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POLARITY REVERSAL OF THE OPTICAL ROTATION SIGNALS WITH CHANGE IN DIRECTION OF IMPULSE CONDUCTION ALONG THE LOBSTER NERVE

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SUMMARY

1. The optical rotation signal of nerve associated with excitation was recorded from peripheral nerve taken from a walking leg of a spiny lobster and its properties were analysed.

2. The polarity of the optical rotation signal was reversed when the site of stimulation was changed with reference to the site of optical recording, so that the direction of impulse conduction was reversed, in most of the preparations.

3. Apart from the main response, which is associated with the conducted impulse, a pre-response was found to exist, which manifested itself on anodic stimulation, in a tetrodotoxin-treated nerve, or during the refractory period of the nerve, when the site of stimulation was close to the site of optical recording. The polarity of the pre-response was also reversed when the site of stimulation was changed with reference to the site of optical recording.

4. When the nerve was inclined from the horizontal level, so that the angle of incidence of light to the nerve was changed, the main response changed its amplitude and sometimes its polarity, whereas the pre-response remained practically unchanged. Thus the dependence on the angle of incidence was different between the pre-response and the main response.

5. It is suggested that the dependence of amplitude and polarity of the main response on the angle of incidence of light cannot be explained by the change in molecular axes of the membrane macromolecules, but can only be explained by their conformational change; and therefore the main response can be used as a monitor for the molecular conformation.

INTRODUCTION

Change in optical activity of a macromolecular system is generally regarded as an extraordinarily sensitive indication of molecular conformational change (e.g. Freifelder, 1982; Creighton, 1984). Since the molecular basis for excitation of axonal membrane is believed to be due to conformational changes of the membrane macromolecules (Hille, 1984), it seems worthwhile to examine whether a change in

optical activity is taking place during nerve excitation. In a previous paper (Watanabe, 1987), it was reported that a rotation signal could indeed be recorded from lobster nerve when it was excited. However, the significance of the signal remained obscure, mainly because the polarity of the signal was variable according to preparations; it could be either an increase or a decrease of dextrorotation, and the reason for this variability in polarity of the signal was not explored.

The main purpose of the present paper is to search for causes of this variability. It was found that the polarity of the optical rotation signal is influenced by several factors. The most conspicuous among them is that the polarity changed according to the direction of impulse propagation along the nerve. A possible mechanism for this phenomenon is that the membrane molecules change their directions according to the spatial gradient of the membrane potential before they undergo molecular conformational change associated with the excitation process. Preliminary reports have appeared (Watanabe, 1988, 1989).

METHODS

Materials. The nerve was taken from a walking leg of a spiny lobster (*Panulirus japonicus*). Whenever possible, larger animals weighing more than 700 g were preferred because they gave nerve preparations of larger diameters. Usually a walking leg of the second or third pair was obtained from an animal living in the aquarium by taking advantage of autotomy. The isolated leg was brought to the laboratory and the nerve was dissected from its meropodite. It was found (see Results) that the tension applied to the nerve is an important factor for determining the polarity of the optical rotation signal. To describe the grade of stretch of nerve, the following procedures were taken. Part of the carapace of the meropodite was removed and the leg nerve was exposed. Then, before the nerve was taken out of the carapace, it was tied at two locations, separated by about 45 mm, with two pieces of thread. The distance between the tied portions was measured with the help of a pair of compasses, and recorded as the length *in vivo* (l_v) of the nerve. The nerve was then dissected out, and mounted on a chamber. The distance between the tied portions was again measured and recorded as the mounted length (l_m). The grade of stretch was calculated by $(l_m/l_v)-1$ and expressed as a percentage after multiplication by 100.

Chambers. Several types of chambers were made, all from black lucite plates (Fig. 1). The stretch chamber was the one most often employed. The chamber was furnished with a stretching device, which was constructed after the design described by Eyzaguirre & Kuffler (1955) except that the overall size was smaller and it could be attached to the main part of the chamber with two screws. The nerve could be stretched by turning the knob of the stretching device. The inclination chamber was employed for the inclination experiments (see Results). This consisted of a main part and a base. The main part, which accepted the nerve, had two short brass bars stuck out from two opposite sides of the chamber and acted as the axis of rotation. Two pillows from the base held each of the brass bars. The main part could thus be inclined from the horizontal position by about ± 10 deg.

Optical set-up. This was essentially the same as that described in a previous paper (Watanabe, 1987). A schematic is shown in Fig. 2. The optical elements employed were listed below in the order along the path of the light. (1) Light source, a 300 W halogen–tungsten lamp (Kondo-Sylvania, JC24V). (2) A cold filter (Nihon Shinku, Tokyo, Japan, CF-A). (3) A condenser lens (Spindler & Hoyer, Goettingen, Germany, 03500). (4) A shutter, made of aluminium plate driven by a rotary solenoid. (5) Interference filters (Nihon Shinku, Tokyo, Japan, type IF-S, BP-2 or BP-3), mounted on a brass frame which was movable on an optical bench. (6) A plain mirror, which deflected light from the horizontal direction to the vertical direction. (7) A heat-cut filter (HOYA, Tokyo, Japan, HA-30). (8) A red-suppression filter (HOYA, Tokyo, Japan, CM-500). (9) A depolarizer (Karl Lambrecht, Chicago, USA, SQLO 25). (10) A polarizer, made of Polaroid dichroic filter of the type HN-22 or HN-32. (11) A photoelastic modulator (Nihon Bunko, Tokyo, Japan, PEM-40, or HINDS, Portland, Oregon, USA, FS-5 or FSA). (12) A condenser lens (Spindler & Hoyer, Goettingen, Germany, 063010). (13) The biological specimen mounted in a chamber. (14) An

analyser, similar to the polarizer described above. (15) A photodetector (HAMAMATSU, Hamamatsu, Japan, S1337-66BR).

In many experiments, optical elements were employed which were less strict in selecting incident light to the nerve than those employed in the previous paper (Watanabe, 1987). For example, interference filters of the type BP-3 had a bandwidth of 25 nm, which was much wider than IF-

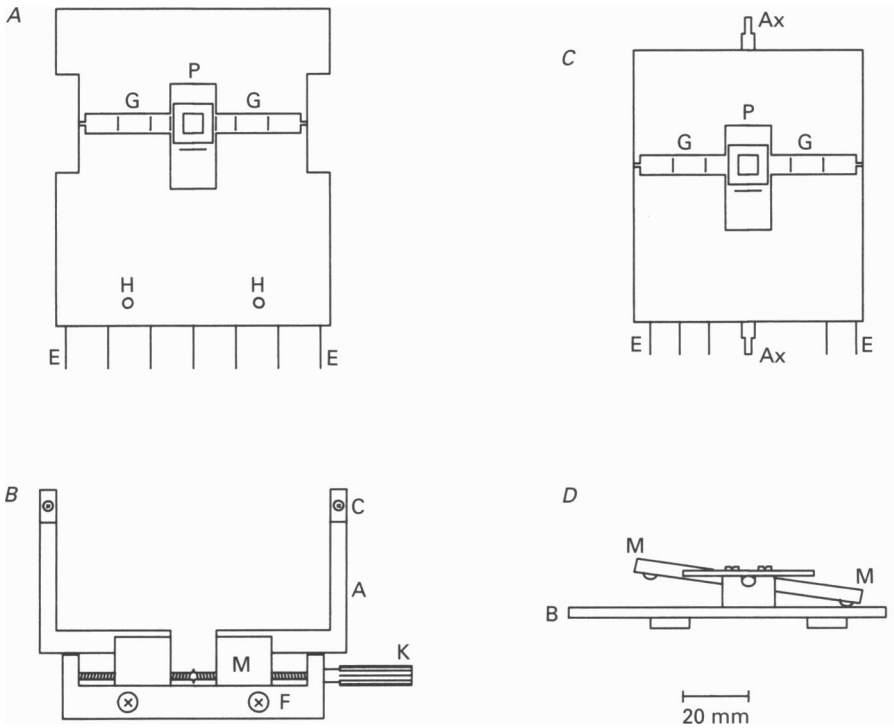

Fig. 1. Experimental chambers. *A*, main part of the stretch chamber, which was milled from 5 mm thick blade lucite. P, the central pool of 4 mm depth. The pool had a square elevation of about 1 mm in height, which received the coverslip, and a square window, opened at the centre of the square elevation, which allowed the incident light to enter. The horizontal bar below the square elevation represents the ground electrode. G, grooves of 2 mm depth to accept ends of the nerve. Six short vertical bars represent electrodes made of platinum wire. H, two threaded holes to accept screws of the stretching device shown in *B*. E, ends of seven pieces of platinum wire, which were continuous with the seven platinum electrodes in the central pool and the side grooves. They served as connection points to the external circuits. *B*, the stretching device of the stretch chamber, made mainly with brass except for the clamps. F, main frame, to be fixed to the main part of the stretch chamber with two screws. M, a movable block of brass by a threaded bar. K, a knob on the threaded bar. A, an arm extended from the movable block. C, a clamp made of pieces of lucite and a lucite screw. *C*, the main part of the inclination chamber. P, the central pool. G, the side grooves. E, ends of platinum wire continuous to electrodes. Ax, two brass bars which worked as the axis of rotation of the main part. *D*, a front view of the inclination chamber. M, the main part of the inclination chamber shown in *C*, which is here shown inclined by -10 deg from the horizontal position. B, the base of the inclination chamber. At about the centre two pieces of lucite plate were stuck vertically to accept the brass bars of the main part of the inclination chamber.

S with a half bandwidth of 8 nm. The results were an enormous increase in light intensity and some improvement in signal-to-noise ratio. On the other hand, the apparatus did not work as an ideal polarimeter, in the sense that the value of the optical rotation calculated from the output of the

lock-in amplifier and the light intensity was less than the real optical rotation of the sample. In practice, the size of the signal was first assessed with the formula

$$\alpha' = 90L/\pi G\Omega V, \tag{1}$$

where α' is the tentatively estimated optical rotation in degrees, L is the lock-in output in volts, G is the gain of the lock-in amplifier, Ω is a mathematical constant 0·625, and V is the output of the current–voltage converter in volts. After the experiment, the apparatus was calibrated with the

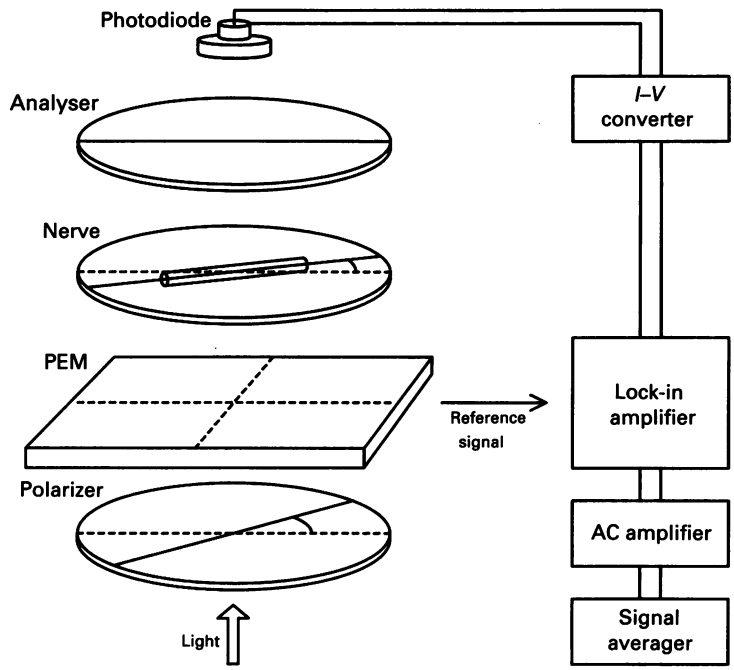

Fig. 2. A schematic of the optical set-up. Stages for the polarizer, nerve and the analyser were made from the specimen stages manufactured for the Nikon polarizing microscope, and were rotatable by 360 deg with an accuracy of 0·1 deg. PEM, the module of the photoelastic modulator which was fixed. The dashed lines on the photoelastic modulator indicate direction of mechanical vibration of a fused silica plate in the module. A reference signal of 100 kHz was sent to the lock-in amplifier from the photoelastic modulator.

sucrose solution filled in a quartz flow cell. The concentration of the sucrose solution was changed and at each concentration the value of α' was calculated. The standard value of the optical rotation was obtained with the formula

$$\alpha = 0{\cdot}0741\,DC/(\lambda^2 - 0{\cdot}0213), \tag{2}$$

where α is the optical rotation in degrees, D is the optical path of the sample in millimetres, C is concentration of sucrose in moles per litre, and λ is the wavelength in micrometres. The formula is based on Lowry (1935, p. 131). The calibration ratio (R_L) was then calculated:

$$R_L = \alpha'/\alpha. \tag{3}$$

The tentative value α' was divided by R_L to get the real value of the signal. The value of R_L varied between 0·6 and 0·9 depending on experimental conditions.

For the polarizer, only the linearly polarizing Polaroid filter was employed, and the use of the circular Polaroid filter was discontinued, because of several advantages of the linear polarizer. One of them was that approximate values of the resting retardation could be easily assessed with the use of the linear polarizer. The procedure was as follows. The photoelastic modulator was turned off. The nerve was placed with its longitudinal direction at azimuth 0 deg. The azimuth of the analyser transmission axis was fixed at 45 deg. Firstly, the azimuth of the polarizer transmission axis was also set at 45 deg. This was called the parallel setting, and the light intensity, I_p, at this setting was recorded. Then the polarizer was rotated by 90 deg and the azimuth of its transmission axis was brought to -45 deg. This was called the cross-polar setting, and the light intensity, I_x, at this setting was recorded. Finally the polarizer was rotated back and the parallel setting was resumed, and again I_p was recorded. When the second I_p was different from the first by more than 2%, the measurement was repeated. Assuming that the resting optical rotation is small in comparison with the resting retardation, the resting retardation was calculated with the use of the following equation (Jerrard, 1948, his eqn (5)):

$$I/I_0 = \cos^2(a-p) - \sin 2a \sin 2p \sin^2(\delta/2), \tag{4}$$

where I is the intensity of the transmitted light, I_0 is the intensity of incident light, a the azimuth of the analyser, p the azimuth of the polarizer, and δ the retardation of the sample. From eqn (4) one can deduce that $I_p/I_0 = 1-\sin^2(\delta/2)$ and $I_x/I_0 = \sin^2(\delta/2)$ and therefore

$$\delta = 2\sin^{-1}[I_x/(I_x+I_p)]^{\frac{1}{2}}. \tag{5}$$

For recording of the optical rotation signal, the azimuth of the transmission axis of the polarizer was usually set at -45 deg (Watanabe, 1987, see p. 230). However, in some experiments adopted in this paper, the azimuth was set at an angle between -50 and -70 deg (see Fig. 9). The procedure was tried because of an expectation that it might increase the signal-to-noise ratio of the apparatus; it reduced the amount of light accepted by the photodetector without too much reduction of the lock-in output (see Gillham, 1956). In practice, however, this procedure decreased the signal-to-noise ratio of my apparatus, and therefore was discontinued after some period of trial.

The normal saline employed was an artificial sea water (mM): NaCl, 470; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 50, Tris-Cl buffered, pH 8·0–8·5). The experiment was performed in a temperature-regulated room. Usual experimental temperature was 10–15 °C, measured by a thermistor probe inserted in the saline of the chamber and placed at several millimetres from the nerve.

RESULTS

Stimulus site-dependence of signal polarity

Dependence of the polarity of optical rotation signal on the site of stimulation

In most of the nerve preparations examined, the polarity of the optical rotation signal depended on the place of stimulation. In Fig. 3, one example is presented. When the stimulus was applied at the distal end of the nerve (Fig. 3*A*), a sharp positive-going signal was obtained, which was followed by a smaller negative phase. When the stimulus was applied at the proximal end (Fig. 3*D*) a reversed signal appeared, which looked almost a mirror image of the signal elicited with the distal stimulation. Birefringence signals taken from the same nerve had the same polarity irrespective of the site of stimulation (Fig. 3, lower traces of *C* and *F*).

When the stage for the chamber was rotated slightly so that the azimuth of the longitudinal direction was changed, the optical rotation signal was superimposed on a birefringence signal (Watanabe, 1987). In Fig. 3*B* and *E*, the azimuth of the nerve was changed by 0·25 deg counter-clockwise from those of *A* and *D*. The slow downward deflections, which appeared at the later phase, were due to birefringence change. In spite of the reversed polarity of the optical rotation signals in Fig. 3*B* and

E, the contribution from the birefringence change appeared with the same polarity. It strengthens the idea that the optical rotation change and the birefringence change are independent of each other. In Fig. 3*C* and *F* the nerve was rotated clockwise by 0·25 deg from the position for *A* and *D*. The slow birefringence components appeared

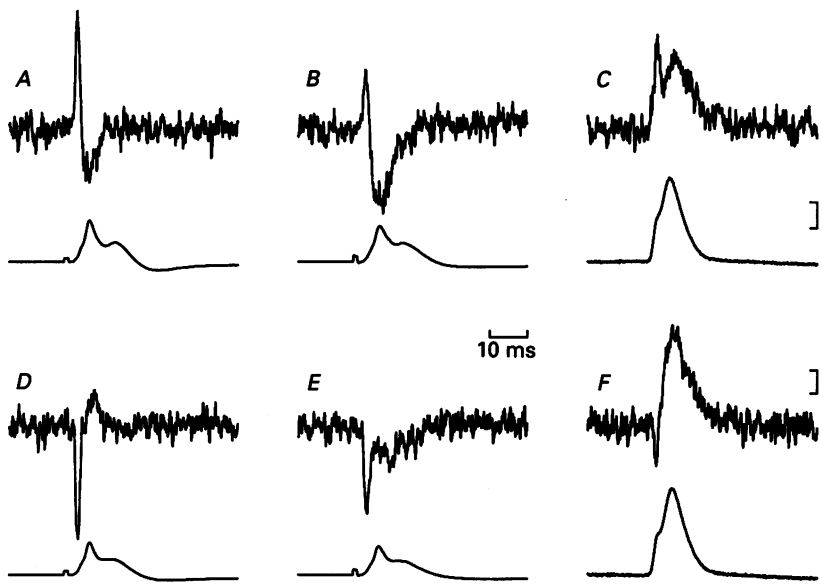

Fig. 3. Optical rotation signals elicited by proximal and distal stimulations. *A*, *B* and *C* are responses to distal stimulation. *D*, *E* and *F* are responses to proximal stimulation. Azimuth angle of nerve was 2·00 deg in *A* and *D*, 2·25 deg in *B* and *E*, and 1·75 deg in *C* and *F*. Upper traces show the optical rotation signal. Lower traces in *A*, *B*, *D* and *E* show the externally recorded action potentials. Lower traces in *C* and *F* show the birefringence signal. Vertical bar in *F* shows 2×10^{-5} deg, applicable to all the optical rotation records (upper traces in *A*–*F*). Vertical bar in *C* shows 10^{-4} of the background light intensity, applicable to the birefringence signals (lower traces in *C* and *F*). 550 nm. Resting retardation, 72·8 deg. Stretch, 6·7 %. 10·5 °C. (In this and the following figures, an upward deflection of the optical rotation signal represents an increase in dextrorotation, unless otherwise noted; and an upward deflection of the birefringence signal or absorption signal represents a decrease in intensity of light received by the photodiode.)

as upward deflections, again with the same polarity irrespective of the difference in site of stimulation, in contrast with the optical rotation signals which are seen to be reversed.

Effects of rotating the nerve by 180 deg

It was suspected that the dependence of the response polarity on the site of stimulation might be due to some lack of symmetry of the optical system rather than the one inherent in the biological preparation. To examine this possibility, the chamber was turned by 180 deg so that the whole of the optical system (except for the chamber) was reversed with reference to the preparation. Figure 4 shows an example of the result of such an experiment. In this preparation, proximal

stimulation always elicited an optical rotation signal with a positive–negative polarity, whereas distal stimulation elicited the one with a negative–positive polarity. It did not depend on the position of the nerve relative to the laboratory. The experiment shows that the difference in the optical rotation signal has a cause

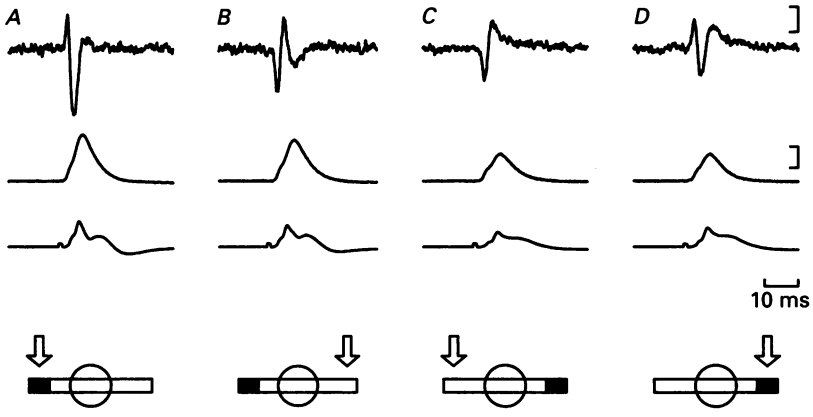

Fig. 4. Effect of 180 deg azimuth change on the phenomenon of conduction reversal. *A* and *D*, proximal stimulation. *B* and *C*, distal stimulation. The azimuth of the nerve was 1·0 deg in *A* and *B*, and 181·0 deg in *C* and *D*. Upper traces: optical rotation signals. Calibration in *D* represents 10^{-4} deg. Middle traces: birefringence signals. Calibration in *D* represents 2×10^{-4} of the background light intensity. Lower traces: externally recorded action potentials. The schematics at the bottom of the figure show experimental arrangements. The open horizontal bar with a filled end represents the nerve. The filled end shows the proximal end. The middle circle shows the site of illumination for the optical recording. The arrow indicates the site of stimulation. 550 nm. Resting retardation, 74 deg. Stretch, 10%. 14 °C.

in the nerve. For brevity of description the phenomenon will later be called the 'conduction reversal' of the optical rotation signal, since the reversal took place when the direction of impulse conduction was reversed.

Disappearance of the optical rotation signal during refractory period

Because the nerve taken from the walking leg of the lobster is composed of multiple nerve fibres, it is possible that proximal or distal stimulation elicited excitation in two different groups of nerve fibres, with different optical properties, exhibiting a positive or negative optical rotation response. To examine this possibility, the nerve was stimulated at both proximal and distal ends with some interval so that the later stimulus was applied during the refractory period caused by the earlier stimulus. Figure 5 shows an example, showing that the later stimulus produced no response except for tiny residual signals, which were shown in Fig. 5*C* and *G*, as the differences of the above traces, respectively. It is concluded that the conduction reversal is not a result of stimulating different groups of nerve fibres by proximal or distal stimulation. In each of the nerve fibres conduction reversal must be taking place.

The residual responses (Fig. 5*C* and *G*) are probably the 'pre-responses', which will be described below. There it will be shown that they survive even during the refractory period of the preceding response.

Addition and subtraction procedure

The conduction reversal of the optical rotation signal was observed in about 85% of the preparations examined. However, the reversal was often not so complete as

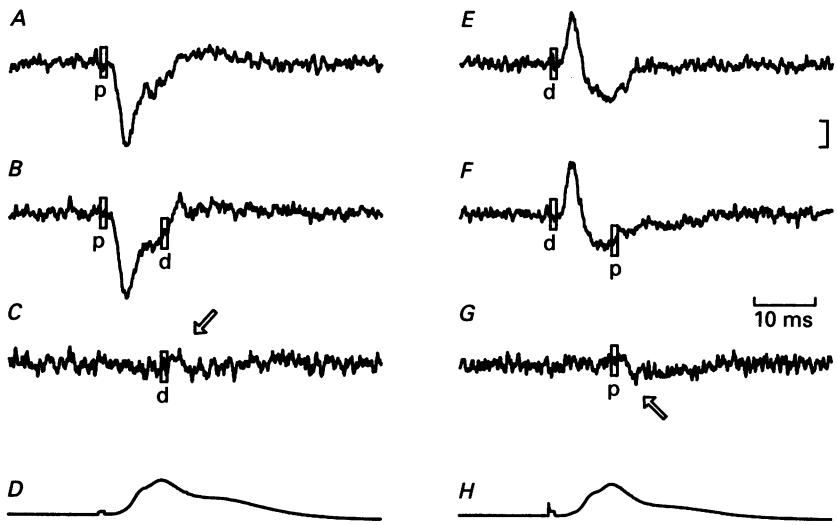

Fig. 5. Effect of refractoriness on the optical rotation signal. *A*, *B*, *E* and *F* are optical rotation signals. *A*, proximal stimulation. *B*, proximal stimulation followed by distal stimulation. *E*, distal stimulation. *F*, distal stimulation followed by proximal stimulation. *C* is obtained by subtracting record *A* from record *B*. *G* is obtained by subtracting record *E* from record *F*. Arrows in *C* and *G* point to the 'residual responses'. Time and duration of the stimulus are shown by open bars, with letter 'p' (proximal stimulation) or letter 'd' (distal stimulation). Calibration between *E* and *F* represents 5×10^{-5} deg. *D*, action potential recorded externally at the same time as *A*. *H*, action potential recorded externally at the same time as *E*. 550 nm. Resting retardation, 51·8 deg. Stretch, 4·3%. 8 °C.

that seen in Fig. 3. In many preparations the absolute values of the amplitudes were different according to the site of stimulation, as seen in Fig. 4. Moreover, about 15% of the preparations did not show any recognizable conduction reversal and were therefore classified as exceptions (Fig. 6).

To get some insight for these variabilities, a simple assumption was adopted. Each optical rotation signal is regarded as a summation of two components: one of them shows no dependence on the direction of impulse conduction, while another shows a perfect conduction reversal. The separation of a signal into these two components can be achieved by adding and subtracting the two signals, one recorded with proximal stimulation and another with distal stimulation, and by dividing them by two. For brevity, the former will be called the sum signal, and the latter the

difference signal. Examples of the result of such manipulations are presented in Fig. 6. They exhibit the natural tendency that, when the conduction reversal is almost complete, the difference signal is much larger than the sum signal, and when the conduction reversal is only poorly manifested, the difference signal becomes

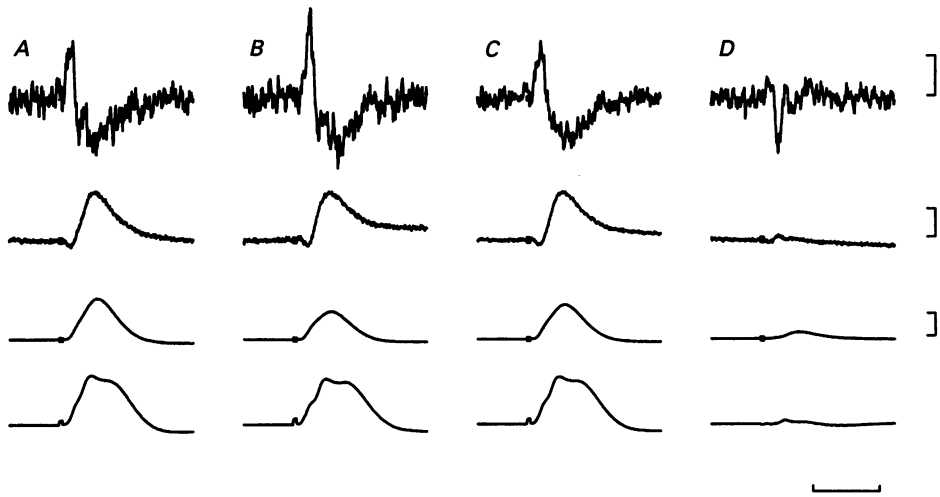

Fig. 6. Optical rotation signals which did not show the conduction reversal. *A*, proximal stimulation. *B*, distal stimulation. *C*, the sum signals, constructed by addition of each of the records in *A* with the corresponding records in *B* and by division by two. *D*, the difference signals, constructed by subtraction of each of the records in *B* from the corresponding records in *A* and by division by two. Top traces, optical rotation signals. Calibration in *D* shows 5×10^{-5} deg. Second traces, absorption signals. Calibration in *D* shows 5×10^{-5} of the background light intensity. Third traces, birefringence signals. Calibration in *D* shows 2×10^{-4} of the background light intensity. Bottom traces, externally recorded action potentials. 550 nm. Resting retardation, 49 deg. Stretch, 8·7 %. 10 °C.

comparable with the sum signal. In all the preparations examined, however, the difference signal never became very small in comparison with the sum signal. This is in sharp contrast with the birefringence signal, which always showed the difference signal with amplitudes of less than several per cent.

It is therefore possible to state generally that the existence of a large difference signal is a characteristic of the optical rotation signal. The existence of the difference signal probably indicates that the events taking place in the membrane are not exactly the same when the direction of impulse conduction is different.

In the later stage of the experiment, it was experienced that the rate of appearance of the exceptions for the conduction reversal was considerably reduced from the previous stage. Thus among the last twenty-three preparations the exceptions appeared only twice (8·7 %). The recognizable differences in experimental conditions were twofold. Firstly, animals of larger size (around 900 g) could be used, which supplied nerve bundles with larger diameters. Secondly, the tension applied to the nerve was limited to less than 3 %. Care had also been taken not to distort, twist or

bend the nerve bundle. It seems that change in gross shape of the nerve from the *in vivo* situation tends to increase the rate of exceptions, probably by increasing inhomogeneity of the illuminated portion of the preparation.

Pre-responses

Effects of anodic pulse on the optical rotation signal

In some preparations, an anodic shock, applied at an electrode near the site of optical recording, produced an optical response which took the opposite direction to

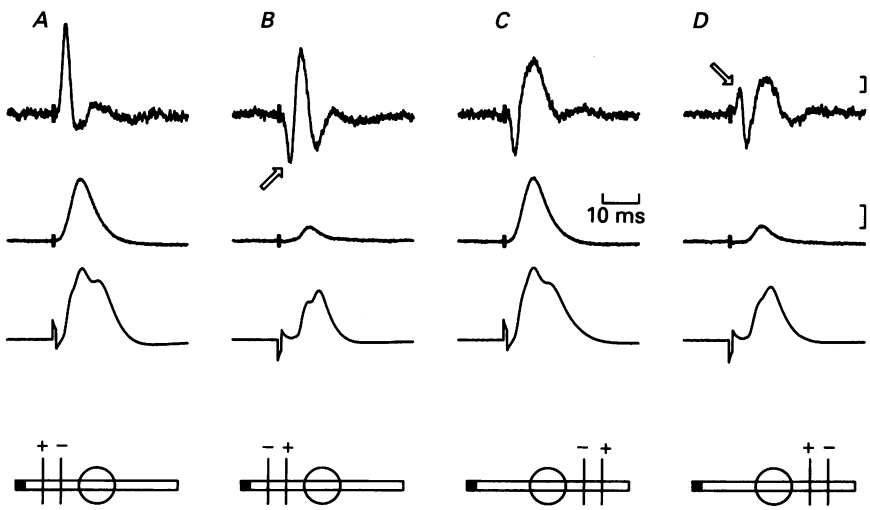

Fig. 7. The example of the anodic pre-response. *A* and *B*, proximal stimulation. *C* and *D*, distal stimulation. In *A* and *C* the stimulus is cathodic. In *B* and *D* the stimulus is anodic. Upper traces, optical rotation signals. The arrows in *B* and *D* point to the anodic pre-responses. Calibration in *D* represents 10^{-4} deg. Middle traces, birefringence signals. Calibration in *D* represents 10^{-4} of the background light intensity. Lower traces, externally recorded action potentials. The schematics at the bottom show experimental arrangements with symbols similar to those in Fig. 4. Two vertical bars indicate stimulating electrodes. 550 nm. Resting retardation, 104 deg. Stretch, 6·8%. 10 °C.

responses evoked by cathodic shocks. One example is shown in Fig. 7. A cathodic shock applied on the proximal side produced a positive optical rotation signal. On flipping the polarity of the stimulus, a roughly triphasic response was recorded. The initial phase was negative with a brief latency. The later phases were similar to the response to cathodic stimulation, which are probably to be interpreted as the one associated with the anode-break excitation. The distal end stimulation evoked a similar series of events, but the polarities were reversed to those evoked by proximal end stimulation at least for their initial phases.

It was often difficult to interpret the waveform of the response to the anodic shock, especially its later phase, because the compound nature of the nerve bundle had to be taken into consideration. However, it is certain that anodic pulse produces a quick signal which is opposite in polarity to that in response to cathodic pulse. In the following the response will be called the 'anodic pre-response'.

Check for artifacts

Before proceeding further it was thought desirable to examine if the anodic pre-response might not be some kind of artifact. It was similar to an electrical artifact because its latency was short, it appeared with anodic pulse, and its size was

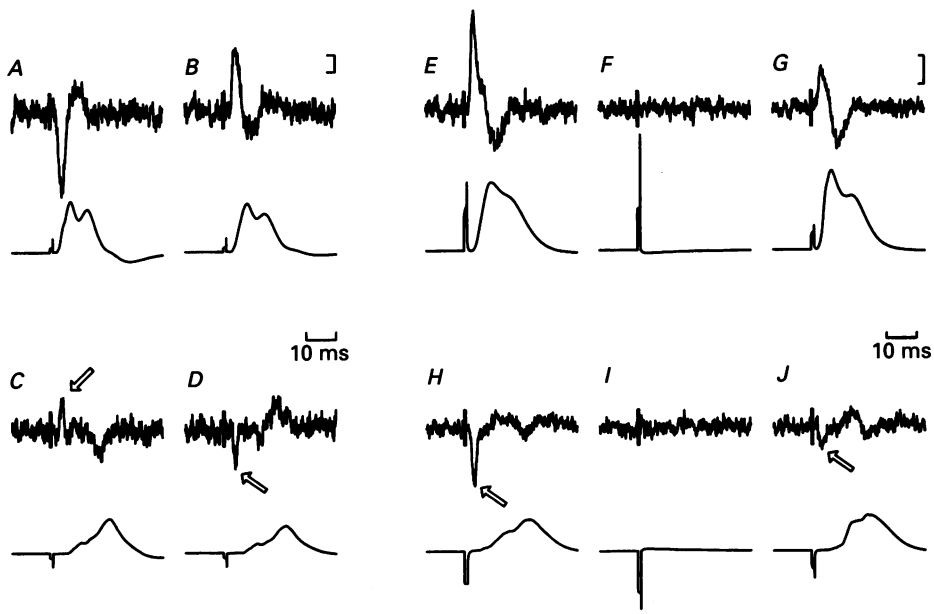

Fig. 8. Tests of artifacts for the pre-response. *A–D* shows an experiment in which the polarizer azimuth was changed from −65 to +65 deg. *A* and *C*, signals taken with polarizer azimuth at −65 deg. *B* and *D*, signals taken with polarizer azimuth at 65 deg. (Note that in *B* and *D* an upward deflection represents a decrease in dextrorotation.) The stimulus was cathodic in *A* and *B*, and anodic in *C* and *D*. Upper traces show the optical rotation signals. Arrows in *C* and *D* point to the anodic pre-responses. Calibration in *B*, 2×10^{-5} deg. Lower traces show externally recorded action potentials. 550 nm. Resting retardation, 84 deg. Stretch, 2·8%. 10 °C. *E–J* show an experiment in which the external medium was replaced with potassium-rich solution. In *E*, *G*, *H* and *J* the external medium was artificial sea water (pH 8·3). In *F* and *I* the external medium contained 150 mM potassium glutamate, 4 mM $MgCl_2$ and 828 mM sucrose (pH 7·9). Upper traces show the optical rotation signals. Lower traces show the externally recorded action potentials. In *E*, *F* and *G* the stimulus was cathodic. In *H*, *I* and *J* the stimulus was anodic. The arrows in *H* and *J* point to the anodic pre-responses. Calibration in *G* represents 5×10^{-5} deg. 550 nm. Resting retardation for records in *E* and *H* was 108 deg. Stretch, 12%. 9 °C.

approximately proportional to the stimulus intensity. Figure 8*A–D* shows that the response produced by anodic pulse reversed when the azimuth of the polarizer was set at an angle which is opposite to the axis of nerve. Since the procedure of changing the polarizer azimuth is to rotate the round metal stage with a piece of Polaroid film at the centre, it would not change the electrical environment around the nerve significantly. In fact, no change in stimulus artifact is noticed in the action potential records (compare action potential records in Fig. 8*A* and *B*, or *C* and *D*). On the other hand, the theory for this apparatus predicts that the procedure reverses some of the

optical signals (Watanabe, 1987). The experiment therefore shows that the origin of the signal is optical rather than electrical. But its rapid appearance and its size preclude the possibility that it comes from other optical signals, e.g. the birefringence signal or the zero-degree scattering signal, rather than the change in optical rotation.

In Fig. 8*E–J*, another source of the artifact was examined. It shows that the anodic pre-response reversibly disappears when the outside medium was replaced by a potassium-rich solution. The result suggests that the pre-response is not the one produced from some direct action of the stimulating current to the axoplasm of the nerve. The response comes from the membrane, because when potassium depolarized the membrane the response stopped appearing.

The experiments described above indicate that the anodic pre-response is a kind of optical rotation signal. Although it reveals its existence with application of an anodic pulse, the same kind of the response with reversed polarity probably consists of the initial part of all the optical rotation signals. For example, the optical rotation signal shown in Fig. 8*A* consists of two parts, one which is the mirror image of the anodic pre-response, and another, which superposes on the former to create a larger positive deflection. These two components will hereafter be called the 'pre-response' and the 'main response', respectively.

Effects of tetrodotoxin

Tetrodotoxin at a concentration of 1 μM abolished the conducted action potential, but the pre-response was not suppressed with this drug. In Fig. 9*A–D*, one such example is presented. In this preparation, too, a K^+-rich external solution eliminated the pre-response, and some recovery was obtained when the K^+-rich solution was replaced by the tetrodotoxin-containing artificial sea water. An incomplete recovery of the action potential and optical rotation signal was noticed after washing with the artificial sea water for about 2 h.

Distance between electrodes and the site of optical recording

The pre-response is probably produced by an electrotonic spread of the stimulating current toward the region of the nerve at the site of the optical window. It should therefore be reduced if the stimulating electrodes would be withdrawn to some distance away from the site of the optical recording. In Fig. 9*E–H*, one such experiment is shown. In Fig. 9*E* and *G*, the electrodes were closer to the site of the optical recording. The response to the cathodic shock appeared with a short latency, and the response to the anodic shock was clearly visible. In Fig. 9*F* and *H*, the electrodes were about 6 mm further away from the site of the optical recording, and the latency of the optical rotation signal in response to a cathodic shock (Fig. 9*F*) was about 2 ms longer than that of the record in Fig. 9*E*. This corresponded to a conduction velocity of about 3 m/s. The optical rotation response to the anodic pulse was not observable in the record in Fig. 9*H*, showing that the anodic pre-response is a result of the electrotonic spread of the stimulating current from the electrodes. The remaining optical rotation responses, which appeared in Fig. 9*F* and *H*, must be the main responses produced by the conducted impulses.

Appearance of pre-response in refractory period

When two shocks were applied successively with a short interval at an end of the nerve, the second stimulus often elicited a small optical rotation signal, even when the nerve was in the state of absolute refractoriness. Figure 10*A–C* shows an

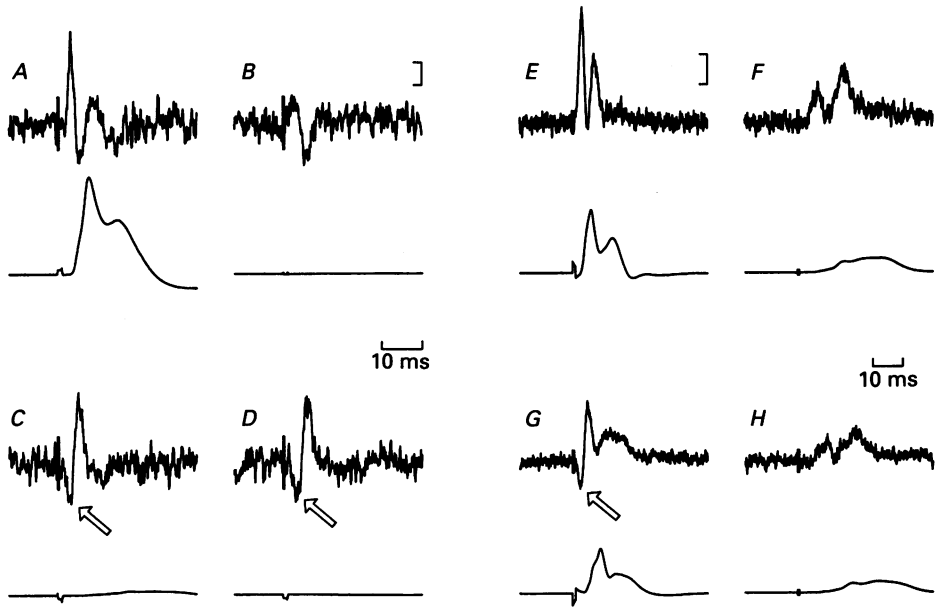

Fig. 9. Properties of the pre-response. *A–D* show the effect of tetrodotoxin on the optical rotation signal. Upper traces are optical rotation signal. Calibration in *B* represents 2×10^{-5} deg. Lower traces are externally recorded action potentials. The external medium was tetrodotoxin-free artificial sea water in *A* and *C*, and 1 μM tetrodotoxin-containing artificial sea water in *B* and *D*. The stimulation was cathodic in *A* and *B*, and anodic in *C* and *D*. The two arrows in *C* and *D* point to the initial phase of the anodic pre-response. 550 nm. Resting retardation in the artificial sea water was 75 deg. Stretch, 4·9%. 10 °C. *E–H* show disappearance of the pre-response by an increase in distance between the site of optical recording and the site of stimulation. The distance between the closer stimulating electrode and the centre of the optical window was about 7 mm in *E* and *G*, and about 13 mm in *F* and *H*. Stimulation was cathodic in *E* and *F* and anodic in *G* and *H*. Upper traces show optical rotation signals. Calibration in *E* represents 10^{-4} deg. An arrow in *G* points to the anodic pre-response. No corresponding response is seen in *H*. Lower traces show externally recorded action potentials. Time of stimulation is marked by thick vertical lines except for lower traces in *E* and *G*. 550 nm. Resting retardation, 79 deg. Stretch, 1·7%. 10 °C.

example. In Fig. 10*B*, the two stimuli were applied with an interval of 5 ms. Neither the birefringence signal nor the externally recorded action potential responded to the second stimulus, showing that the nerve was in the state of refractoriness. A tiny peak appeared, however, on the optical rotation record (arrow), with an amplitude comparable to the anodic pre-response, shown in Fig. 10*C* (arrow).

Polarity of the pre-response coincided with the polarity of the main response in most preparations. Among 127 examples, 14 (about 11%) showed that the polarity

of the pre-response was opposite to that of the main response. Figure 10*D* and *E* shows an example of the case in which the pre-response showed the polarity opposite to that of the main response.

Polarity of pre-response and the site of stimulation

The appearance of the pre-response does not require the excitation process in the nerve, as is evident from, for example, the experiment using tetrodotoxin.

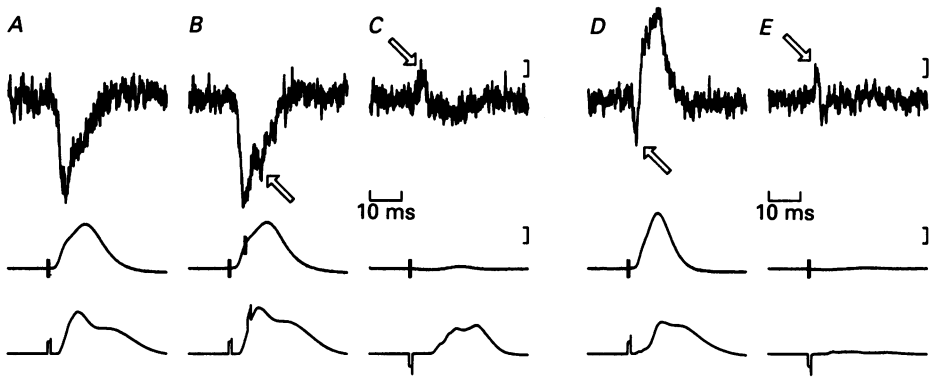

Fig. 10. Pre-responses in refractoriness and the variation of direction of the pre-response. *A–C* shows a pre-response in refractoriness. Upper traces show optical rotation signals. Calibration in *C* represents 2×10^{-5} deg. Middle traces show birefringence signals. Calibration in *C* represents 2×10^{-4} of the background light intensity. Time and duration of stimuli are marked by thick bars. Lower traces show externally recorded action potentials. *A*, responses to a single cathodic stimulus. *B*, responses to two successive cathodic stimuli. The arrow in the upper trace points to a deflection produced by the second stimulus. *C*, responses to a single anodic stimulus. The arrow in the upper trace points to an anodic pre-response. Stimulation was all applied at the proximal end. 550 nm. Resting retardation, 52 deg. Stretch, −3·1 %. 10 °C. *D–E* shows an example of the pre-response with polarity opposite to that of the main response. Upper traces, optical rotation signals. Calibration in *E* represents 2×10^{-5} deg. Middle traces, birefringence signals. Calibration in *E* represents 2×10^{-4} of the background light intensity. Lower traces, externally recorded action potentials. *D*, responses to distal cathodic stimulation. *E*, responses to distal anodic stimulation. The arrow in *D* points to a cathodic pre-response. The arrow in *E* points to an anodic pre-response. 550 nm. Resting retardation was 84 deg. Stretch, 8·9 %. 10 °C.

Nevertheless the polarity of the pre-response is dependent on the site of stimulation in most of the preparation (see Fig. 7*B* and *D*). Among fifty-two preparations in which the pre-response could be observed with both proximal and distal stimulations, forty-two preparations (about 81 %) showed that their polarities were reversed when the site of stimulation was different. The polarity of the pre-response is explained if molecules in or near the membrane are sensitive to the spatial gradient of the membrane potential, because this gradient is reversed when the site of stimulation is changed.

One might consider that the main response could also be explicable on the same basis. Thus the main response might be a result of the spatial potential gradient

produced by the conducted action potential. However, this explanation conflicts with the results obtained by the inclination experiment, which will be described below.

Inclination experiments

A simple hypothesis which would explain the dependence of polarity of the optical rotation signal on the site of stimulation is to suppose that the molecular axes of the

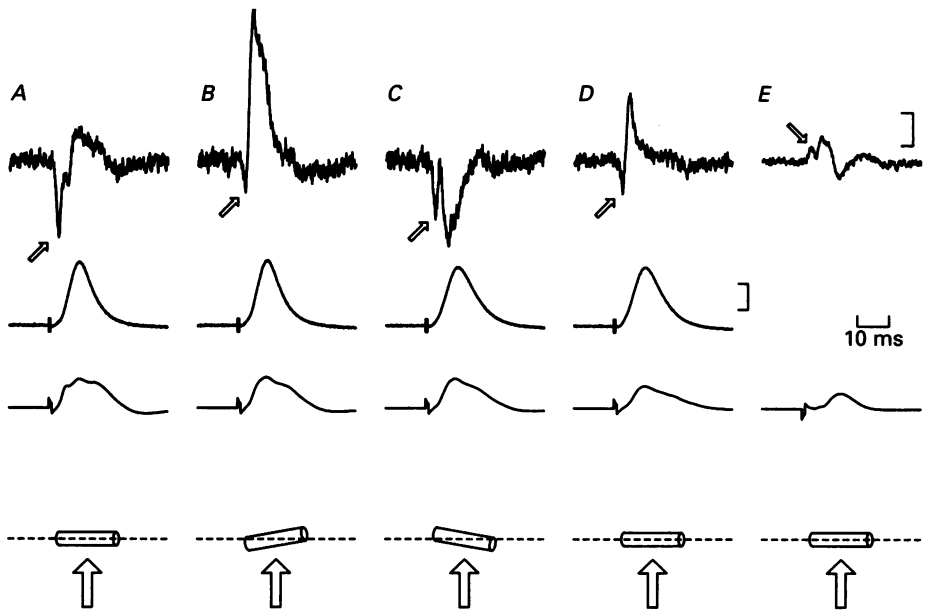

Fig. 11. An example of the inclination experiment. Upper traces show optical rotation signals. The arrows on the upper traces point to pre-responses. Calibration in *E* represents 5×10^{-5} deg. Middle traces show birefringence signals. The time and duration of stimulation are marked by thick bars in the middle traces. Calibration in *D* represents 10^{-4} of the background light intensity. Lower traces show externally recorded action potentials. Schematics at the bottom show inclination angles of the chamber. Arrows indicate direction of incident light. The angle of inclination was zero in *A*, *D* and *E*, 10 deg in *B* and −10 deg in *C*. The stimulus was applied on the distal side, was cathodic in *A–D*, and anodic in *E*. 550 nm. Resting retardation changed from 95 to 89 deg. Stretch, 10%. 10 °C.

macromolecules in the membrane change their directions according to the gradient of the membrane potential along the longitudinal direction of the nerve fibre, since it is known that the optical rotatory power of a molecule depends on the molecular axis of the optically active material (see Discussion). If such a hypothesis could have some meaning, then one might expect that, if the longitudinal axis of the nerve were to be inclined with reference to the incident beam, the optical rotation signal might undergo some important changes, because the starting angle of the assumed change in molecular axis changes with this procedure.

Experiments were performed using the inclination chamber, described in the Methods, which allowed inclination of the chamber by about 10 deg in either

direction from the horizontal position. In the following description, when the chamber is inclined in the direction so that the right-hand side is higher than the left-hand side, the angle of inclination will be designated as positive. The inclination angle was mostly 10 deg. It must be noticed that the inclination angle of the chamber

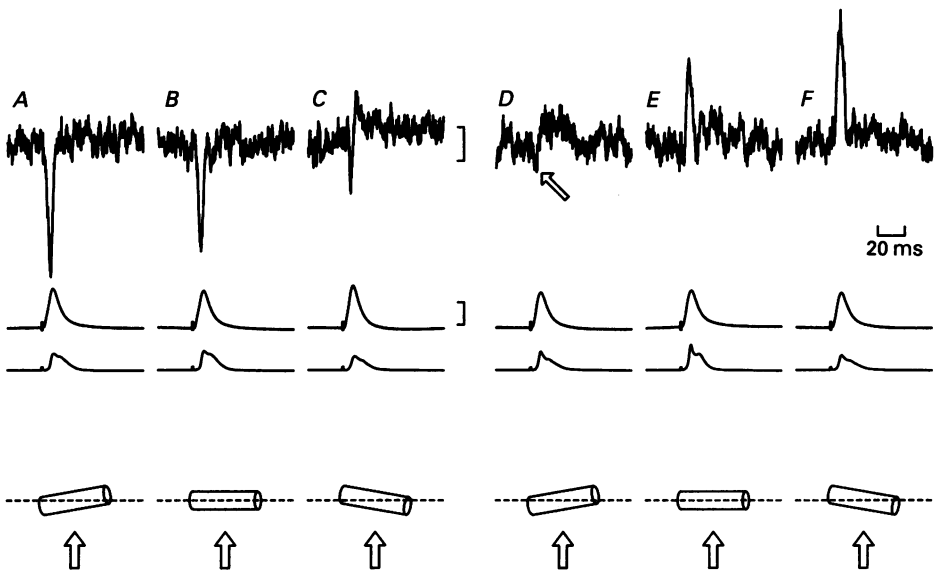

Fig. 12. Effects of inclination on responses elicited by proximal and distal stimulations. Upper traces show optical rotation signals. Calibration between *C* and *D* represents 10^{-5} deg. The arrow in *D* points to the reversed optical rotation signal. Middle traces show birefringence signals. Calibration represents 2×10^{-4} of the background light intensity. Lower traces show externally recorded action potentials. The schematics at the bottom show the inclination of the chamber in the same way as in Fig. 11. Stimulation was applied on the proximal side in *A–C*, and on the distal side in *D–F*. Experiments were performed in the order of *E*, *B*, *A*, *D*, *F* and *C* (see the decay in amplitude of the action potential). 550 nm. Resting retardation, 51–52 deg. Stretch, 2·1 %. 13 °C.

does not represent the change in angle of incidence of the beam to the nerve, because of the refraction which occurs at the boundary between air and the chamber. If one uses the sea water refractive index of 1·334 (Utterback, Thompson & Thomas, 1933), then calculation with Snell's law gives a value of incidence of 7·45 deg rather than 10 deg.

An example of the result is shown in Fig. 11. In Fig. 11*A*, the nerve was held horizontally, and the optical rotation signal consisted of a sharp downward deflection followed by a small upward deflection. When the chamber was inclined to +10 deg, the initial negative deflection was followed by a large positive deflection. This was not a birefringence signal; one can observe from Fig. 11*B* that the peak of the optical rotation signal precedes some 4 ms to the peak of the birefringence signal. More convincingly, when the azimuth of the nerve was changed by rotating the chamber stage, the birefringence contribution came in at the tail of the large positive deflection, which is not shown in this figure. If the chamber was inclined by −10 deg

(Fig. 11*C*), the initial negative deflection was followed by a medium-sized negative deflection. Again it is possible to show that this later component did not come from the birefringence change. In Fig. 11*D*, the chamber resumed its horizontal position. The optical rotation signal did not show a complete reversibility; a small positive-going response appeared following the initial negative response, probably because of a slight error in setting the azimuth angle. In Fig. 11*E*, the polarity of the stimulating pulse was reversed. The initial negative-going signal reversed its polarity, showing that the initial response can be identified as the pre-response described above.

The experiment shows that the major change takes place in the main response by inclination of the nerve with reference to the incident beam. The pre-response is much more refractory with this procedure.

Another characteristic of the effect of inclination was that the change always took place asymmetrically; the effect of +10 deg inclination was opposite to the effect of −10 deg inclination. However, the direction varied among preparations. In Fig. 11 the inclination to a positive angle enhanced the positive-going optical rotation signal. In other preparations the opposite change was observed.

Effects of inclination on the responses elicited by proximal and distal stimulations

As described before, the optical rotation signal elicited by proximal stimulation usually has the polarity which is opposite to the optical rotation signal elicited by distal stimulation. Comparison of the effect of inclination on these responses might therefore be useful for elucidating mechanisms underlying the effects of inclination on the optical rotation signal.

Figure 12 shows a result of such trials. When the nerve was placed horizontally, proximal stimulation produced a negative optical rotation signal (Fig. 12*B*) whereas distal stimulation produced a positive optical rotation signal (Fig. 12*E*). Inclination to the positive angle increased the amplitude of the optical rotation signal elicited by proximal stimulation (Fig. 12*A*), but it decreased the amplitude of the optical rotation signal elicited by distal stimulation (Fig. 12*D*). Inclination to the negative angle produced effects which were opposite to the effects produced by inclination to the positive angle.

Thus the inclination procedure changes the peaks of the optical rotation signals toward the same direction, irrespective of the site of stimulation.

In this preparation, anodic shocks produced negative responses on proximal stimulation, and positive responses on distal stimulation. Therefore, the pre-responses had the same polarity as the main responses, and they did not come out distinctly. In Fig. 15*D* responses of anodic and cathodic pulses are plotted against the angle of inclination. It is clear that the anodic responses were very insensitive to the procedure of inclination. In the same way as the example shown in Fig. 11, only the main response changed its amplitude with inclination procedure.

Effects of stretching the nerve

Stretching the nerve longitudinally sometimes reversed the polarity of optical rotation signal. In Fig. 13*A* and *B*, one example is shown.

The polarity change took place, however, only in some preparations. In others, the polarity remained the same, although in general the shape of the response changed considerably; for example, change in amplitude, change in duration, or appearance or disappearance of some of the subsidiary peaks was often observed. These changes

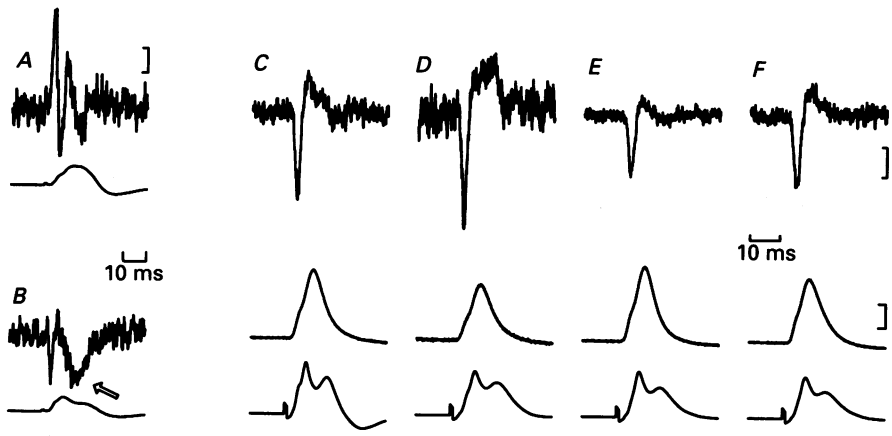

Fig. 13. Effects of stretch and change in wavelength on the optical rotation signal. *A* and *B* show an example in which stretching the nerve changed the polarity of the optical rotation signal. *A*, stretch 0%. *B*, stretch 7%. Upper traces, optical rotation signal. Calibration in *A* represents 5×10^{-5} deg. A slow negative deflection in *B* (arrow) probably came from the birefringence change. Proximal stimulation. 550 nm. Resting retardation in the stretched state was 54 deg. 9 °C. *C–F*, effects of change in wavelength. *C*, 550 nm. *D*, 450 nm. *E*, 650 nm. *F*, 550 nm again. Top traces, optical rotation signals. Calibration in *F*, 5×10^{-5} deg. Middle traces, birefringence signals. Calibration in *F*, 2×10^{-4} of the background light intensity. Lower traces, externally recorded action potentials. Proximal stimulation. Resting retardation, 97 deg at 450 nm, 76 deg at 550 nm, and 63 deg at 650 nm. 11–12 °C.

(including the polarity change) were mostly irreversible, in the sense that on relaxing the nerve the shape and polarity of the optical rotation signal did not resume the original state. A part of the reason for this irreversibility must be the friction between the nerve and the chamber, especially at portions where petroleum jelly was applied, so that the tension at the central part of the nerve did not return to the original value. Other possible reasons for the lack of reversibility would be the change in effective intensity of the electric stimulating current because of the decreased sealing resistance of the petroleum jelly partitions, change in the gross shape of the illuminated portion of the nerve, and damage of some groups of nerve fibres due to the stretch.

Studying the effects of stretch in a systematic way was soon proved to be a difficult task, because of the irreversibility and variability of the phenomenon, and no extensive study on this phenomenon was performed. Nevertheless, it was realized that the tension applied to the nerve was an important parameter for determining the polarity of the optical rotation signal. In later experiments, therefore, care was taken not to exert an excessive stretch on the nerve, in order to keep one of the experimental parameters approximately constant.

Unrelated conditions

Several conditions examined were found unrelated to the polarity of the optical rotation signal. They will be briefly described below.

Sidedness of walking legs

Chiral phenomena in living things might be correlated with the sidedness (left or right) of the body, as the one found in circular polarization in emission from the lantern of firefly larvae (Wynberg, Meijer, Hummelen, Dekkers, Schippers, & Carlson, 1980). Experimental records were analysed statistically to assess if there might be a significant correlation between the polarity of the optical rotation signal and the side of walking legs from which the nerve was taken. No significant correlation was found.

Wavelength of incident light

Optical rotation of a substance depends on the wavelength employed, and the polarity of the optical rotation can change at particular wavelengths where absorption and circular dichroism take place prominently (Jirgensons, 1973). Lobster nerve does not show any distinct colour, and therefore the existence of an absorption band or a circular dichroism band is improbable. Nevertheless the wavelength dependence of the rotation signal was examined in the range of 450–650 nm (Fig. 13*C–F*). No reversal of the polarity of the optical rotation signal was observed. The signal usually increased in size with the use of shorter wavelength. This is in common with the tendency observed in the optical rotatory dispersion of many substances (Lowry, 1935, p. 106). Nevertheless, the signal-to-noise ratio was optimum at about the wavelength of 550 nm, because of the decrease in light intensity at shorter wavelengths available for illuminating the preparation.

Influence of the resting retardation of nerve

As a possible cause of the reversal of the optical activity, the influence of the resting retardation of nerve has to be considered. (1) When the nerve is homogeneous, the resting retardation of more than 180 deg should produce the reversal of the optical rotation signal. (2) When the nerve is non-homogeneous, a part of the nerve bundle might act as a wave-plate, and might create a reversal of the optical rotation signal, even with 90 deg retardation (Watanabe, 1987). However, these two possibilities now seem much less likely, as a result of further experiments, in which the resting retardation of the nerve was routinely measured in each preparation. In no example, was the measured retardation of the nerve close to 180 deg (the maximum was 136·7 deg; average $\pm$ S.D., 77·7 $\pm$ 20·4 deg; $n = 168$). Therefore, the first possibility can safely be excluded as a cause of variability of the signal polarity. Since about 75% of the preparations showed retardations of less than 90 deg, the second possibility can also be dismissed in this group of preparations. For other preparations which showed retardations of more than 90 deg, the second possibility still remains, but in the face of other elucidated causes like the conduction reversal, it seems that the second possibility might not play an important role in determining the polarity of the optical rotation signal.

Colchicine is one of the antimitotic drugs and depolymerizes the microtubules inside the axon. Because microtubules are highly birefringent colchicine reduces the axon birefringence when applied to the axon. External application of colchicine at a concentration between 0·5 and 2 mM to three lobster nerve preparations with retardations of more than 90 deg reduced the retardation of the nerves to a value less than 90 deg after several hours without causing loss of excitability. The polarity of the optical rotation signals did not change with this procedure, however. Thus, at least in these three preparations, the value of retardation did not influence the polarity of the optical rotation signal.

DISCUSSION

Sources for the variability of the polarity of optical rotation signal

Several sources for the variability have been identified. The polarity depends on the site of stimulation with reference to the site of optical recording. The tension applied to the longitudinal direction of the nerve has also been found influential to the optical rotation signal polarity. However, even when the site of stimulation was fixed, and the tension to the nerve was regulated to a small value, the polarity of the optical rotation signal was still not constant. In a following paper (Watanabe, 1993), another source is described, which is the direction of illumination with reference to the preparation. Thus the polarity of the optical rotation signal is variable among preparations because of many experimental conditions which are not necessary discernible to the experimenter.

The hypothesis of molecular axes tipping

The phenomenon of conduction reversal may be explained by stating that the polarity of the optical rotation signal depends on the spatial gradient of the membrane potential along the longitudinal direction of the nerve fibre. The explanation, however, does not help elucidating the mechanism by which the conduction reversal takes place. A hypothesis has already been put forward before (see *Inclination experiments*), which assumes that the membrane macromolecules change the direction of their molecular axes when the potential gradient is developed in the longitudinal direction of a nerve fibre. One has to admit, however, that the hypothesis is a peculiar one, and some more reasoning is needed to justify its introduction.

The phenomenon of the conduction reversal indicates that the state of the membrane is not exactly the same when the direction of impulse conduction is different. But it is very difficult to consider that the difference in direction of impulse conduction creates totally different excited states. For example, it is impossible to think that the proximal stimulation excites a selected group of membrane channels and the distal stimulation excites some other group. Such an idea is in conflict with the currently established theory of excitation that the membrane channels enter excited states when the membrane is depolarized. Moreover, the idea does not explain the experimental result that during the state of refractoriness, after e.g. proximal stimulation, the optical rotation signal was not elicited by distal stimulation. Thus in spite of the difference in polarity, the optical rotation signals

evoked by proximal and distal stimulations are produced by the same group of molecules in the membrane. Therefore, before the channel molecules go into the state of excitation, the molecules are put under a certain condition, which depends on the direction of impulse conduction, but which is separate from the process of excitation. This is the conclusion which can safely be deduced from the experiment.

The hypothesis put forward before really means that the difference in the condition of membrane molecules would most conveniently be visualized as the difference in the direction of molecular axes of the membrane macromolecules. The hypothesis assumes that the membrane macromolecules rotate and change their direction when the spatial gradient of the membrane potential is set up by local or stimulating currents. The hypothesis is convenient firstly because it supplies a simple pictorial representation. Secondly, it allows us to make some predictions which can be examined experimentally. The inclination experiment is an example. Thirdly, the hypothesis can be examined in the light of the knowledge of the optical activity of the oriented systems (see Charney, 1979; chap. 9). A substance is said to be an oriented system of molecules when the molecular axes are aligned in special directions, in contrast to an isotropic system, like a homogeneous protein solution, in which the solute molecular axes are randomly oriented. There should be little doubt that the membrane macromolecules of living axons form an oriented system. Their molecular axes are supposed to be approximately vertical to the membrane surface, and the shape of the membrane is, to a first approximation, cylindrical. In the oriented system, the chiral–optical effects are highly dependent on the propagation direction of the measuring light with respect to the orientation of the molecular axes of the optically active material (Charney, 1979). This is what we actually saw in the inclination experiment. Among the systems examined in detail by chemists, probably the one most pertinent to the present problem is a solution of a synthetic polypeptide whose molecules take the conformation of the α-helix. When a high electric voltage is imposed at both ends of the solution container, the molecules align themselves because of their intrinsic dipole moment and form a partially oriented system, as shown by Tinoco (1959) and Hammond & Jennings (1980). Their results show that when the molecules are illuminated along the helical axis a dextrorotation is observed with a specific rotation, $[\alpha]$, of 475 deg cm^2 g^{-1}, whereas when they are illuminated vertically to the helical axis, a laevorotation is observed with $[\alpha]$ of -227 deg cm^2 g^{-1}. But the optical rotation of the isotropic solution is only very weakly dextrorotatory, with $[\alpha]$ of 1 deg cm^2 g^{-1} (data from Hammond & Jennings, 1980, at 515 nm wavelength). Biological membranes are known to contain abundant α-helices (see Singer, 1971). In the axonal membrane, channel molecules contain α-helical (or 3_{10}-helical) structures which penetrate vertically the lipid bilayer of the membrane (Noda *et al.* 1984). It is therefore probable that a living nerve preparation can show much stronger optical rotation than that observed in an isotropic solution which contains the same amount of channel molecules, but its value critically depends on the angle between the direction of the nerve and the direction of the incident light.

An explanation of the conduction reversal

For simplicity, let us assume that, for small angles, the angle of tipping of the molecular axis is linearly correlated with the optical rotatory power of the membrane molecules (Fig. 14, line r–r). When the stimulating electrodes are close to the site of

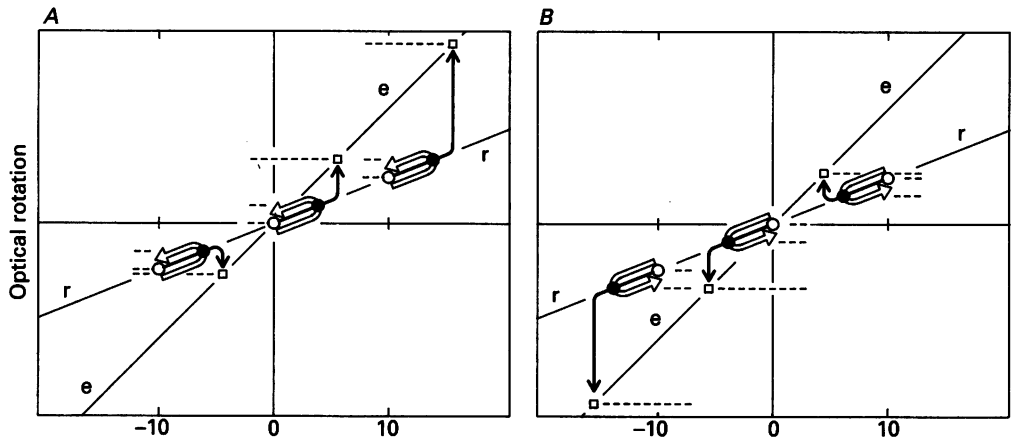

Fig. 14. An explanation of the result of the inclination experiment. *A*, distal stimulation. *B*, proximal stimulation. Ordinate, optical rotation of the axonal membrane. Abscissa, angle between the molecular axis on the membrane and the direction of incident light (which will be called the angle of incidence). Open circles show the resting optical rotation of the membrane at three different angles of the chamber inclination. Filled circles show the final points of the optical rotation when the subthreshold stimulation is applied to the axon, so that the spatial gradient of the membrane potential is created along the longitudinal direction of the axon. The line r–r shows the linear relationship between the angle of incidence and the optical rotation when the membrane is not in the exited state. Thick open arrows show the course of change when a subthreshold pulse is applied at an end of the preparation. The vertical distance between the open and filled circles represents the polarity and magnitude of the pre-response. The line e–e shows the linear relationship between the angle of incidence and the optical rotation of the membrane when the membrane is in the excited state. The squares on the line e–e show the final points of optical rotation when the action potential sweeps over the axon. Thin filled arrows show the course of change during the rising phase of the main response. The vertical distance between the open circle and the square represents the polarity and the magnitude of the main response.

optical recording, the stimulating current spreads electronically to the site of optical recording, producing some spatial gradient of the membrane potential. This in turn produces a tipping of molecules and a change in optical rotation, which we observe as the pre-response. When the action potential approaches the site of optical recording, the spatial gradient of the membrane potential increases, and the change in optical rotation also increases. Then some of the molecules undergo some conformational change which is associated with the process of excitation. However, without the results obtained with the inclination experiment, we are unable to tell if the conformational change of molecules makes any contribution to the observed optical rotation signal. In other words, all the optical rotation signal may well be

explained as a result of the change in spatial gradient of the membrane potential. But with the help of the inclination experiment, we can go a little further. The main response changes its amplitude and (sometimes) its polarity when the nerve is inclined. This finding is explicable only when we assume that in the excited state the

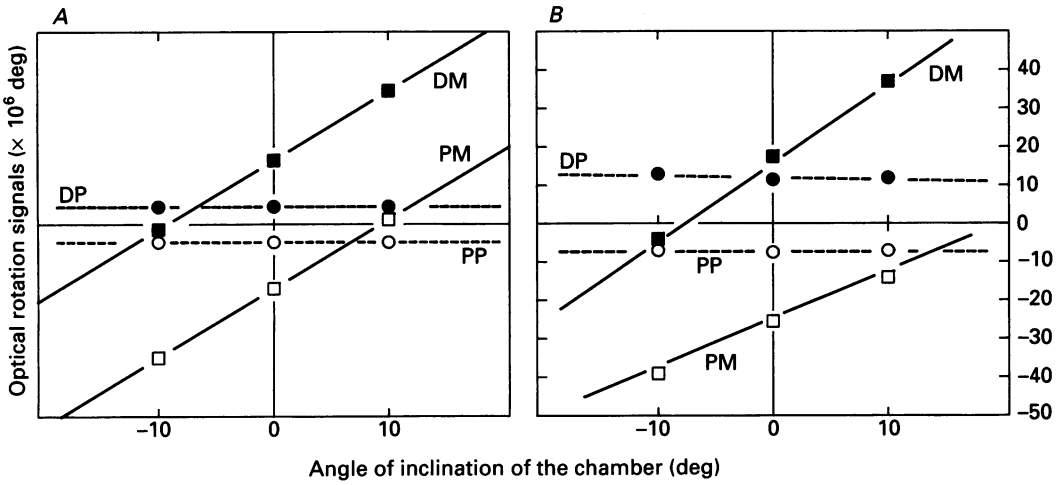

Fig. 15. Comparison of the predicted and observed size and polarity of the optical rotation signal during the inclination experiment. *A*, prediction from the hypothesis described in Fig. 14 and in the text. *B*, the observed values plotted from the experiment shown in Fig. 12. Ordinate, polarity and amplitude of the optical rotation signals. Abscissa, angle of inclination of the chamber. ●, pre-responses by distal stimulation. ○, pre-responses by proximal stimulation. ■, main responses by proximal stimulation. Dashed lines (for the pre-response) and continuous lines (for the main response) are regression lines. DP, distal stimulation, pre-response. DM, distal stimulation, main response. PP, proximal stimulation, pre-response. PM, proximal stimulation, main response. In *B*, the data at 0 deg inclination are the averages of the two records; only the first of them is shown in Fig. 12.

relationship between the angle of incidence of light and the optical rotatory power follow a different relationship from that in the resting state. If it were to remain the same, the main response should not change with inclination of the nerve, but should remain constant, like the pre-response does, because with inclination of the nerve the change in spatial gradient of the membrane potential should remain the same, and the change in optical rotation should accordingly remain the same. In Fig. 14 the relationship between the angle of incidence of light and the optical rotation in the excited state is depicted with a line labelled by e–e, again assuming that it is linear, but with a slope which is different from that of the resting state. It will be seen that the result of the inclination experiment can satisfactorily be explained with this model. Moreover, the same relationship can supply explanations for the results obtained with stimulation applied to the other end of the nerve. This time the longitudinal potential gradient is opposite, and therefore the angle of tipping of the molecular axes is also reversed (Fig. 14*B*). The resultant change in optical rotation signal is plotted against the angle of inclination of the nerve in Fig. 15*A*. This reproduces the results shown in Fig. 15*B*, which comes from the preparation shown

in Fig. 12, explaining why the peaks of the optical rotation signals, produced by proximal and distal stimulations, change in the same direction. In summary, the hypothesis described above can explain four experimental observations: (1) the phenomenon of conduction reversal, (2) the constancy of the pre-response on chamber inclination, (3) the change in amplitude and polarity of the main response on chamber inclination, and (4) the agreement in direction of the change in main responses produced by proximal and distal stimulations when the chamber is inclined. The above analysis suggests that the major part of the main response of the optical rotation signal is to be explained as a result of the conformational change of the membrane macromolecules during excitation.

Conclusions

The characteristics of the optical rotation signal, displayed in the inclination experiments, have been reproduced with the use of the hypothesis of the molecular axis tipping. In spite of this encouraging sign, it is believed that some other confirming evidence is needed to treat the hypothesis as a fact. A serious shortcoming is the difficulty in identifying the cause of the molecular tipping when the spatial gradient of the membrane potential is established along its longitudinal direction. Possible candidates for the cause include the intracellular longitudinal current, contraction of cytoskeletal structure underneath the membrane, and the thickness change of the fibre membrane associated with the change in membrane potential (Tasaki & Iwasa, 1982; Terakawa, 1985). However, any of them seems to produce tipping of molecular axes of an order comparable to that of the angle of inclination of the nerve preparation. It is therefore safer to regard the hypothesis as still a tentative one until it is examined and confirmed with some other independent method.

It has been suggested that the major part of the main response is a result of the conformational change of the membrane macromolecules. This is based on the difference in behaviour of the pre-response and the main response in the inclination experiment, and at present there seems no proper alternative explanation. The study of the optical rotation signal of nerve thus adds evidence for the proposition that the conformational change of the membrane macromolecules should be the molecular mechanism underlying the excitation process of the axonal membrane.

At present the low signal-to noise ratio prevents application of the present method to single fibre preparations. However, with improvement the technique would probably be able to supply a useful way to monitor the time course of the molecular conformational change which takes place during excitation.

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