THEORY OF THE FLOW OF ACTION CURRENTS IN ISOLATED MYELINATED NERVE FIBERS, XI*

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In this and in the following communication, the information already presented[1] will be supplemented, by analysis of experiments on direct electrical stimulation of isolated segments of fiber, to the extent which is necessary to arrive, in a later communication, at a satisfactory explanation of the essential differences that exist between the modes of propagation of the nerve impulse in isolated and in normal nerve fibers (cf. refs. 1a, Fig. 3; 1i, p. 1613).

Technique.—To the details already given, the following should be added. In all the experiments to be discussed below, exposed node N_1 was placed at or near to the center of the central pool, the width of which was increased from 270 μ (ref. 1a, Fig. 1) to 700 μ, while the width of the vaseline gaps (550 μ) was kept unchanged. The purpose of widening the central pool was to include in this pool the points of lowest threshold of the P_d segment of *Int. 1* and of the P_p segment of *Int. 2* (cf. ref. 1a, Fig. 3). Under conditions such as these, according to the argument presented in reference 1b, p. 945, the stimulating device, acting as a low-resistance (2 megohm) shunt across the first gap, would not prevent—on the contrary it would facilitate—the jump into the central pool of impulses initiated in the proximal pool as well as it facilitates the jump of the impulse in the opposite direction.

The results of direct electrical stimulation have been modified by three different procedures. The first is progressively increasing or decreasing the magnitude of the stimulating current. The second is modifying the resting membrane potential, i.e., by applying continuous electrical polarization by means of a second stimulating device placed across the stimulating gap. When two stimulating devices were used, each one was connected to the preparation through a 4-megohm resistance, whereby, since the internal resistance of the stimulating devices is negligible, the resistance of the shunt across the stimulating gap was kept constant. The polarity of the applied currents, stimulating or polarizing, will always be given in reference to the flow of membrane current in the segment of fiber in the central pool. Thus, for example, cathodal current is one that produces an outward flow of membrane current in that segment. The third procedure is the placing of low-resistance shunts across the recording gap. As a rule, the duration of the rectangular pulses of stimulating current was made slightly longer than the duration of the oscillograph's sweep.

In this communication, only action potentials initiated by cathodal currents will be considered. As should be expected, direct electrical stimulation results in activation of the same zones of internode (ref. 1c, Fig. 3, IV, a, b_1, b_2, c) which become active when the isolated segment of fiber is invaded by a traveling nerve impulse. In the latter case, zone a is always the first to become active and the three other zones usually become active in this order, b_1, b_2, c, although under certain conditions the temporal order of activation may become a, c, b_2, b_1 (ref. 1g, Fig. 1). When direct electrical stimuli are used, the order of activation of the four zones depends both upon the magnitude of the applied current and upon the distribution of stimulation thresholds in the isolated segment of fiber (cf. ref. 1a, Fig. 3).

In work with self-propagating nerve impulses, the responses of zones b_1 and b_2 (ref. 1c, Fig. 3, IV) follow one another in a succession which is so rapid that as a rule the two responses cause the appearance of a single upward peak in the action potential, but in experiments on direct electrical stimulation, zone b_2 is found to have a lower stimulation threshold and to produce a higher action potential than zone b_1. This difference is referable to the fact that the applied, rectangular cathodal current initiates simultaneously two different processes in the excitable nerve membrane. One process leads to excitation, i.e., to the initiation of the nerve impulse. The other process leads to cathodal depression, i.e., to an increase of the threshold of stimulation and to a decrease of the magnitude of the action emf. The rate at which cathodal depression

develops increases markedly with the rate of establishment of the catelectrotonus.[2] Since in zone b_1 the catelectrotonus is established at a higher rate than in zone b_2, cathodal depression is created in zone b_1 before the action emf is initiated, and therefore zone b_1 responds later and produces a smaller action potential than zone b_2.

The third fact that must be taken into account is as follows. After they have been created, zones b_2 and c propagate themselves continuously toward the center of the recording gap, but both propagations occur with decrement so strong that the two active zones become extinguished before collision, with the result that there is permanently in the recording gap an inactive segment of internode which can supply two flows of longitudinal action current, in opposite directions, toward the active zones (ref. 1*i*, p. 1613). According to equation (2) in reference 1*b*, the amplifier across the second gap measures the difference between the potential differences created by those two flows.

Analysis of Poliphasic Action Potentials.—Records *1–4* (Fig. 1) illustrate frequently observed types of action potentials. Threshold or near-threshold stimuli initiated triphasic action potentials (Fig. 1, *1,2*), but after a slight increase of the stimulus, depending upon the ever-present cyclic variations of the stimulation threshold, there appeared either a diphasic action potential having a small, terminal upward peak (Fig. 1, *3*) or a triphasic action potential (Fig. 1, *4*). After a further slight increase of the stimulus, only action potentials such as that in record *3* were produced.

The genesis of the action potentials in Figure 1, *1,4*, is readily understandable. In the cases of records *1,2,4* (Fig. 1), the four zones a, b_1, b_2, and c (ref. 1*c*, Fig. 3, *1V*) became active in this order, b_2, c, a, b_1, while in the case of record *3* (Fig. 1) the anelectrotonus prevented the jump of the impulse from zone b_2 into zone a, and consequently the action potential displayed only a small terminal upward peak, which was referable to the creation of a small action emf in zone b_1.[3] As can readily be noted, the large, second upward peaks in records *1,2,4* (Fig. 1) correspond to the upward peak in record *3*. Those peaks were large because the electrotonic spread of action currents was enhanced by the presence of a low-resistance shunt across the first gap (cf. ref. 1*h*).

Additional information is presented in Figure 1, *9–24*. A low-resistance shunt placed across the recording gap exerted its customary, spectacular effect upon the results of electrical stimulation (cf. ref. 1*f*, Fig. 2, *13–20*, and ref. 1*g*, footnote 2). In the presence of the shunt, even cathodal currents of large magnitude were able to initiate only small monophasic action potentials (Fig. 1, *9–12*) which doubtlessly were referable to the creation in the central pool of small action emf's which were unable to propagate themselves. When the applied currents exceeded a certain large magnitude, active zones were created in the distal pool, and the records displayed large downward deflections (Fig. 1, *13–15*). From the large magnitude of those deflections, it may be concluded that the active zone created by the stimulating current in the distal pool (zone c) had propagated itself decrementally backward toward or even into the recording gap, a conclusion which is supported by the following facts. The transition from record *14* to record *15* (Fig. 1) was perfectly smooth in the sense that when the magnitude of the stimulating current was progressively decreased, the latency of the downward phase increased progressively, even though the height of the downward phase remained constant. The transition from record *15* to record *16* was all-or-nothing, i.e., no matter how slightly the applied current was varied, either record *15* or record *16* was obtained. The explanation of such transition was given in reference 1*f*. The small downward deflection
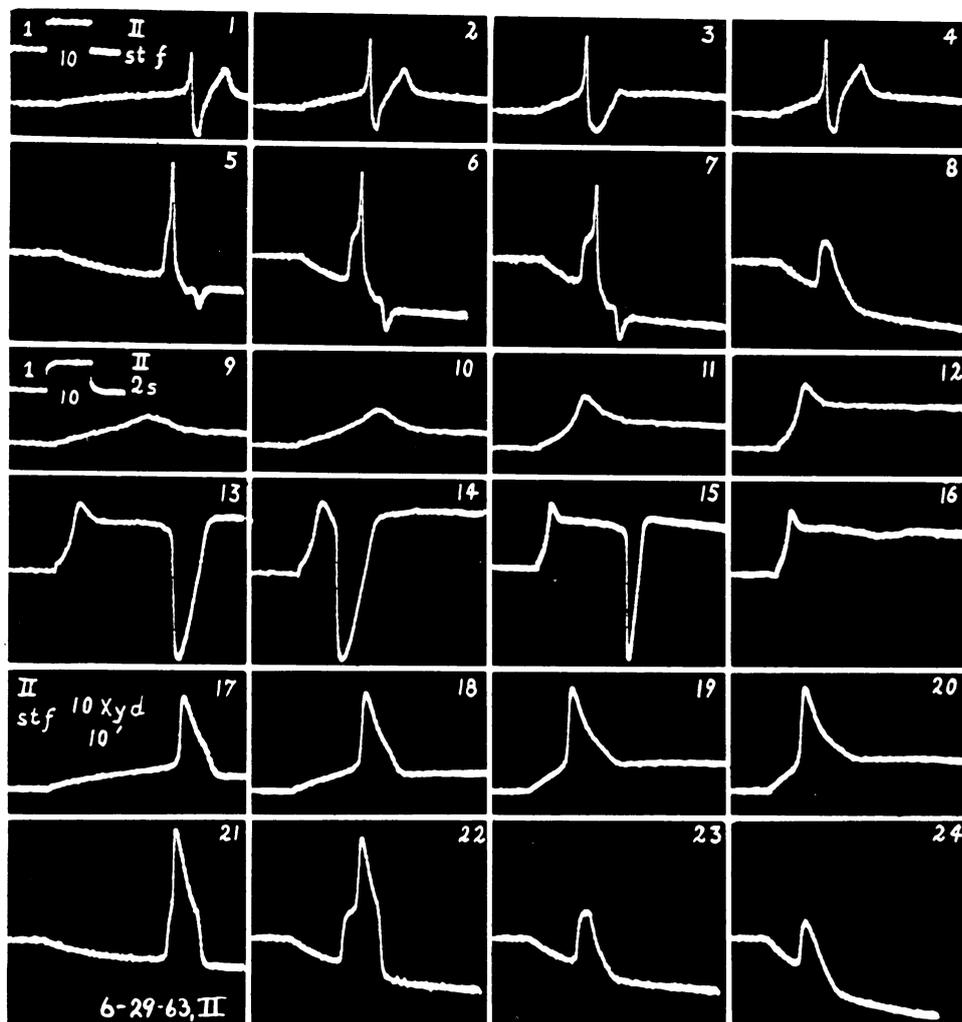
Fig. 1.—Action potentials initiated by direct electrical stimulation of the isolated fiber with rectangular pulses of current.   The sign of the electrotonic potential indicates whether the cathode or the anode was connected to the central pool.   Node N_1 at the center of the 700-μ-wide central pool and the also exposed node N_2 in the distal pool.   The stimulating device was placed across the first gap, and the amplifier across the second gap.   Records *9–16* were obtained in the presence of a 2-megohm shunt across the second gap.   Records *17–24* were obtained, in the absence of a shunt, beginning 10 min after the introduction of 10 mM xylocaine in the distal pool.   The calibrating signal in record *1* is 1 msec wide and 10 mv high; it applies to *1–8* and *17–24*.   The calibrating signal in record *9* applies to *9–14; 15* and *16* were obtained at 2.5 times smaller sweep speed.

in record *16* was referable to the creation in the distal pool of an action emf which was too small for propagation, but an increase, however small, of that action emf resulted in propagation and consequently in the appearance of large downward phases of fixed height.   Within a reasonable degree of approximation, the downward phases in records *13–15* (Fig. 1) measure the contribution made to the action potentials in records *1–4* by the flow of action current from right to left during backward propagation of the active zone *c* which had been created in the distal pool.

On the other hand, on the basis of the records obtained after the introduction of

xylocaine in the distal pool it is possible to estimate directly the contribution made to those action potentials by the longitudinal action current from left to right during decremental propagation of the active zones which had been created in the central pool. As is always the case, the anesthetic produced a dual effect (cf. ref. 1c), and by its secondary action the anesthetic modified the response of the untreated segments of the isolated fiber. Comparison of records *1,2* and *17,18* (Fig. 1) reveals that after the introduction of the anesthetic the impulse could no longer jump from the central into the proximal pool, a sufficient explanation for this failure being the fact that the secondary action of the anesthetic had caused a reduction of the magnitude of the action potential initiated in the central pool. The absolute crest height of the action potential (i.e., the height measured from the initial base line of the records) is greater in record *21,22* (Fig. 1) than in record *17,18* which shows (cf. ref. 1a, Fig. 2, *22–27*) that the anodal current had relieved the depression caused by the anesthetic and had increased the action potential, probably to normal height.

Under conditions such as these, it is clear that, except for the small, terminal upward peak, the action potential in record *3* (Fig. 1) was the result of the summation [ref. 1b, eq. (2)] of the effects of two simultaneous flows of longitudinal action current in the recording gap—one flow from left to right, which was somewhat greater than, but had the same temporal course as, the flow which gave rise to the trapezoidal action potential in record *17* (Fig. 1), and the other flow from right to left which had the same temporal course as the flow which gave rise to the downward phase in record *13* (Fig. 1).

In the experiment illustrated by Fig. 2, the anelectrotonus was sufficient to prevent activity in the proximal pool even when threshold currents were used. In the case of record *1*, the creation of an action emf in the central pool (zone b_2) was quickly followed by the creation of an active zone in the distal pool (zone c). The action potential, however, displayed only a small downward phase because a second action emf was created in the central pool (zone b_1), which increased the flow of action current from left to right in the recording gap. When the applied current was increased, the response of zone b_1 became greater, with the result that the downward phase of the action potential had a much reduced magnitude (Fig. 2, *2,3,5*). After a further but still slight increase of the stimulus, a diphasic action potential appeared (Fig. 2, *4*) which had a large downward phase of long duration. The simplest explanation of this change is that when the stimulus was increased, action emf's were established simultaneously in zones b_1 and b_2 in the central pool. This explanation is in agreement with results that were obtained later (Fig. 2, *18–24*).[4]

Two procedures were used for analysis of the action potentials. Records *6–11* (Fig. 2) were obtained by applying at 2-sec intervals the stimulus used for record *5* during the continuous flow of a subliminal cathodal current which was made approximately 1 sec before obtaining record *6*. At the time when record *6* was obtained, cathodal depression had already developed to such an extent that (1) the action potential referable to the low-threshold patch (zone b_2) in the central pool had a reduced height, and (2) the response of the high-threshold patch (zone b_1) failed to be produced—hence the appearance of a large downward phase. The depth of the cathodal depression increased progressively with advancing time, and consequently the initial upward phase of the record decreased progressively in height (Fig. 2, *7,8*) until it disappeared (Fig. 2, *9*) and the record presented only the
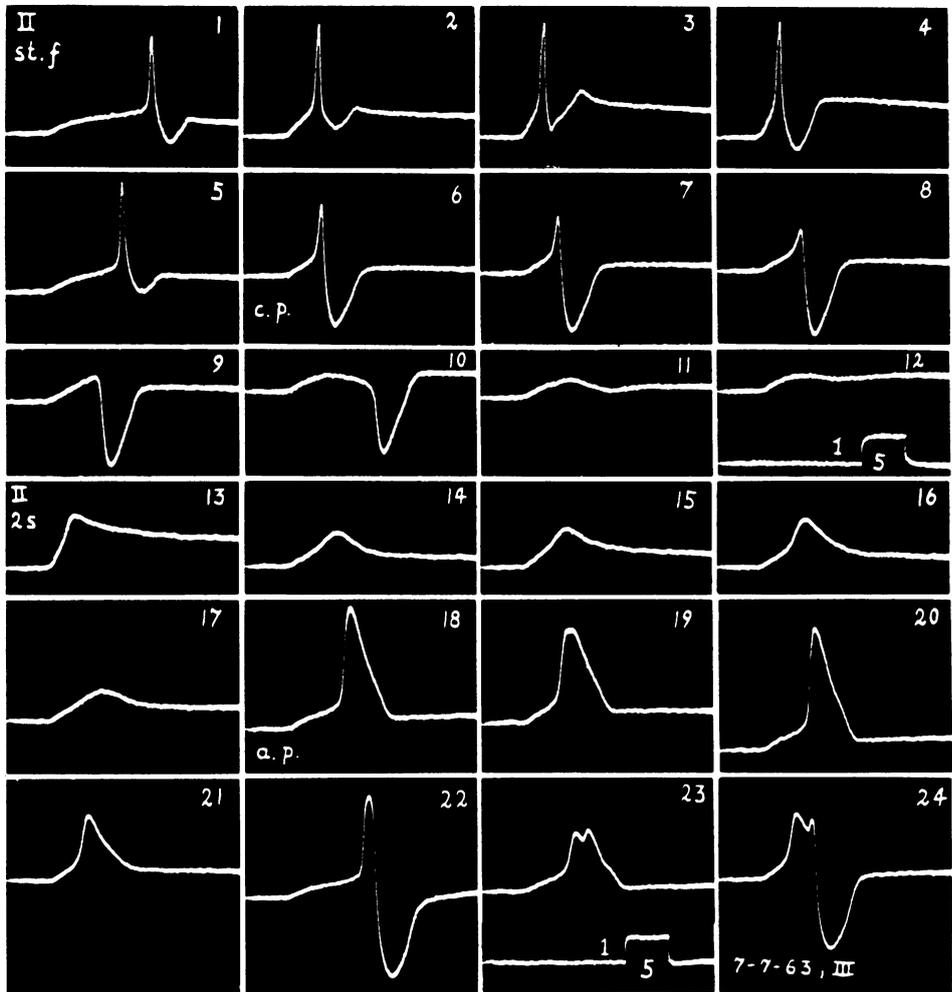
Fig. 2.—(See legend of Fig. 1.) Records *6–12* were obtained during continuous cathodal polarization (*c.p.*). Records *13–24* were obtained in the presence of a 2-megohm shunt across the second gap, and records *18–24* during continuous anodal polarization (*a.p.*). The calibrating signals are 1 msec wide and 5 mv high.

downward phase produced by backward decremental propagation of active zone *c*. Later, the downward phase appeared after a delay and had a reduced magnitude (Fig. 2, *10*), which indicated that cathodal depression was spreading toward the distal pool and was reducing the extent to which active zone *c* could propagate itself. Finally, cathodal depression became deep enough to prevent propagation entirely, and the large downward phase in record *10* was replaced, in an all-or-nothing fashion, by a small downward deflection (Fig. 2, *11*) which was referable to the creation in the distal pool of an action emf which was too small for propagation, i.e., an action emf which occupied a length of internode shorter than the liminal length needed for propagation (ref. *1f*, p. 1063).

Since record *9* (Fig. 2) measures with reasonable accuracy the contribution made to the action potentials in records *1–5* by the longitudinal flow of action cur-

rent produced by the active zone created in the distal pool (zone *c*) in a first approximation, the contributions made to those action potentials by the longitudinal flows produced by the active zones in the central pool can be evaluated by measuring the difference between the downward phase in record *9* and the action potentials in records *1–5* (Fig. 2).

Figure 2, *13–17*, shows that in the presence of a 2-megohm shunt across the recording gap only small monophasic action potentials could be elicited. The explanation of this remarkable phenomenon was given in reference *1g*, footnote 2. The effect of the low-resistance shunt was readily prevented by raising the membrane potential by means of continuous anodal polarization, because in the presence of the existing anelectrotonus the stimulating current failed rapidly to produce cathodal depression. Records *18–24* (Fig. 2) present, in the order in which they were observed, the main types of action potentials which were produced at 2-sec intervals during a 3-min period of observation, during which the magnitudes of the polarizing and stimulating currents were frequently varied. In all probability the large action potentials in records *18–20* measure the contribution made to the action potential in record *4* by the two active zones created in the central pool. The broad crest and slightly reduced height of the action potential in record *19* indicate that the two patches of internode in the central pool had responded in quick succession, while in the case of records *18* and *20* both patches responded simultaneously. The existence of two responding patches in the central pool appears with greater clarity in records *21–24* (Fig. 2). At times, only one patch responded (Fig. 2, *21*), and at other times, the two patches responded either in succession (Fig. 2, *23,24*) or simultaneously (Fig. 2, *22*). The presence of large downward phases in records *22* and *24* shows that in those cases the flow of action current had been sufficient to create an action emf in the distal pool, which was able to propagate itself decrementally backward.

The type of action potentials illustrated by Figure 3, *1–8*, is observed only when, probably owing to differential injury during the isolation process, the stimulation threshold is lower in the distal than in the central pool. The small downward deflection that appears in Figure 3, *1*, superposed upon the catelectrotonus was referable to the creation of small unpropagated action emf in the distal pool (zone *c*). After a slight increase of the stimulating current, backward propagation occurred and the action potential displayed a large, initial downward phase (Fig. 3, *2*). The flow of action current was sufficient to complete stimulation of patches of internode in the central pool, so that the downward deflection was replaced by an upward peak, which reached a large height because the impulse executed a second backward jump from the central into the proximal pool. After a further increase of the stimulus, activity set in earlier in the central pool, and consequently the height of the downward phase was reduced (Fig. 3, *3,4*). The presence of a large second upward phase in record *4* indicated that the impulse still was able to execute the jump from the central into the proximal pool, but after a further increase of the stimulus, the anelectrotonus in the proximal pool became sufficient to prevent the jump, and the action potential (Fig. 3, *5*) displayed only a small downward deflection. The only possible interpretation of the downward deflection in record *5* is that action emf's had been created simultaneously in the distal and in the central pool and that in the recording gap the potential difference established by the flow of
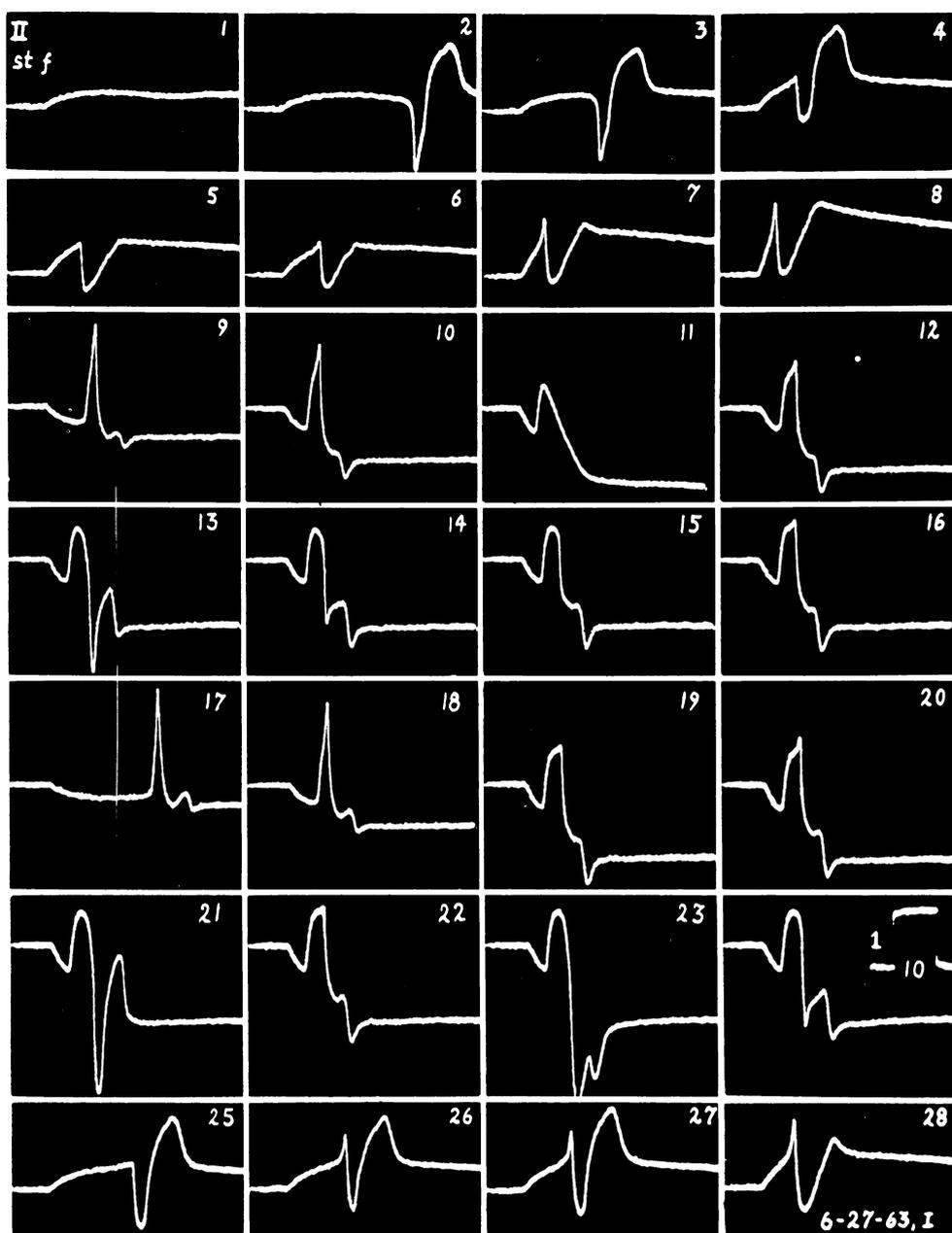
FIG. 3. (See legend of Fig. 1.) In this experiment neither a shunt across the second gap nor continuous polarization was used.

longitudinal current from right to left permanently was slightly greater than the potential difference established by the flow in the opposite direction. After further increases of the stimulus, activity began earlier in the central than in the distal pool, and therefore the action potentials displayed an initial upward phase (Fig 3, 6–8).

Repeated application of anodal pulses of current (Fig. 3, *9–24*) improved the state of the segment of fiber in the central pool whereby the difference between the stimulation thresholds in the distal and in the central pool was decreased. Thus, although threshold cathodal currents still produced action potentials beginning with a downward phase (Fig. 3, *25*), a slight increase of the current was sufficient for the creation of activity earlier in the central than in the distal pool, and consequently triphasic action potentials were recorded (Fig. 3, *26–28*). From the shape of the records, the temporal order of activation of zones a, b_1, b_2, c (ref. 1c, Fig. 3, *IV*) can easily be deduced. In the case of record *25* (Fig. 3), the temporal order was c, b_2, a, b_1, and in the cases of records *26* and *27* the order was b_2, c, a, b_1, while in the case of record *28* the anelectrotonus prevented activation of zone a and the action potential was produced solely by successive activation of zones b_2, c, and b_1.

The action potentials in reference 1f, Figure 2, *1–4*, also were produced by successive activation of several patches of internode. In the cases of records *1* and *2*, the four patches of internode became active in this temporal order, b_2, c, b_1, a. In the case of record *3*, the larger stimulus created anelectrotonus in the proximal pool which was sufficient to prevent activation of zone a, and since it also created cathodal depression in zone b_1, this zone responded late and produced only a small action emf. Finally, with the use of a much larger stimulus the cathodal depression became sufficient to prevent the response of zone b_1, and the action potential (ref. 1f, Fig. 2, *4*) was referable solely to successive activation of zones b_2 and c.

The presentation of the theory of the isolated fiber will be continued.

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[1] (a) Lorente de Nó, R., and V. Honrubia, these PROCEEDINGS, **53**, 757 (1965); (b) *ibid.*, p. 938; (c) *ibid.*, p. 1384; (d) *ibid.*, **54**, 82 (1965); (e) *ibid.*, p. 388; (f) *ibid.*, p. 770; (g) *ibid.*, p. 1061; (h) *ibid.*, p. 1303; (i) *ibid.*, p. 1606; (j) *ibid.*, **55**, 91 (1966); (k) *ibid.*, p. 321.

[2] In the following communication the exceedingly important fact will be discussed that patches of the central part of an internode which readily produce an action emf in response to the rather slowly rising flow of action currents always fail to produce an action emf in response to an applied rectangular pulse of cathodal current.

[3] The attempt could be made to explain the presence of a small terminal peak in Fig. 1, *3*, in a different manner. Both the downward action potential in records *13* and *15* and the upward action potential in record *17* have small second phases which were referable to the temporary hyperpolarization during the R_1 process, which process brought the deflection beyond the level of electrotonic potential existing at the time of initiation of the action potential (below in the case of record *17* and above in the cases of records *13* and *15*). Therefore, the possibility could be considered that the terminal upward peak was caused by superposition of the effects of the R_1 processes in the two active zones. However, that terminal peak is too brief to be explained in this manner. On the other hand, the explanation given in the text is in better agreement with the results of other experiments, and it is not in disagreement with the fact that a second upward peak does not appear in record *17* (Fig. 1), because responses of high-threshold patches of internode are readily prevented by the secondary action of an anesthetic.

[4] The fact that an indeed slight increase of the stimulating current was sufficient to cause the transition from record *3* to record *4* (Fig. 2) excludes the alternative explanation, namely, that in the case of record *4* the stimulating current by creating severe cathodal depression had prevented the response of zone b_1. In this connection, it should be emphasized that the susceptibility of isolated fibers to cathodal depression varies within wide limits depending upon the value of the resting membrane potential. The susceptibility decreases with increasing value of this potential.