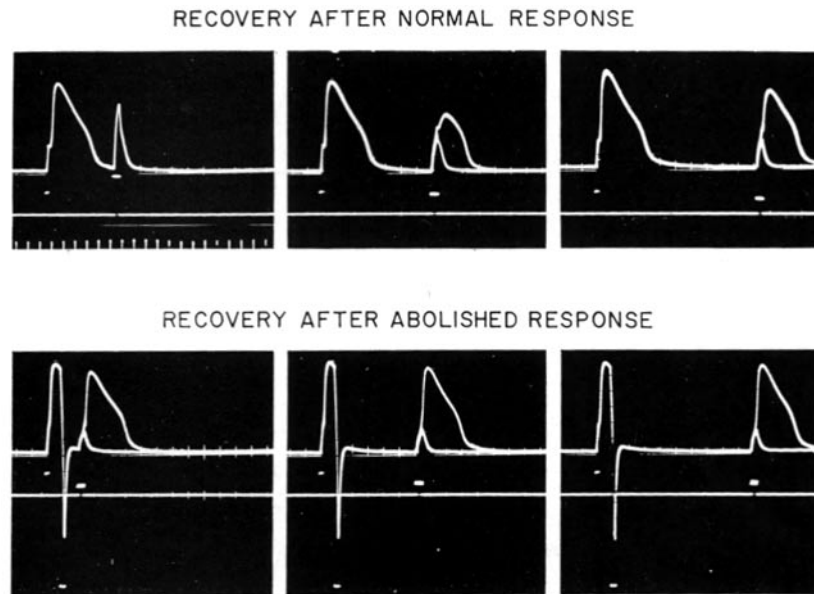

RECOVERY AFTER ABOLISHED RESPONSE

FIG. 8. Recovery in spike height and in duration of the action potential of a single
node after a normal and an abolished response. The amplitude of the last rectangular
pulse in each record indicates the threshold intensity at each moment. Time marks
in milliseconds. The same nerve fiber as in Fig. 7.

The above mentioned observation on the effect of strong current pulses
upon the active nodal membrane gives a satisfactory explanation of the pre-
vious results obtained by the old shock test method (Tasaki (13), Fig. 7). Since
this old method of determining the time course of the action potential of a
single node is indirect and tedious, the previous observation by this method is
not entirely accurate.

In the automatically beating Purkinje fiber of the heart, Weidmann (20)
showed that the action potential could be switched off by a strong anodal pulse.
Apparently Weidmann has seen the phenomenon of abolition in a cable-like
excitable tissue capable of carrying impulses. Abolition of a propagated action
potential would be in general impossible, because the applied current does not
spread along an active (depolarized) membrane.

5. Recovery Process Following Abolition of Action Potential.—The time course of recovery from refractoriness after an abolished action potential was investigated by means of another pulse of outward current. The node to be studied was excited by the first pulse of outward current. The second pulse of strong inward current abolished the action potential. The third pulse was ad-
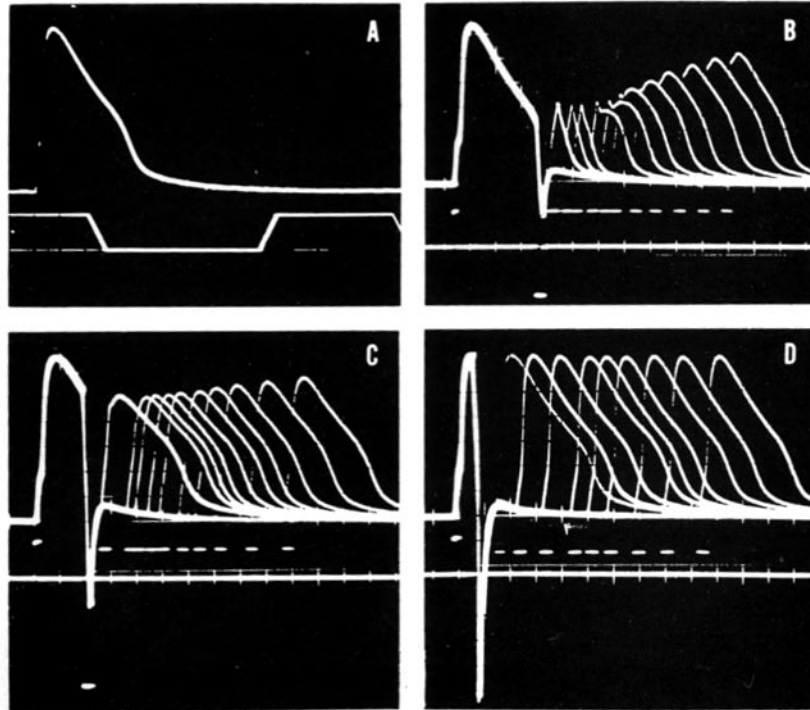

FIG. 9. A, action potential of a single node (top) and a truncated 60 cycle wave indicating 100 mv. level in applied (stimulating and abolishing) pulses. B, C, and D, superposed recordings showing recovery after an abolished response. The same single fiber preparation as in the two preceding figures.

justed to threshold at various times after the abolition to map out recovery in spike height and in excitability.

The records in Fig. 8, top, show the recovery process of a node following a normal, unabolished action potential as a control. Immediately after the end of the spike response, the nodal membrane is completely inexcitable (*cf.* 14, p. 88). Later, a small and short second response can be evoked at a high threshold voltage. The height of the spike inducible in the refractory period recovers approximately exponentially as the time after the end of the first, conditioning action potential.

The pattern of this normal recovery was clearly modified by abolition of the action potential in its falling phase. The nodal membrane was found to be excitable at any moment after abolition. The magnitude of the response inducible immediately after abolition was determined by the time at which the abolishing pulse was delivered: it was approximately equal to the potential level at the moment when the abolishing pulse was delivered to the node (Figs. 8 and 9). The recovery in spike height starts from this finite level instead of zero as in the case of an ordinary recovery after an unabolished response. The course of the following recovery seemed to be approximately exponential, the time constant with which the spike height approached the normal value being about the same as that of the recovery curve after a normal action potential.
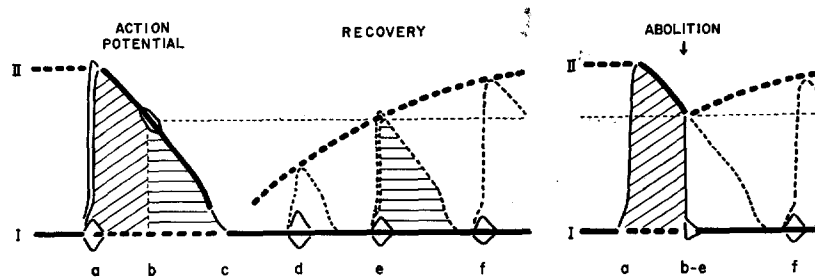

FIG. 10. Diagram summarizing the observations on initiation and abolition of the action potential of a single node. Description in text.

DISCUSSION

The experimental results dealing with initiation and abolition of the action potential of a single node of Ranvier are summarized in Fig. 10. The thick solid line represents the more or less "tenacious" portion of the potential level observed in these experiments. These portions are "tenacious" in the sense that the effect of small disturbances in the potential level, caused by passing short inward or outward current pulses through the nodal membrane, soon disappears and the potential inside the node goes back to its original level when the pulse is withdrawn. The small deflections shown in the diagram at the moments labelled "*a*," "*b*," "*d*," etc. represent these disturbances. The potential level in the interval between the start of a stimulating pulse (at *a*) and the peak of the evoked action potential is not tenacious in the sense described above. Nor is the potential level at the very end of the action potential (see Fig. 4 of this paper and Fig. 3 in the preceding paper (17)). But the major portion of the falling phase of the action potential is definitely tenacious.

The thick broken lines in the figure represent the potential levels that can be attained by "transition" from the potential level shown by the thick solid lines. Transition can be induced by application of relatively strong current pulses

to the nodal membrane in proper directions. This level is also tenacious, since the intensity of the pulse causing the transition does not to any appreciable extent affect the potential level to which the nodal membrane eventually settles. It has been mentioned under Results that abolition of an action potential is practically all-or-none. Initiation of action potentials in the relatively refractory period is also all-or-none.

The response elicited from a relatively refractory node is smaller in height and shorter in duration than a normal response of a resting node. The rate of potential decline in the falling phase is not much different in normal and refractory nodes. The abrupt fall of potential near the end of the falling phase exists in subnormal responses elicited from a refractory node as well as in normal responses. This means that the falling phase of a subnormal response is practically superposable upon the posterior portion of a normal response. In the diagram the shaded area in the interval between b and c is superposable upon the shaded area following e. The property of the nodal membrane just mentioned indicates that the rate of potential fall is independent of how this potential has been reached, namely, whether by the automatic decay of potential from the peak of a normal response or by a transition from the resting potential level in the relatively refractory period.[3]

When an action potential is abolished in its falling phase, recovery starts from the potential level at which the response is abolished. The recovery curve after an abolished response is a close replica of the late portion of a normal recovery curve. This is shown diagrammatically in the figure. The courses of the thick lines in the right-hand diagram are represented by the courses of the lines in the left-hand diagram in which the whole period between b and c has been contracted and replaced by the duration of the short abolishing pulse.

The diagrams in Fig. 10 represent, with a slight simplification, nothing more than what has actually been observed in the experiments. These diagrams, however, can be interpreted as indicating the existence of two "equilibrium states" in the excitable system consisting of the axoplasm, the nodal membrane, and the external fluid medium. The equilibrium state "I" determines the resting potential level shown by the heavy straight line I in the figure. This level remains unchanged during activity, since in abolishing an action potential the potential inside the node jumps back to this level. The equilibrium state "II" determines the active potential level indicated by the thick line II in the figure consisting of broken and solid pieces of lines. When the potential of the axoplasm (referred to the potential of the outside fluid medium) goes above the threshold voltage, the system jumps from equilibrium state "I" to "II." When the system is kept in state "II," the potential level de-

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[3] It might be added in this connection that small responses inducible from a node immersed in a low sodium–Ringer or in a dilute cocaine–Ringer show approximately the same configuration as the responses induced in the relatively refractory period.

termined by the system suffers a gradual fall, which one might reasonably attribute to a chemical combination of some metabolic product of activity with a part of the membrane responsible for maintaining level II. When the potential is lowered, by this automatic process or by an abolishing pulse, down to the critical level, the system jumps from state II to I. The recovery process can then be interpreted as the dissociation of the metabolic product from the membrane.

An unsuccessful attempt was made to interpret these experimental results in terms of the sodium theory of nerve excitation (8).

SUMMARY

1. Using single node preparations of the bull frog or the toad, observations were made on the variation of the voltage across the nodal membrane under various experimental conditions.

2. The time constant of the variation in the membrane voltage caused by a long subthreshold rectangular pulse was of the order of 0.1 msec.

3. The action potential was initiated when the potential inside the node was raised stimulating pulses above a threshold level of approximately 15 mv. for a node in normal Ringer; it was greater in a relatively refractory node and in a partially narcotized node.

4. The variation of the membrane voltage caused by long stimulating pulses of subrheobasic strengths was in general proportional to the strength of the applied pulse. A non-linear behavior of the membrane voltage was observed with barely subthreshold stimulating pulses.

5. The early portion of the action potential of a node was not modified by a direct current which was strong enough to produce measurable potential changes (IR drops) across the resting membrane.

6. A strong pulse of inward current applied to the node during activity abolished the portion of the action potential following the pulse in all-or-none manner.

7. There was no refractory period after a response abolished in its early phase. Following a response abolished later, the recovery in the spike height started from the level of the action potential at the time of abolition.

8. Initiation and abolition of action potentials at a single node are interpreted as "transitions" between the two "equilibrium potential levels" at the node.

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