Resistivity of Axoplasm

II. *Internal Resistivity of Giant Axons of Squid and Myxicola*

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ABSTRACT The specific resistivity of the axoplasm of giant axons of squid and *Myxicola* was measured utilizing a single metal microelectrode subjected to alternating current in a circuit in which the voltage output varies with the conductivity of the thin layer of fluid at the exposed electrode tip. The average specific resistivity of stellar axons of *Loligo pealei* was 31 Ωcm (1.55 times seawater [× SW]) while for *Loligo opalescens* it was 32 Ωcm (1.30 × SW). Smaller giant axons had a higher average resistivity. *Myxicola* giant axons had a resistivity of 68 Ωcm (2.7 × SW) in normal seawater, and 53 Ωcm (2.1 × SW) in a hypertonic high-Mg^{++} seawater. The temperature dependence of squid axon resistivity does not differ from that of an equally conductive dilution of seawater.

The internal resistivity of the giant axon of the squid was first measured by Curtis and Cole (1938) who reported a value 4.2 times that of seawater (×SW). Subsequent studies have reported a range of values for axoplasmic resistivity, all less than 4.2 × SW. In intact axons Cole and Hodgkin (1939) reported resistivity to be 1.4 × SW while Cole and Moore (1960) found a value of 1.2 × SW. Whereas the above studies were performed on intact axons, measurements have also been made on extruded axoplasm. Taylor and Cole (reported by Cole, 1968) found axoplasmic resistivity equal to that of seawater, while Cole (1975) has found an average resistivity of 1.35 × SW.

In this study we report measurements of resistivity of the giant axons of squid and *Myxicola*, the marine annelid, using a different technique. The measurement is a modification of the technique described by Bak (1967) for testing metal microelectrodes and is made through a single metal microelectrode, insulated except for the tip. Alternating current, usually at 100 kHz, is passed between the microelectrode tip and an extracellular reference.

The voltage output reflects the ability of a thin layer of solution at the tip of the microelectrode to carry current. Although other factors, such as electrode polarization impedance, contribute to the voltage output, these factors can be accounted for by individual electrode calibration and the technique can be used to measure intracellular resistivity. Use of this procedure has been described in reports of studies of *Aplysia* neurons (Carpenter et al., 1971, 1973). In this investigation, we attempt to resolve the dispute as to the true average resistivity of squid axon, to compare this value to that of another giant axon, and to apply additional tests to a technique which has potential application to any large cell for measurement of intracellular resistivity.

METHODS

Experiments on *Loligo pealei* were performed at the Marine Biological Laboratories, Woods Hole, Mass. All other studies were done in Bethesda, Md. *Loligo opalescens* were obtained from Pacific Biomarine Supply Co., Venice, Calif. and were maintained in aerated artificial seawater (5°C) until used. *Myxicola infundibulum* were obtained from Mr. Arthur Mackay, Deer Island, New Brunswick and were maintained at 5°C until used. Axons were prepared as described by Binstock and Goldman (1969). In Woods Hole, animals were maintained in and experiments performed using natural seawater which has a resistivity of 20 Ωcm. All experiments in Bethesda utilized seawater made from Instant Ocean salts, which had a resistivity of 25 Ωcm as determined on a conductivity meter.

Axons were dissected under flowing seawater, cleaned, ligated, and removed to a Lucite chamber where they lay over a pair of Ag-AgCl electrodes for stimulation. In experiments utilizing the stellate ganglion, the giant axons were dissected for a distance of approximately 1 cm from the ganglion, ligated, and cut. The ganglion with attached axons was pinned to a layer of Sylgard (Dow Corning Corp., Midland, Mich.) in a Lucite chamber and maintained under flowing seawater.

Microelectrodes were made from etched 0.004-inch platinum-iridium (80:20) wire. The exposed tip was about 1 μm in diameter. Glass insulation was applied over the wire to within 10–20 μm of the tip with a De Fonbrune microforge. At this distance the electrode diameter was 8–15 μm. The electrode was held in hypodermic tubing and the exposed tip was platinized.

All axons (except those attached to the stellate ganglion) were recorded from both with the metal microelectrode and a glass pipette, filled with 3 M KCl. Penetration was made under direct visual control. The metal electrode was inserted as nearly parallel to the axon as possible and for a distance of at least 100 μm. Thus, the exposed tip (less than 20 μm in length) was without question totally in the axon. The electrode diameter at the membrane was about 75 μm when fully inserted. The glass pipette recording (DC coupled) of membrane potential and action potentials utilized an Electronics for Life Sciences (Rockville, Md.) (Bak) DC high-impedance amplifier. Axons were not studied if they showed indications of injury (action potentials of less than 80 mV, resting potential of less than 40 mV) after penetration with both electrodes. The output from the metal microelectrode was led simultaneously to the

resistivity-measuring circuitry and to a DC electrometer for recording of action potentials through the metal electrode. The output of the electrometer was amplified through an AC-coupled Tektronix 3A3 preamplifier (Tektronix, Inc., Beaverton, Ore.) with a cut-off frequency of 15 Hz. Although this cut-off frequency passes the brief action potentials, there was some distortion due to the high impedance of the metal electrodes and the fact that the amplifier was not impedance matched. The reference electrode for all measurements was a large Ag-AgCl wire in the bath.

Resistivities were determined only with electrodes on which complete calibration curves in dilutions of seawater were obtained and where the electrode showed identical readings before and after penetration. In many axons multiple penetrations at different sites were made with the metal electrode and results averaged.

All resistivity measurements were made at a frequency of 100 kHz except in those experiments in which frequency was varied. In such experiments a Krohn-Hite model 4200 oscillator (Krohn-Hite Corp., Cambridge, Mass.) was used and frequencies varied from 25 to 300 kHz. Plots of output voltage as a function of frequency were obtained with frequency on the X axis input and voltage on the Y axis input of a Tektronix 502 oscilloscope. Records were photographed on Polaroid film from the oscilloscope face. All other records of voltage output, membrane potential, and temperature were recorded on a Brush Mark 200 penwriter (Gould, Inc., Palo Alto, Calif.) after suitable amplification. Temperature was changed by flowing the seawater through coiled tubing in an ice bath and was monitored by a small thermistor placed near to the axon. All experiments were performed at room temperature unless specified.

The voltage output of the experimental apparatus is given by Eq. 1 (Discussion). The output varies with source voltage and feedback resistance as well as electrode impedance which is, in turn, a function of frequency and several electrode properties in addition to local conductivity. Under the experimental conditions all of these factors but local conductivity are kept constant. In our previous publications (Carpenter, et al., 1971, 1973), voltage output was defined as "equivalent capacitance" and numerical values were obtained by calibrating with known capacitors. This term is, unfortunately, without meaning, and thus in this and future publications the output voltage will be plotted directly. The specific resistivity of axons was obtained by determining the dilution of seawater with the same voltage output measured in the axon, and measuring the resistivity of that solution in a conductivity cell.

RESULTS

Figure 1 illustrates an experiment recording resistivity and electrical activity from a stellar axon of *Loligo opalescens*. Part A shows the recording of resistivity. The electrode is initially in seawater and the voltage output is about 10 mV. When the electrode is lowered against the axonal membrane, the output falls momentarily, but at the arrow marked "in," the electrode was seen to pop through the membrane into the axoplasm. However, voltage output in the axon was about 10% less than when the electrode was in seawater. On withdrawal of the electrode the output returned to the control

level. The action potentials recorded through the glass pipette and metal electrode (B) indicate that the axon was healthy. In C resistivity values obtained from five different axons are superimposed on the electrode calibration curve obtained from dilutions of seawater. The resistivities of these axons range from 28 to 32 Ωcm.

Table I summarizes the values of axoplasmic resistivity obtained. Isolated stellar axons were studied from 15 *Loligo pealei* and 13 *Loligo opalescens*, and the averaged resistivities were 31 and 32 Ωcm, respectively. For both groups there was a considerable variation from one preparation to another. This

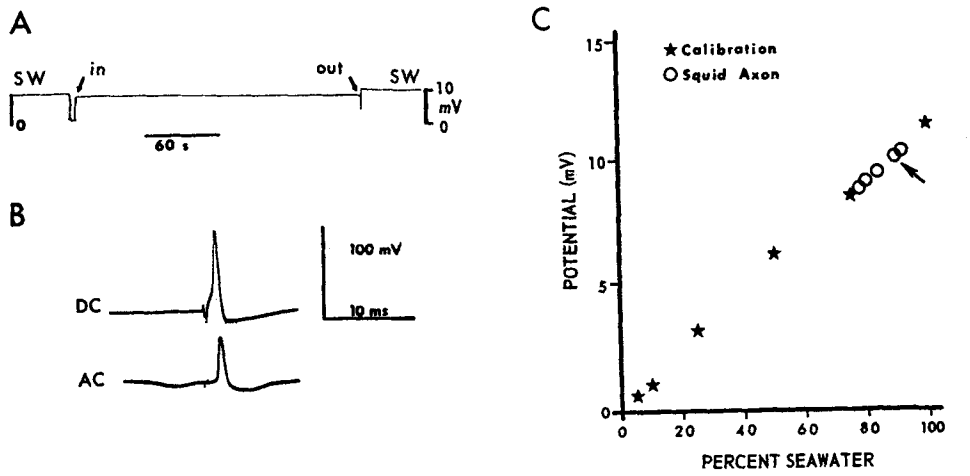

FIGURE 1. Resistivity of an isolated axon of *Loligo opalescens*. Part A shows record of resistivity measurement on penetration (in) and withdrawal (out) of the metal electrode. The transient fall in voltage output before penetration probably results from a tight indentation of membrane by the metal microelectrode such that seawater is excluded. The axon potential recorded from glass (DC) and metal (AC) electrodes is shown in B. C shows the electrode calibration (stars) and values recorded in five axons with this electrode (open circles). The arrow indicates the axon illustrated in A and B. Records in A are from penwriter traces, while B was photographed from an oscilloscope face.

variation could not be correlated with any other fiber property. Moreover, multiple penetrations at different sites of one axon with both the same and with different electrodes gave very consistent resistivity values. Although the explanation for the variability is not apparent, it appears to be real.

Only relatively few axons were studied in experiments on the stellate ganglion, but these experiments suggest that the resistivity of smaller axons is on the average greater than those of larger axons.

Fig. 2 shows the temperature dependence of the voltage ouput of a resistivity microelectrode in an axon of *Loligo pealei*, along with that of the calibrating solutions of seawater and 50% seawater in distilled water. The re-

TABLE I

VALUES OF AXOPLASMIC RESISTIVITY

Preparation	Number of preparations	Equivalent isotonic salt solution (average and range)	Resistivity relative to seawater (average)	Specific resistivity (average)
		%		Ω cm
Seawater (Woods Hole)		100	1.00	20
Loligo pealei				
Stellar axon	15	65 (49–105)	1.55	31
In Stellate ganglion:				
Stellar axon	5	69 (55–82)	1.45	29
Smaller axons	6	55 (45–70)	1.80	36
Seawater (Instant Ocean)		100	1.00	25
Loligo opalescens	13	78 (61–90)	1.3	32
Myxicola infundibulum				
In normal SW	6	37 (30–44)	2.7	68
In hypertonic SW (high Mg^{++})	13	47 (39–60)	2.1	53

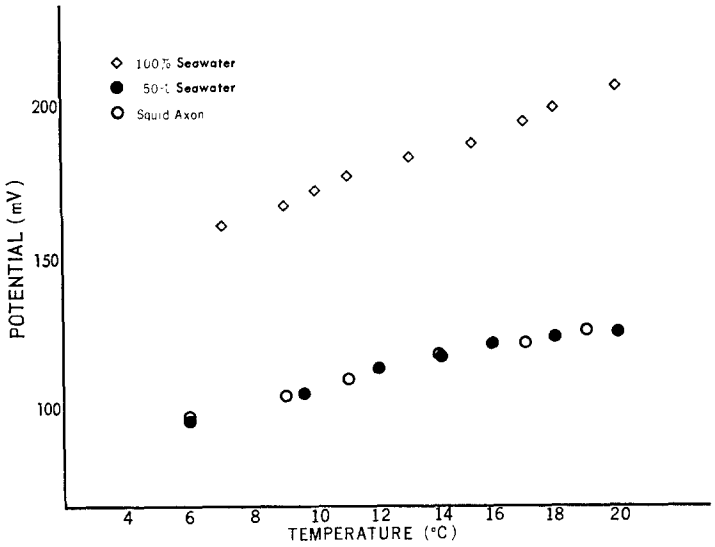

FIGURE 2. Temperature dependence of resistivity of an axon of *Loligo pealei* and calibrating solution. Temperature was changed by running the perfusing seawater through an ice bath.

sistivity of this axon was almost identical to that of the 50% seawater over all the temperature range.

In order to test that the electrode properties did not change on penetration of the axon, experiments were performed where the voltage output was re-

corded during a frequency sweep. One such experiment on an axon from *Loligo pealei* is shown in Fig. 3, where frequency was swept over the range 25-130 kHz. Part A shows the plot recorded in seawater before penetration and in the axon. Similar plots obtained from the calibrating solutions are shown in B. This axon had a resistivity equal to that of 50% seawater, and the frequency plot in the axon is identical over the entire frequency range studied to that obtained in the calibrating solution. The plot on withdrawal of the electrode to seawater was superimposable on the control.

Fig. 4 shows results of an experiment on an axon from *Myxicola*. In this experiment the axon was perfused with seawater to which 100 mM $MgCl_2$ was added to depress the excitation of adherent muscle fibers when the axon

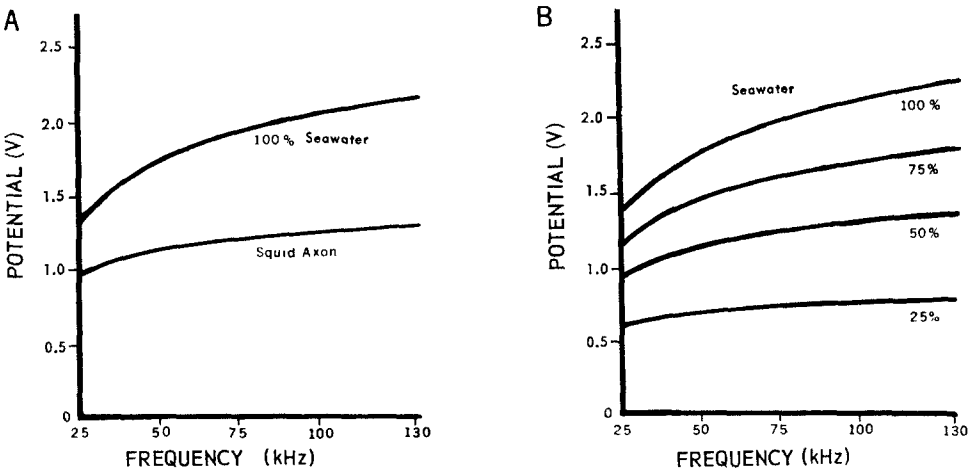

FIGURE 3. Voltage output as a function of frequency from an experiment of *Loligo pealei* (A). The trace labeled "100% seawater" was taken just before penetration. B shows similar frequency sweeps in calibrating solutions.

discharged. The addition of the $MgCl_2$ increased osmolarity from 1,025 to 1,300 mosmol but without it the muscular contraction after action potentials usually dislodged the electrodes and damaged the axon. Part A shows the resistivity measured, part B shows the action potential recorded through the glass and metal electrodes, respectively, and part C shows calibration of the electrode (stars) with resistivity values from three *Myxicola* axons recorded with the same electrode in high-Mg^{++} seawater. These axons had resistivities between 39 and 50 Ωcm.

As seen in Table I the average resistivity of *Myxicola* axons was 53 Ωcm in high-Mg^{++} seawater but 68 Ωcm in normal seawater. Although action potentials were not recorded in the axons in normal seawater (to check their viability) the preparations all showed contractions before placement of the

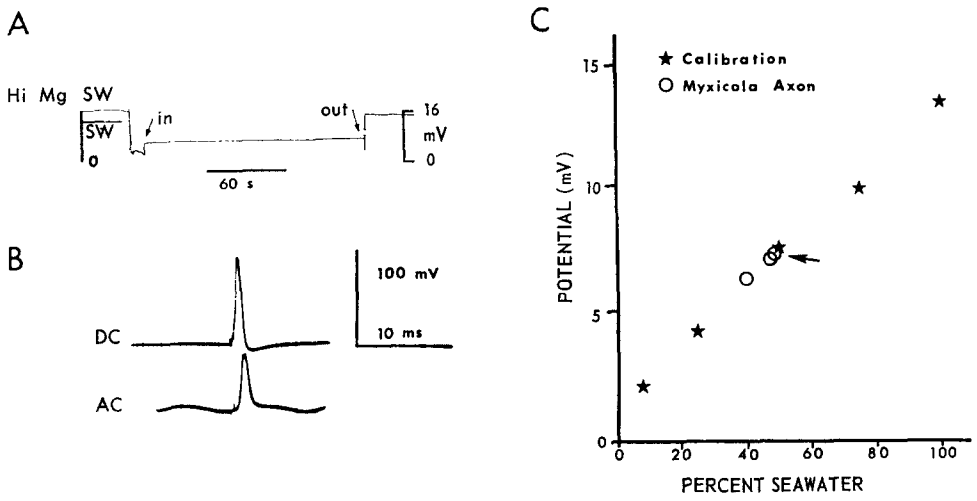

FIGURE 4. Resistivity of an isolated *Myxicola* giant axon. The axon was perfused with seawater containing 100 mM added $MgCl_2$ to suppress muscular contraction when the axon discharged. Traces illustrated are comparable to those in Fig. 1 for the squid axon. In C, resistivities from three axons studied with this electrode are plotted on the electrode calibration curve. The axon illustrated in A and B is indicated by the arrow

electrodes and can be assumed to be healthy. This observation suggests that the normal resistivity of *Myxicola* axons is 68 Ωcm, and that the value obtained in those studied in the hypertonic high-Mg^{++} solution is lower as a result of osmotic loss of water from the axon.

DISCUSSION

Possible Errors Resulting from Use of This Technique for Resistivity Measurement

The voltage output, V_o, for the recording system used in these experiments is:

$$V_o = (R_1/|Z_x|)\,V_s, \tag{1}$$

where R_1 is a feedback resistance, V_s is the source voltage, and $|Z_x|$ is the input impedance of the microelectrode in its local environment (Carpenter et al., 1973). Since R_1 and V_s are held constant, V_o varies inversely with $|Z_x|$.

The input impedance of the electrode has the form

$$Z_x = \frac{\rho}{4\pi a} + \frac{\bar{\gamma}}{4\pi a^2 (j\omega)^{\alpha}}, \tag{2}$$

where the first part represents the electrode impedance and the second the polarization impedance; (ρ is specific resistance of the medium in ohmcenti-

meters, a is electrode radius, $\bar{\gamma}$ is specific polarization impedance, ω is frequency, and the exponent, α, is characteristic of a given electrode system and has a value of 1 for a pure capacitance, 0 for a pure resistance or values in between for combinations, and j is equal to the square root of -1). Use of this technique is valid if the only difference between calibrating solutions and tissue measurements is in ρ, and not R_1, V_s, α, $\bar{\gamma}$, ω, or a. A serious difficulty in keeping these factors constant is in maintaining the coating of platinum black. The application of the platinum black is important to increase surface area and particularly to reduce the polarization impedance (Schwan, 1963). Unfortunately it is fragile and easily rubbed off with electrode use. It is, however, easy to detect electrodes in which this has occurred, since they show a decreased voltage ouput on withdrawal from the axon into the seawater as compared to the initial reading. Data obtained with such electrodes were discarded.

The degree to which the polarization impedance influences V_o is the ratio of polarization to electrode impedance and is equal to

$$\Delta = \frac{\bar{\gamma}}{\rho a (j\omega)^{\alpha}}. \tag{3}$$

Thus, the polarization impedance will have little effect if $\bar{\gamma}$ is small, conductance $(1/\rho)$ is small, ω is high, and a is large. Although it is very much preferable to have polarization impedance small, the use of this technique to measure conductivity is still valid in the presence of appreciable polariaztion impedance, provided that it is not different in calibrating and testing measurements.

The data illustrated in Fig. 3 provide evidence that the polarization impedance does not increase on penetration of an axon. Axoplasmic or solution resistivity, being a purely resistive component of the total impedance, is frequency independent whereas the polarization impedance (the second part of Eq. 2) is a function of frequency and decreases as frequency increases. The observation that the voltage output-frequency curve is identical in every respect in the axon and in an appropriate calibrating seawater dilution is definitive evidence that the electrode polarization impedance as well as other electrode properties have not changed on penetration. Under these circumstances the voltage outputs vary only with local conductivity, and as long as careful calibrations with each electrode are made in solutions of known conductivity, the method is a valid one for measurements of this parameter in cells.

Resistivity of Axoplasm

The average values for resistivity of squid axoplasm obtained in these experiments are in good agreement with other measurements on intact axons. The

values are slightly greater than those obtained on extruded axoplasm (Cole, 1975), possibly because of a small contamination of extruded axoplasm with seawater. The temperature dependence of resistivity of axoplasm is no different from that of the external salt solutions of equal resistivity, in agreement with the view that axoplasm may be considered functionally to be a dilute salt solution over a physiological temperature range.

These experiments confirm the variability in axoplasmic resistivity from one squid axon to another which is suggested by studies by several authors giving values ranging from 1.0–1.6 × SW (Cole and Hodgkin, 1939; Cole and Moore, 1960; Cole, 1968). Moreover, such a variability is also confirmed by totally independent experiments on extruded axoplasm (Cole, 1975). The explanation for this variability is not obvious, although the observation that smaller axons have a greater average resistivity suggests that axon size may be one important variable.

The resistivity of *Myxicola* axoplasm is nearly twice that of the squid. It is likely that this is a real species difference, since most of the *Myxicola* axons were as large as many of the squid axons. Furthermore, it is not likely that this difference is explicable on the basis of internal ion concentration. Gilbert and Shaw (1969) report Na^+ and K^+ concentration of 23 and 322 mmol/liter fiber H_2O, respectively, for extruded *Myxicola* axoplasm. These values compare with Na^+ and K^+ ranges of 44–101 and 267–369 mmol/liter fiber H_2O, respectively, for *Loligo pealei* (summarized by Brinley, 1965), and has been confirmed by estimates of E_{Na} and E_K obtained from voltage clamp studies of *Myxicola* axons (Goldman and Binstock, 1971; Binstock and Goldman, 1969). Carpenter et al. (1973) have shown depression of conductivity of salt solutions by addition of various carbohydrates and proteins, and have suggested that differing protein concentrations and composition may account for the very high intracellular resistivities found in *Aplysia* neurons. Differences in proteins may also explain the differing internal resistivities of these otherwise very similar giant axons.

Some of these experiments were performed as guests in the laboratories of Drs. K. S. Cole and W. J. Adelman, Jr., at the Marine Biological Laboratories, Woods Hole, Mass. We are particularly grateful to Dr. Cole for advice and support. Mr. William Burris and Mr. Harry Thompson provided valuable technical assistance. We are also grateful to Mr. L. Binstock for showing us the *Myxicola* preparation.

Received for publication 21 February 1975.

REFERENCES

BAK, A. F. 1967. Testing metal micro-electrodes. *Electroencephalogr. Clin. Neurophysiol.* **22**:186.

BINSTOCK, L., and L. GOLDMAN. 1969. Current- and voltage-clamped studies on *Myxicola* giant axons. Effects of tetrodoxin. *J. Gen. Physiol.* **54**:730.

BRINLEY, F. J., JR. 1965. Sodium, potassium and chloride concentrations and fluxes in the isolated giant axon of *Homarus*. *J. Neurophysiol.* **28**:742.

CARPENTER, D. O., M. M. HOVEY, and A. F. BAK. 1971. Intracellular conductance of *Aplysia* neurons and squid axon as determined by a new technique. *Int. J. Neurosci.* **2**:35.

CARPENTER, D. O., M. M. HOVEY, and A. F. BAK. 1973. Measurement of intracellular conductivity in *Aplysia* neurons: evidence for organization of water and ions. *Ann. N.Y. Acad. Sci.* **204**:502.

COLE, K. S. 1968. Membranes, Ions and Impulses. University of California Press, Berkeley, Calif.

COLE, K. S. 1975. Resistivity of axoplasm. I. Resistivity of extruded squid axoplasm. *J. Gen. Physiol.* **66**:133.

COLE, K. S., and A. L. HODGKIN. 1939. Membrane and protoplasm resistance in the squid giant axon. *J. Gen. Physiol.* **22**:671.

COLE, K. S., and J. W. MOORE. 1960. Liquid junction and membrane potentials of the squid giant axon. *J. Gen. Physiol.* **43**:971.

CURTIS, H. J., and K. S. COLE. 1938. Transverse electric impedance of the squid giant axon. *J. Gen. Physiol.* **21**:757.

GILBERT, D. S., and T. I. SHAW. 1969. Extrusion and perfusion of the giant nerve fibre of *Myxicola*. *J. Physiol.* (*Lond.*). **204**:28P.

GOLDMAN, L., and L. BINSTOCK. 1971. Rectification in instantaneous potassium current-voltage relations in *Myxicola* giant axons. *J. Physiol.* (*Lond.*). **217**:517.

SCHWAN, H. P. 1963. Determination of biological impedances. *In* Physical Techniques in Biological Research. W. L. Nastuk, editor. Academic Press, New York **6**:323.