Further Studies of Rapid Mechanical Changes in Squid Giant Axon Associated with Action Potential Production

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Abstract Mechanical changes in the squid giant axon associated with the production of an action potential are examined further by using piezo-electric and optical methods. The peak of swelling of the axon coincides with the peak of the action potential recorded internally at the site of mechanical recording. Mechanical changes produced by a train of action potentials do not summate. Repetitively fired action potentials induced by lowering the external Ca-ion concentration are preceded by a gradual swelling of the axon. An inward current through the membrane causes shrinkage and an outward current produces swelling of the axon. An inward current enhances and an outward current depresses the mechanical changes associated with the action potential. There is a transient shortening followed by an elongation of the axon when an action potential travels along the axon. It is argued that the experimental results obtained are consistent with the colloid chemical, or macromolecular, theory of excitation.

Key Words: squid axon, rapid mechanical changes, action potential, current pulse.

In recent papers (TASAKI and IWASA, 1980, 1982; IWASA and TASAKI, 1980) we have described several aspects of rapid mechanical changes that take place in the squid giant axon when electrically excited. The present paper deals with the results of our further studies of these rapid mechanical changes in the squid axon associated with action potential production. We show that the new results throw considerable further light upon the rapid structural and physicochemical changes that take place in the nerve membrane during excitation.

MATERIALS AND METHODS

Giant nerve fibers taken from squid, Loligo pealei, available in the Marine Biological Laboratory, Woods Hole, were employed in all the experiments described in this paper. After dissection in running seawater, small nerve fibers at-
tached to the portion of the giant fiber used for mechanical recording were care-
fully removed under a dissecting microscope. After cleaning, the giant fiber
was mounted in a plastic chamber described in our previous paper (TASAKI et al.,
1980). Unless otherwise stated, the axon was stimulated extracellularly by using
a pair of platinum (or silver) wire electrodes. Action potentials were monitored
with a pair of extracellular metal electrodes. When intracellular recording of
action potentials was required, a 50 μm-diameter enameled silver wire with a 100
μm-long uninsulated tip was introduced longitudinally into the axon through an
incision made at one end of the axon. A voltage-follower, constructed with an
operational amplifier (AD 515), was employed to record variations of the intra-
cellular potential referred to the potential of the extracellular medium.

As in previous studies, a modified Fotonic sensor was used for detecting small,
rapid displacements of the axon surface. The setup employed for recording rapid
pressure changes in the axon was either a Gulton bender (TASAKI et al., 1980) or a
new device constructed by using polyvinylidene fluoride film as piezoelectric
material (see below). The surface electrodes on the piezoelectric material was
connected to the input of an AD 515 charge-amplifier (see Fig. 1). The output
of the amplifier was led to a signal averager (Nicolet Instrument Co.) through a
capacity-coupled preamplifier (Tektronics, Type 122) with a gain of 1,000 in the
frequency range between 0.8 and 10,000 Hz. The dwell-time (i.e., the time re-
solution) of the signal averager was usually 50 μsec. Most of the records were
obtained after averaging over 512 or 1,024 trials.

Polyvinylidene fluoride (abbreviated to PVDF) is a synthetic polymer material
which becomes highly piezoelectric and pyroelectric when stretched at a high
temperature in the presence of a strong electric field (Kawai 1969; Murayama,
et al., 1976; Robinson, 1978). The 9 μm-thick PVDF film used in the present

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Fig. 1. Left: schematic diagram illustrating the setup used for demonstrating the simultaneity
between the peak of pressure rise and the peak of the internally recorded action potential.
PVDF indicates a piece of polyvinylidene fluoride film. A nylon string held in a nearly
horizontal position was employed to keep the PVDF film under tension. A stylus (B)
fixed vertically at the end of the lower end of the PVDF film pressed against the surface
of the axon. R indicates the potential recording electrodes. The axon was excited by
brief shocks applied through electrodes marked S. Right: the upper trace shows the
mechanical response; the lower trace, the action potential taken at the site of mechanical
recording. 21°C.

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studies was a generous gift of Kureha Chemical Industry Co., Tokyo. The film was already stretched and poled; on each surface of the film, there was an approximately 30 nm-thick aluminum layer. In order to detect rapid pressure changes in the axon by using this film, a device schematically illustrated in Fig. 1 was constructed. The upper end of the film (approximately 3 mm wide and 35 mm long) was fixed to a small plastic plate which could be moved vertically by means of a screw. The lower end of the film was attached to the middle of a (roughly 20 mm long) nylon string of about 0.35 mm diameter and also to a stylus made with bristles in the manner illustrated in the figure. After applying a slight tension to both nylon string and the PVDF film, the two ends of the nylon string were rigidly fixed. The stylus had a diameter of about 0.5 mm and a length of about 10 mm. For the purpose of ensuring a stable contact with the surface of the giant nerve fiber, which was kept immersed in the saline, the lower end of the stylus was slightly enlarged and flattened so that the tip formed a disc of 1 mm diameter.

The piezoelectric transducer (bender or film) was placed, together with the operational amplifier, in an electrically screened teflon box. By means of a calibrated microscrew, the teflon box was lowered toward the nerve chamber mounted on a horizontal stage. Measurements of pressure changes in the axon were carried out after lowering the stylus approximately 100 μm below the point of initial contact with the nerve fiber surface. The transducer was calibrated by determining the voltage shift at the output of the operational amplifier generated by the sudden removal of forces applied to the tip of the stylus.

With the detectors with PVDF elements we could improve the signal-to-noise ratio about three-fold over the detectors with ceramic bender elements. With these two types of pressure detectors we obtained quantitatively consistent results. To show that the records obtained from these detectors are indeed pressure changes, we attached larger discs (e.g., 2 mm in diameter) at the lower end of the stylus and observed that the signal recorded from crab nerve bundles are roughly proportional to the area of contact, in addition to other examinations already reported (TASAKI and IWASA, 1982).

Mechanical changes induced by transmembrane electric current were examined by using either extracellular electrodes (see TASAKI et al., 1980) or a pair of platini zed platinum electrodes introduced longitudinally into the axon (see Fig. 4, top). In one series of experiments, changes in axon birefringence associated with repetitive firing of action potentials were examined by the use of the technique described by COHEN et al. (1968).

Artificial seawater and isotonic salt solutions were prepared in accordance with the Formulae and Methods issued by the Marine Biological Laboratory. The pH of the solutions was adjusted to 8.0 by using 1–5 mM tris(hydroxymethyl)- aminomethane buffer. Most of the experiments were conducted at room temperature (19–21°C).
RESULTS

1. The simultaneity between the peak of swelling and the peak of the action potential

In the preceding paper (TASAKI and IWASA, 1982), we reported that the peak of the pressure developed by the squid giant axon, detected with a Gulton bender, coincides fairly accurately with the peak of the intracellularly recorded action potential. This finding strongly suggests to us that the swelling which takes place during the action potential is not a mere epiphenomenon associated with potential changes or ion fluxes. Since the temporal relationship between the mechanical and electrical events is of prime importance in analyzing the causal relationship between these two events, we have re-examined the previous results by using our new mechano-electric transducer described in MATERIALS AND METHODS.

The diagram in Fig. 1, left, shows the arrangement of the mechanical and electrical recording systems employed. The stylus for detecting pressure changes was located directly above the tip of the intracellular recording electrode. Mechanical and electrical responses of the axon were recorded alternately using stimulating shocks of the same intensity. By shifting the base-lines of the two types of records, it was shown that there was nearly perfect coincidence between the peaks of the two responses. However, the downward deflection of the mechanical response, representing shrinkage of the axon, was far more pronounced than the undershoot of the action potential (see DISCUSSION).

Judging from the frequency response of the mechanical and electrical recording systems employed, the time resolution of these measurements is approximately 0.1 msec. These findings are quite consistent with those of our previous studies.

2. Mechanical changes induced by high-frequency stimulation of the axon

Prolonged, high-frequency stimulation of the axon is known to bring about a small increase in the axon diameter (HILL, 1950; LIEBERMAN, 1969). It is therefore significant to study mechanical responses associated with bursts of action potentials. In the present study, the movement of a small piece of gold leaf placed on the surface of the axon was detected by the use of a modified Fotonic sensor.

A giant axon was mounted in a nerve chamber with a convex bottom. The cleaned surface of the axon was kept approximately 2 mm below the surface of the seawater in the chamber. The distance between the gold leaf and the tip of the sensor was adjusted to the most sensitive portion of the output-distance curve (see TASAKI et al., 1980). Through a pair of metal wire electrodes placed near one end of the axon, bursts of electric shocks were delivered to the axon. The interval between individual shocks in the burst was varied in the range between 3 and 5 msec. The bursts were repeated at intervals of about 200 msec. The amplifier connected to the detector had a 60 db gain in the frequency range between 0.08 and 10,000 Hz.

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Fig. 2. Left: movements of the axon surface associated with five propagated action potentials evoked at 5 msec intervals. Right: similar record taken (from a different axon) at a higher frequency of stimulation. An upward displacement of the trace indicates an outward displacement of the axon surface.

As can be seen in the two examples of the records obtained by this procedure (see Fig. 2), the time-course of the mechanical response of the axon associated with the second, third,... action potentials was not significantly different from that accompanied by the first. At the end of a burst of action potentials, a slow recovery of shrinkage followed by a small transient swelling, which lasted for about 10 msec, was frequently observed (see the left-hand record). In many other axons, however, no clear optical sign of cumulative after-effects of repetitive stimulation was observed (see the right-hand record).

It should be noted that optical signals which last much more than about 100 msec could not be detected by the present experimental procedure because the mechanical responses were averaged by repeating at 200 msec intervals. Nevertheless, it seems safe to conclude that the mechanical responses we have described are not directly related to the phenomenon reported by Hill (1950). (Note that Hill (1950) reported that 12,000 impulses produced an approximate 0.2 µm diameter increase in the cuttlefish axon of 240 µm diameter; this effect is about 0.2 nm for 12 stimuli, which is below the noise level of our measurement.)

3. Mechanical responses associated with chemically induced, recurrent action potentials

Many chemical stimulants, applied to the external surface of the giant axon, induce action potentials that repeat at a frequency between 100 and 150 Hz at room temperature. The simplest technique of inducing recurrent action potentials is to reduce the external divalent cation concentration. It is relatively easy to record mechanical responses of the axon associated with such recurrent action potentials. When the signal averager is triggered by the onset of amplified action potentials, averaged records of mechanical responses can be obtained every 10 to 20 sec. In freshly excised axons immersed in a solution containing approximately 2 mM CaCl₂ and 500 mM NaCl (at pH 8.0), repetitive firing of action potentials usually lasts for several minutes.

Record A in Fig. 3 was obtained by using our modified Fotonic sensor for detection of mechanical changes. It is seen in the figure that, during sustained repetitive firing, the pattern of the mechanical responses that appeared on the
Fig. 3. A: movement of the axon surface associated with repetitively fired action potentials. Two traces of the mechanical responses, taken at an interval of about 40 sec, are shown in the figure, in order to demonstrate that there was little progressive change in the state of the axon under study. B: pressure changes accompanied by repetitive firing of action potentials. C: the upper trace represents changes in birefringence during repetitive firing. Upward deflections indicate swelling (for left and middle records) or a decrease in light intensity transmitted through the axon (right record). The lower trace represents extracellularly recorded action potentials. Approximately 20°C.

Record B in Fig. 3 was obtained by using a Gulton bender for detection. The amplitude of the first phase of the mechanical response (representing a rise in pressure) was slightly enhanced by the decrease in the concentration of external calcium. Nevertheless, the general pattern of the response was not significantly different from that of the response associated with a single propagated action potential. It is to be noted, however, that the rapid rise in pressure seen in the record is also preceded by a slow, continuous rise in pressure during the interval between successive responses.

The upper trace in Fig. 3, C, shows a record of birefringence responses of an axon immersed in the same salt solution (containing 2 mM CaCl₂ and 500 mM NaCl at pH 8.0). The axon under study was placed between two Polaroid sheets (HN series) arranged in a crossed position, and the long axis of the axon was fixed at about 45° to the polarizing axes of the Polaroid sheets. The downward deflection of the upper (optical) trace represents a decrease in the light intensity. The lower trace in the figure represents the electrical responses recorded externally near the site of optical recording. It is interesting to note that the birefringence responses are also diphasic (see Cohen et al., 1968). Again, we see that the rapid change in the axon birefringence was preceded by a slow gradual change. A plausible physicochemical basis for the observed diphasicity is presented in DISCUSSION.
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4. Mechanical changes generated by transmembrane electric current

We now examine rapid mechanical changes generated by application of a pulse of electric current through the axon membrane. To apply electric currents to the axon, we employed either intracellular or extracellular electrodes. The setup using a pair of intracellular electrodes is illustrated schematically in Fig. 4, top. The current electrode was a 50 µm-thick enameled platinum wire, of which a 12 mm-long portion near the tip was platinized after removing the enamel. Changes in the membrane potential generated by the applied current pulses were recorded with another enameled platinum wire with a 1 mm-long scraped (and platinized) portion located in the middle of the current electrode. The set of these electrodes was held with a micromanipulator and was inserted longitudinally into a 30 mm-long giant axon. The tip of the stylus for detecting pressure changes was placed on the axon surface above the site of potential recording. Measurements of pressure changes were carried out after lowering the stylus to a point about 100 µm below the point of initial contact with the axon surface. Changes in the membrane potential were monitored by connecting the potential electrode to an oscilloscope through a voltage-follower.

It is seen in the figure that, in response to a pulse of constant current directed inwardly through the axon membrane, the pressure exerted by the axon on the stylus fell gradually. The time course of the observed pressure change was not very different from the change in the membrane potential. The amplitude of the pressure change increased with the current intensity. Upon termination of the current pulse, an action potential was generated (break excitation). As expected, a markedly diphasic mechanical response accompanied the action potential.

The mechanical change generated by a pulse of outwardly directed current of a subthreshold strength generated a very small rise in pressure; it was difficult
to measure the magnitudes of pressure changes because of the high noise level in the record. In response to a strong pulse of outwardly directed membrane current, an action potential was generated after a short latency. The swelling phase of the mechanical response associated with this action potential was very similar to that of the response accompanied by a propagated action potential. The amplitude of the shrinkage phase of the mechanical response observed under these conditions was significantly reduced by the applied current. The magnitude of the pressure change observed during the passage of a constant outward current (following production of an action potential) was very low. Very similar results were obtained by using a Fotonic sensor instead of a PVDF film. The results obtained with extracellular current electrodes are described below.

5. **Effects of transmembrane current on mechanical responses**

The effect of a constant transmembrane current on the mechanical response of the axon can be studied by superposing a brief, strong current pulse on a long polarizing current pulse. With a view toward circumventing the possible source of artifacts caused by electrolysis at the surface of an intracellular metal electrode, extracellular electrodes arranged in the manner described previously (Fig. 3 in TASAKI et al., 1980) were used in this series of experiments. The detector employed was either a Fotonic sensor or a piezoelectric bender. Figure 5 shows an example of the results obtained with a Fotonic sensor.

The trace marked “1” in the figure represents a diphasic mechanical response associated with a strong brief pulse alone. The trace marked “2” shows the response induced in the same axon by a long current pulse alone: this response is evidently similar to that shown in Fig. 4, left. Trace “3” indicates the results

![Fig. 5](image-url)

**Fig. 5.** Records showing the effects of transmembrane currents on mechanical responses of the axon. The current was applied with extracellular electrodes. The lowest record (marked 1) shows a mechanical response associated with an action potential evoked by a brief shock (delivered at the moment marked) alone; the middle record, 2, was taken with a 0.5 μA current pulse alone; the uppermost record, 3, shows the response obtained when both a brief shock and a constant current pulse were delivered. The direction of current flow was either inward (left) or outward (right). The duration of the constant current pulses, 10 msec. 20°C.
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obtained by delivering both the long and brief pulses without changing either the temporal relation of the two pulses or the pulse intensities. It can be seen that, in the presence of a constant inward current, the amplitude of the mechanical response associated with the action potential evoked by the brief pulse was markedly enhanced. This enhancement was characterized by an increase in the amplitude of the first (swelling) phase without being accompanied by an increase in the size of the second (shrinkage) phase. (Note that, under the influence of an inward current, the rate of development of the action potential declined appreciably.)

The effect of a constant current pulse on the mechanical response of the axon increased with the current intensity. Qualitatively, the results obtained by using a piezoelectric transducer were similar to those described above.

Outwardly directed current pulses through the axon membrane brought about a distinct reduction in the amplitude of the mechanical response associated with the action potential evoked by the brief pulse (see Fig. 5, right). Because of this reduction, it was difficult to obtain accurate measurements of the response amplitudes under these conditions.

6. Changes in tension developed in the longitudinal direction associated with propagated action potential

The device used for measuring changes in the tension developed by a giant nerve fiber in the longitudinal direction during action potential propagation was the same as that employed in a similar measurement carried out on crab nerves (TASAKI and IWASA, 1980). After removing all the small nerve fibers, an approximately 40 mm-long giant nerve fiber was suspended vertically in a plastic chamber. The chamber was provided with three partitions in its lower half. The upper half of the chamber was filled with either artificial seawater or a solution containing 20 mM CaCl₂ and 500 mM NaCl. The lower end of the fiber was fixed to the bottom of the chamber and the upper end was connected to a piezoelectric bender located above the chamber by means of a fine thread. Brief stimulating pulses (50 μsec in duration and 1.5 times the threshold) were delivered across the lowest partition at intervals of about 100 msec. Propagated action potentials were monitored during signal averaging by means of extracellular electrodes placed across the uppermost partition.

It was found by this procedure that, in a wide range of initial tension (0.2–1 g), the axon gives rise to a diphasic tension change, a rise in tension followed by a fall (Fig. 6, left). The peak amplitude of the tension (rise) observed was between 2 and 3 μg at room temperature (19–21°C). The amplitude ratio between the positive and the negative phase of the mechanical response of this type varied in the range between 1:2 and 1:5 at room temperature.

When the temperature of the medium was lowered to about 6°C, there was a marked prolongation of the duration of response, accompanied by an enhancement of the positive (i.e., shortening) phase. There was usually only a slight increase in the amplitude of the negative phase.
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Fig. 6. Left: records showing changes in the tension developed by an axon (in the longitudinal direction) associated with action potential propagation, measured first at 22°C and later from the same axon at 6°C (twice at an interval of about 5 min). Right: records showing the effects of cooling on the mechanical response (detected in the radial direction).

The prolongation of the positive phase of a response of this type by cooling may be attributed to the reduction of conduction velocity in combination with the increase in length of the portion of the axon in the excited state. Reflecting the effect of cooling on the duration of the action potential, the duration of swelling (in the radial direction of the axon) is expected to be markedly increased by cooling. In order to verify this expected temperature effect, the (radial) swelling of the axon was determined at two temperatures (see Fig. 6, right). These measurements showed that the duration of the swelling phase at 6°C was approximately four times as long as that at 20°C. Since the conduction velocity is reduced by a factor of about two under these conditions, it is clear that the length of the axon in the excited state is roughly doubled by cooling to 6°C.

Based on the findings described above, it is inferred that the shortening of the axon is directly related with the swelling. In partially cross-linked polyelectrolyte gels, it is known that radial swelling is accompanied by longitudinal shrinkage (see e.g., p. 10 in KATCHALSKY, 1954).

DISCUSSION

As the starting point of our attempt to explain the experimental results, as described in RESULTS, on physicochemical and morphological bases, we first consider the phenomenon of swelling in inanimate colloidal and polymer gels. It is well known that the swelling of gels (i.e., invasion of gels by water) is caused mainly by the difference between the osmotic pressure of the small ions inside the gels and that of the ions outside (see e.g., p. 563 in HERMANS, 1949; p. 24 in KATCHALSKY, 1954). Replacement of univalent cations by divalent cations in acidic polyelectrolyte gels is known to produce enormous swelling of these gels (see e.g., p. 81 in LOEB, 1922; KATCHALSKY and ZWICK, 1955; p. 103 in HELFFERICH, 1962). Among common univalent cations, the ability to induce swelling in various biocolloids is known to increase in the following order (see p. 564 in HERMANS, 1949; KATCHALSKY, 1954): K<Na<Li. Qualitatively, most of these facts were known to LOEB (1906, 1922) and HÖBER (1926) who proposed the colloid chemical theory of nerve excitation.
According to Loeb, exchange between Ca-ions and univalent cations within the nerve fibers is at the base of the process of nerve excitation. In essence, he postulated that the colloidal material in the superficial layer of the nerve fiber becomes compact when it is rich in Ca-ions; replacement of these Ca-ions by univalent cations loosens up the colloidal structure. Höber (1926), as well as Bethe (1920), emphasized that the portion of the nerve around the extracellular stimulating anode is in a Ca-rich, compact state; the portion around the cathode is, in contrast, in a K-rich, loosened state.

Recently, the biochemistry of Na-, K-, and Ca-ion has been discussed extensively by Williams (1970). He emphasizes at the outset that both K- and Na-ions are highly mobile in protein molecules, while Ca-ions are only semimobile. The existence of a large difference in mobility between the Ca-ion and Na-ion in the superficial layer of the squid axon is well known (Hodgkin and Keynes, 1957; Tasaki et al., 1967).

Next, we consider the axonal structure involved in the production of the mechanical changes described in this paper. According to the consensus reached in a recent international conference on “Structure and Function of Excitable Cells” (see Chang et al., 1982), the integral protein molecules which penetrate the lipid portion of the axolemma are intimately connected to the ectoplasmic layer of the axon. The ectoplasm contains a high density of fibrillar elements consisting mainly of neurofilaments, microtubules and actin filaments. The layer of ectoplasm, in conjunction with the axolemma, plays a crucial role in the maintenance of normal excitability (Metuzals and Tasaki, 1978). The amplitude of the mechanical response is not seriously affected by removal of almost all the endoplasm (Tasaki and Iwasa, 1982).

The results of physicochemical and morphological studies mentioned above provide a reasonable starting point for explaining our experimental results. However, it should be noted that the “regenerative” aspect of the process of initiating an action potential cannot be explained without additional assumptions. We believe that, when the old colloid chemical theory is supplemented by the concept of “cooperative ion-exchange process” involving Ca-ion and univalent cations, the major portion of our experimental findings can be explained. The physicochemical basis of the cooperative processes in the axon has been discussed in some detail in a recent monograph (pp. 265–269 in Tasaki, 1982) and will not be repeated here.

Based on the present state of our knowledge on the physiological properties of the axon, we offer the following tentative explanation of the origin of the mechanical responses. In the resting state of the axon, the integral protein molecules are in a compact, Ca-rich state. In electrical stimulation, a cooperative conformational transition of the integral protein molecule is “triggered” by invasion of intracellular K-ions into the protein molecules. The resulting substitution of the major portion of the Ca-ions in the molecules by Na-ions is expected to bring
about a drastic swelling of these molecules. This swelling, in turn, enhances the mobilities of the cations in the molecules and, consequently, markedly accelerates the influx of extracellular cations and, simultaneously, the efflux of the intracellular cations. Therefore, when an action potential is generated in the axon, the Na-ion concentration in the superficial layer of ectoplasm is expected to rise and fall transiently. The Ca-ion concentration also rises and falls in the layer; however, because of the low mobility of the divalent cations in protein molecules, the rate of rise and fall of the Ca-ion concentration is much lower than that of the Na-ion concentration. From the general properties of polyelectrolytes, therefore, it is expected that the ectoplasm first swells and then shrinks when an action potential is generated in the axon.

The simple explanation of the mechanical response described above can be extended to include the effect of transmembrane electric currents (see Figs. 4 and 5). A strong inward current is carried predominantly by Na- and Ca-ions in the external medium. Within the protein molecules in the superficial layer of the axon, however, Na-ions are the principal charge carrier. As Bethe (1920) had shown a long time ago, this large difference in the transference number leads to a rise in the Ca-ion concentration in the axon. Bethe also pointed out the importance of changes in pH produced by long current pulses. Although the H-ion concentration in the media surrounding the axon membrane is very low, it is probable that the change in the ion concentration, expressed in percentage of the initial concentration, is far greater for the H-ion than for other ions. Thus, the large shrinkage of the axon seen in Fig. 4 is explained as being produced by a rise in the concentration of Ca-ions (and probably of H-ions) in the ectoplasm. When the ectoplasm is in its Ca-rich state, an invasion of a large quantity of Na-ions associated with production of an action potential is expected to bring about a pronounced swelling (see Fig. 5, left).

A weak outward current transports the intracellular K-ions into the axolemma and brings about a partial replacement of K-ions for Ca-ions. In such a slightly swollen state of the axolemma, the mechanical response associated with action potential production is small (see Fig. 5, right).

In a medium containing a low concentration of Ca-ions, there is a gradual rise in the degree of swelling before an action potential is triggered (see Fig. 3). This finding strongly suggests that the phenomenon of swelling plays an important role in initiation of repetitive firing of action potentials. It seems possible that an action potential is initiated when the degree of swelling reaches a critical level. The experimental results shown in Fig. 3 also open up the possibility that birefringence changes associated with production of action potentials can be explained in terms of structural changes in the axolemma-ectoplasm complex.

We emphasize in this connection, that the time-courses of the mechanical responses described in this paper are not simple reflections of the variations in the membrane potentials (see also Tasaki and Iwasa, 1982). In support of our
contention that the swelling and shrinkage of the axon are not produced simply by the changes in the membrane potential, we quote here the results of our recent observations on tetraethylammonium (TEA)-treated axons (IWASA and TASAKI, 1981). We found that injection of TEA does not prolong the swelling phase of the mechanical response appreciably. Following the initial (swelling) phase, the axon shrinks rapidly and progressively. Since the prolonged depolarization phase of the action potential of the TEA-treated axon is accompanied by a large and prolonged shrinkage of the axon, it is evident that the mechanical response cannot be regarded as a simple voltage-dependent phenomenon.

Finally, we see in Fig. 6 that the lowering of temperature decreases the amplitude of shortening (and shrinkage) of the axon at the end of the action potential. We may attribute this effect of cooling to the situation that substitution of Ca-ions for univalent cations is endothermic and unfavorable at low temperatures (see e.g., p. 248 in TASAKI, 1982). Further studies are required to elucidate the complex behavior of Ca-ions in the axon.

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