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Passive Electrical Properties of Squid Axon Membrane

Shiro Takashima and Herman P. Schwan

Department of Bioengineering, Moore School of Electrical Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19174

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Summary. The admittance of the resting membrane of squid axon is investigated in natural and artificial seawater with alternating currents using an internal axial electrode. Corrections for the series electrolyte resistance between electrodes and membranes were applied to the measured admittance and the membrane capacitance and conductance are calculated. The membrane capacity was found to decrease from 1 $\mu F/cm^2$ to about 0.6 $\mu F/cm^2$ between 1 kHz and 50 kHz. The conductivity was found to increase from 1.2 $mmho/cm^2$ to 40 $mmho/cm^2$ in the same frequency range. In addition to the capacitive and conductive components, an inductive element is observed at low frequencies confirming the early work by Cole and co-workers. The effect of tetrodotoxin (TTX) and tetraethylammonium (TEA) on the membrane admittance was investigated. It was found that TTX did not have any effect on the capacitive component but has a profound effect on the inductive element. TEA alters the inductive element even more drastically than TTX.

It appears that present knowledge of the linear electrical properties of biological membranes is still limited. By linear properties are meant membrane capacitance and conductance per unit area surface membrane as detected with weak stimuli, sufficiently small so that the application of the signal does not affect these properties. These properties may be either obtained from studies in the time domain where a step in potential or current is applied or in the frequency domain where the frequency of the applied signal is varied. Both approaches are theoretically equivalent since the results from the one can be converted to the other. The time domain approach is frequently experimentally simpler and faster; the frequency domain approach has greater resolution and better reveals significant dynamic aspects of membrane behavior at a linear level although it is more laborious.

Dielectric dispersions have been reported for muscle, red blood cell and other biological membranes (Schwan, 1954, 1957, 1965; Fatt, 1964). These dispersions are probably not caused by a relaxation process in the membrane

itself but may well result from participation of the tubular system or a frequency-dependent surface admittance element due to counter-ions (Schwarz, 1962; Takashima, 1967).

Several attempts have been made to study if, and under what circumstances, artificial membranes display relaxation behavior. Work by Hanai, Haydon and Taylor (1964) and Mueller, Rudin, Tien and Wescott (1964) is in accord with a frequency-independent capacitance of hydrocarbon and bimolecular lipid films. The capacitance of lecithin membranes has been reported (Schwan, Huang & Thompson, 1966) to be frequency-independent from 20 Hz to 20 kHz with a resolution of better than 0.5%. A strong dispersion of the low frequency properties of vesicle membrane has been observed (Schwan, Takashima, Miyamoto & Stoeckenius, 1970), is probably due to counter-ion movement, and expected for charged vesicles. The membrane properties of uncharged vesicles, on the other hand, indicate only a slight relaxational behavior (Redwood, Takashima, Schwan & Thompson, 1972). A reported dispersion of the properties of bimolecular lipid membranes (Coster & Simmons, 1970) is suspected by us to depend critically on the assumed value of the electrolyte series impedance element between electrodes and membrane.

The electrical properties of squid axon membrane have been probably most extensively investigated. Earlier classical work on its linear properties by Cole and Curtis (1939) and Cole (1968) is interpreted in terms of the constant phase angle element of the membrane (Cole, 1932; Curtis & Cole, 1938; Cole, 1968). Taylor (1965), using an internal electrode, measured the admittance of squid axon membrane. The results he obtained, confirming Cole's interpretation, may be represented from 15 to 70 kHz by a constant phase angle element and a series resistance. More recently, Palti and Adelman (1969) have investigated the squid axon linear properties over the frequency range from 10 Hz to 2 kHz. No significant change in capacitance with frequency has been noted in their limited frequency studies. A dispersion has been noted more recently (Matsumoto, Inoue & Kishimoto, 1970), but it is not apparent that the series impedance element between electrode and membrane has been duly considered. It thus appears that nearly all artificial membranes so far studied are free of any dispersion effects at least as far as linear properties are concerned and that the evidence for such a dynamic behavior in the case of biological membranes is not yet assured or clarified.

In this article squid axon membrane data are presented over a frequency range located between those investigated by Palti and Adelman and by Taylor. A considerable dispersion of both capacitance and conductance is

observed even after the correction of data for the series resistance. The electrode arrangement excludes the possible participation of an admittance arising from the tangential component of the field around the axon. The use of an internal electrode ensures that the observed frequency-dependent admittance is strictly the property of axon membrane.

Experiments

Electrodes

The internal electrode is a platinum wire of 100-μ diameter coated with enamel. The end of the wire is exposed for a length of 15 mm and plated with platinum black. The internal electrode is inserted into the axon with the depth of the insertion about 2.0 cm. Hence, the exposed portion of the wire has no direct contact with the bathing solution. The external and ground electrode is also a platinum wire approximately 3 mm away from the outer surface of the nerve fiber. Stimulating electrodes are parallel platinum wires situated on one side of the trough. Action potentials are recorded at the beginning and the end of the impedance measurement to check normal electrical activity of the axon.

To estimate the contribution of electrode polarization, the following analyses were performed. Generally, the measured capacitance consisted of the capacitance of the sample and the capacitance due to electrode polarization as shown in Eq. (1) (Schwan, 1963).

$$C_t = C_s + \frac{G^2}{\omega^2 C_p} \tag{1}$$

where C_t and C_s are the total measured capacitance and the capacitance of the sample. In the second term, ω is angular frequency and G is the conductance of the sample. C_p is the electrode polarization capacitance. To estimate the magnitude of C_p for each electrode, capacitance and resistance of 0.1 M KCl solution are measured. In this case, the first term (C_s) is measured in picofarads and is negligibly small compared to the second term and can be neglected. Using measured total capacitance C_t and conductance G, we can calculate the magnitude of electrode polarization C_p. The value of C_p is usually of the order of 0.8 to 1.3 μF. Substituting these values in Eq. (1) we can estimate the contribution of electrode polarization to the total measured capacitance. For example, using the data at 500 kHz (*see* the first entry in Table 3), we obtain the magnitude of electrode polarization capacitance to be 0.0064 μF while the total capacitance is 0.2557 μF. Hence, we can safely conclude that the electrode polarization capacitance is negligible.

To minimize the end effect due to the longitudinal current flow, the electrode length was maintained at 15 mm which is sufficiently long and the end effect is considered not serious. However, no systematic study was made to determine the magnitude of the tip capacitance.

Admittance Bridge

All the measurements were carried out with a Wayne-Kerr bridge B-221. The bridge read-out is in terms of parallel capacitance and conductance (C and G) between 100 Hz and 100 kHz with seven dial settings. The range of the capacitance reading is from 0 to 10 μF and the conductance reading is from 0 to 100 mmhos. The wide range of

capacitance and conductance available made this bridge particularly suitable for the present measurement since the axon membrane exhibits a large variation of capacitance and conductance with frequency. A large capacitor of 20 μF was placed in series with the sample admittance to prevent a possible d−c shunt from the oscillator. The small error due to the presence of this capacitor was duly considered (Tasaki, 1968). The temperature of measurements was 20 °C all through these experiments and the bathing solution was not circulated.

Analysis of Equivalent Circuit

The equivalent circuit of axon membrane placed in an electrolyte solution is illustrated in Fig. 1*a*. The membrane is represented as a parallel combination of a conductance G_m and capacitance C_m. The capacitance of external solution including the Schwann cells was neglected and the external solution is represented by a resistance R_0. The Schwann cell is thought to be a very leaky condensor because of the presence of channels between the external media and the membrane. In view of this, neglect of the capacitance component is justified[1]. Likewise, any capacitance of axoplasm was ignored and the resistance of the internal solution is lumped into R_0.

The bridge method employed in this experiment measures two quantities; i.e., C_p[2] and G_p which are shown in Fig. 1*b*. The quantities C_p and G_p are expressed in terms of C_m and G_m and the series resistance R_0 by a simple transformation of circuits and they are given by the Debye equations (2) and (3).

$$C = C_\infty + \frac{C_0 - C_\infty}{1+(\omega T)^2} \tag{2}$$

$$G = G_0 + (G_\infty - G_0)\frac{(\omega T)^2}{1+(\omega T)^2} \tag{3}$$

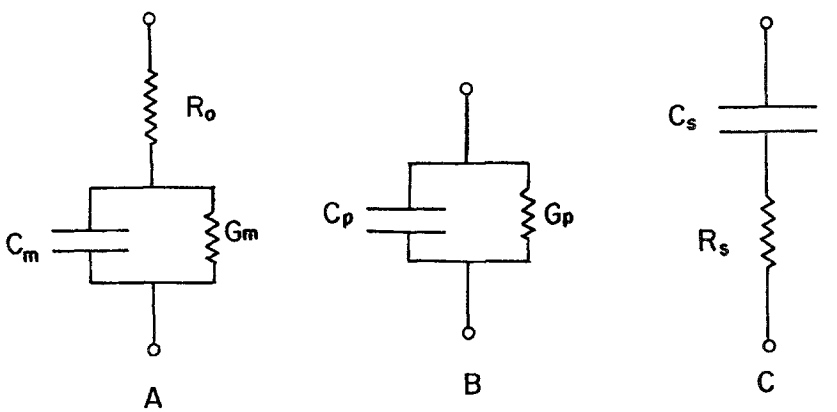

Fig. 1. Equivalent circuits of axon membrane. (*A*) Membrane admittance C_m and G_m with a series resistance R_0; (*B*) admittance representation of the circuit *A*; (*C*) impedance representation of the circuit *A*

1 If the Schwann layers have a small capacitance with a small resistance, the dispersion of capacitance due to Schwann layers should appear in the megahertz region.

2 This C_p should not be confused with electrode polarization capacitance.

where

$$C_0=\frac{C_m}{(1+R_0 G_m)^2} \qquad C_\infty=0$$
$$G_0=\frac{G_m}{1+R_0 G_m} \qquad G_\infty=1/R_0 \tag{4}$$

where $\omega=2\pi f$ and f is frequency. T is the time constant which is equal to the inverse of the center angular frequency of the dispersion:

$$T=\frac{C_m}{G_m+1/R_0}. \tag{5}$$

Only if $R_0 \ll R_m(=1/G_m)$ do C_0 and G_0 approach C_m and G_m and the dispersion shifts towards increasing frequencies.

It must be emphasized that Eqs. (2)–(4) indicate that the measured capacitance and conductance will depend on frequency regardless of the frequency characteristics of C_m and G_m when the series resistance R_0 is not negligible. Therefore, the apparent frequency dependence of measured capacitance and conductance do not necessarily represent the true frequency profile of membrane capacitance and conductance. The values of C_m and G_m at various frequencies are obtained only by solving Eqs. (2) and (3) with the knowledge of the magnitude of the series resistance.

The equivalent circuit of membrane shown by Fig. 1*a* can be put into the series representation in terms of X_s and R_s as shown by Fig. 1*c*. The plot of X_s *versus* R_s is called impedance locus which may trace a circle, an arc, a straight line or more complicated paths depending upon the characteristics of membrane admittance. The intersection between the impedance arc and the abscissa at the high frequency limit is exactly the value of series resistance R_0.

The experimental procedure is to plot the resistance R_s and reactance X_s against each other. For squid axon, the resulting impedance plot is circular *(vide infra)*. By reading the resistance coordinate of the intersection between the circle and the abscissa at the high frequency end, we can obtain the value of the series resistances. Using the value of series resistances in Eqs. (1) and (2) we can solve them for C_m and G_m. The use of the computer facilitates these calculations. An example of these calculations is shown in Fig. 5.

Experimental

All experiments were performed with the giant axon of squid pealii at Woods Hole, Mass. The length of axons was usually about 4 cm and the diameter was 400 to 600 μ. Natural and artificial seawater were used as the bathing solution. The voltage of the applied field for the admittance measurement across the axon membrane was 2 to 7 mV. This was sufficiently low to ensure the linearity of the measured admittance. Capacitances and conductances measured at different applied voltages are tabulated in Table 2. Obviously, the readings are independent of the applied potential in confirmation of the above statement.

The resting membrane potential and action potential were measured with microglass tip electrodes with a resistivity of 5 to 10 MΩ purchased from Transidyne Co. A silver-silver chloride wire was used as the reference electrode. The potential was amplified with an electrometer Keithley 605 and displayed on an oscilloscope. The measurement of the resting membrane potential was performed to confirm that the presence of an

Table 1. Composition of artificial seawater[a]

	ml
NaCl	423.0
KCl	9.0
$CaCl_2 \cdot 2\,H_2O$	9.27
$MgCl_2 \cdot 6\,H_2O$	22.94
$MgSO_4 \cdot 7\,H_2O$	25.50
$NaHCO_3$	2.15

[a] Volume of 1 M solution for one liter of seawater.

Table 2. Measured capacitance and conductance at various input voltages

Frequency (Hz)		Voltage (mV)		
		2	4	8
150	C μF	0.125	0.124	0.126
	G mmho	0.17	0.18	0.165
200	C μF	0.165	0.166	0.167
	G mmho	0.15	0.14	0.14
300	C μF	0.193	0.193	0.193
	G mmho	0.14	0.14	0.14
500	C μF	0.202	0.200	0.198
	G mmho	0.19	0.185	0.19

internal axial electrode (diameter 100 μ) does not disturb the resting potential. It was found that the insertion of an internal electrode does not shift the resting membrane potential within the accuracy of the measurement. We usually recorded a resting potential between −60 and −65 mV. The action potential was measured to be between 90 and 105 mV. The axons whose action potential was lower than 90 mV after the insertion of internal electrodes were discarded. A slight elevation of the threshold voltage was sometimes observed after the a–c measurements. However, the capacitance and conductance values were invariant even after several frequency sweeps over the entire frequency range (100 Hz to 100 kHz).

Results

The measurements of membrane admittance were carried out between 100 Hz and 100 kHz. The measured conductance and capacitance in natural seawater are shown in Fig. 2. As shown, the measured capacitance is strongly dependent upon frequency. Below 300 Hz, the capacitance decreases markedly and inverts into negative values. The conductance on the other hand, shows an inflection at the same frequency and begins to increase

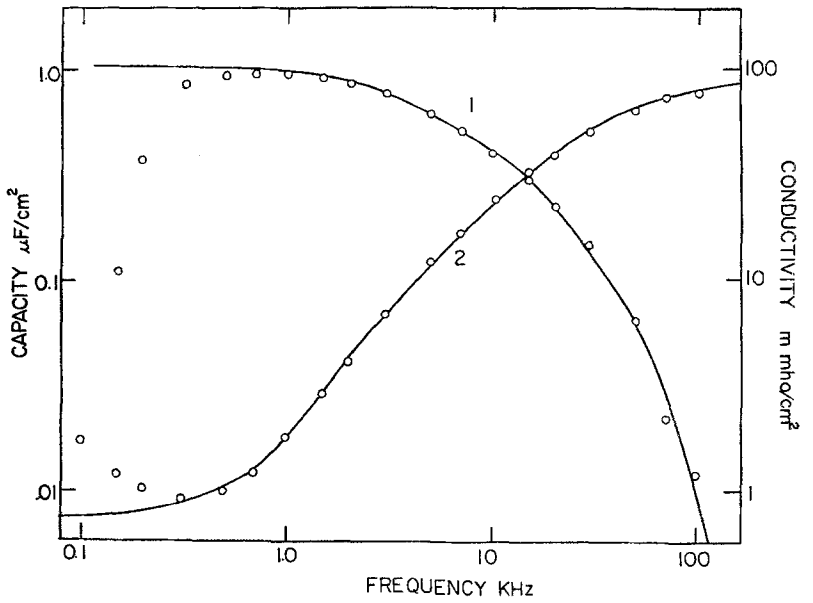

Fig. 2. Double logarithmic presentation of measured capacities and conductivities of axon membrane without the correction for series resistance. Curve 1 is capacity μF/cm² and curve 2 is conductivity mmho/cm². Solid lines are drawn through the experimental points ignoring the low frequency anomalies

as the frequency further decreases. This behavior is called an anomalous inductive reactance (Cole, 1941; Cole & Baker, 1941; Cole & Marmont, 1942; Cole, 1968). Above 300 Hz, the measured capacitance decreases with the increasing frequency and the conductance increases at the same time. The variation of the measured capacitance is from 1 μF/cm² to below 0.01 μF/cm² between 1 kHz and 100 kHz. The frequency dependence of capacitance and conductance, as mentioned before, may be partially or totally due to the presence of series resistance. To determine the magnitude of series resistance, the measured values are converted into the series quantities X_s and R_s and plotted against each other, thus obtaining the impedance plot.

The impedance plot, shown in Fig. 4, resembles remarkably the one obtained by Cole and Baker (1941), Cole (1941) and Cole and Marmont (1942) using external electrodes. The impedance plot consists of an inductive reactance at low frequencies and a capacitive reactance at high frequencies. In Fig. 3, the open circles are measured values and crosses are calculated using the inserted equivalent circuit suggested by Cole (*personal communication*).

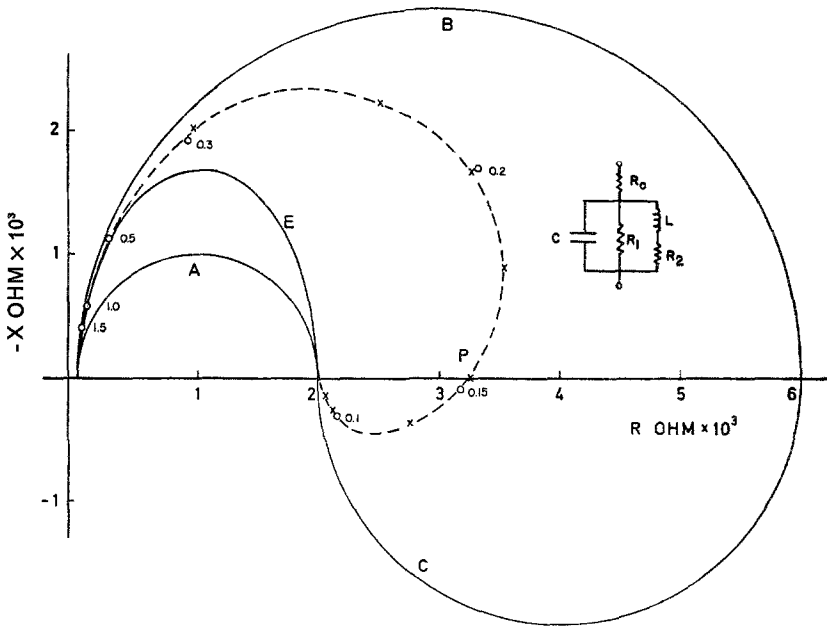

Fig. 3. Impedance loci of squid axon membrane between 100 Hz and 100 kHz. Open circles are measured values and crosses are calculated using the equivalent circuit. Curves *A*, *B* and *C* are semi-circles to show the contributions of the inductive and capacitive elements. For *A*, $L=0$; *B*, $L=\infty$, and *C*, $C=0$. The dashed locus is the pedal curve of the ellipse *E* and the pedal point *P* (Cole, 1968)

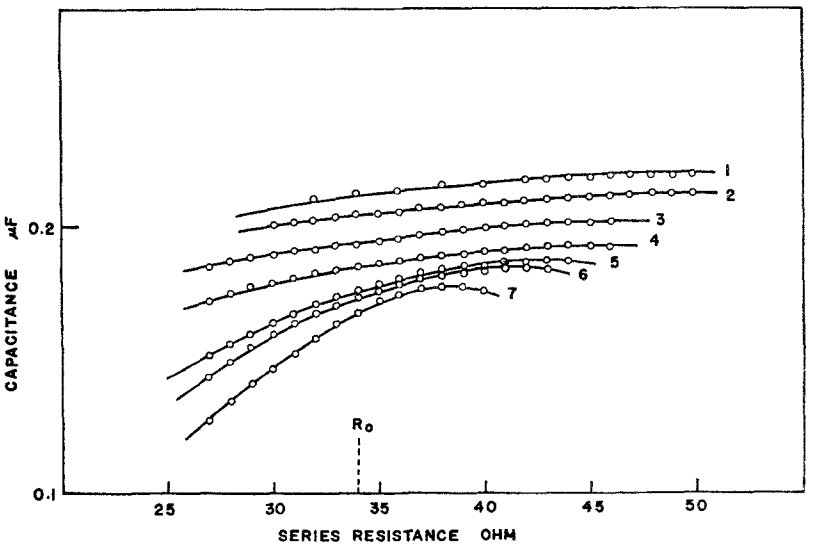

Fig. 4. Correction of measured capacitances for different values of series resistances (abscissa). Curves 1–7 are for frequencies 5, 7, 10, 15, 20, 30 and 50 kHz. Dashed line indicates the value of series resistance obtained from impedance locus

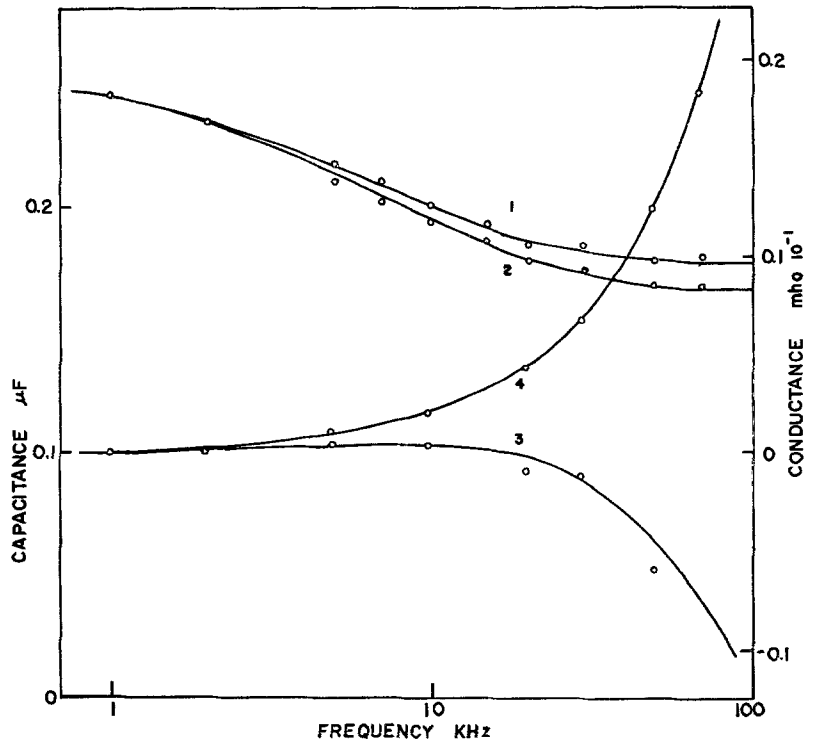

Fig. 5. Membrane capacitance and conductance after correction for the series resistance. Curve 1, capacitance obtained from the limiting values shown in Fig. 4. Curve 2, capacitance obtained by use of R_0 shown in Fig. 4. Curve 3, conductance obtained using the limiting values. Curve 4, conductance with R_0

From the intersection between the arc and the abscissa (resistance coordinate), we can read the value of R_0 which is usually 30 to 40 ohms. This gives rise to a specific resistivity of about 10 $\Omega\,\mathrm{cm}^2$. This value is slightly larger than the usual 7 $\Omega\,\mathrm{cm}^2$ but this is due to the fact that the ground electrode is purposely separated from the membrane.

The series resistance obtained is substituted into Eqs. (2) and (3) and the residual membrane capacitance and conductance are calculated. During the computer correction for series resistance, the value of the series resistance was purposely changed to test the sensitivity of calculated membrane capacitance and conductance. As shown in Fig. 4, the calculated membrane capacitance is very sensitive at high frequencies and is less sensitive at lower frequencies. Nevertheless, the calculated membrane capacitance approaches a limiting value as the series resistance increases. Further increase in the series resistance beyond these limiting values causes either no change or

Table 3. Capacitance and conductance of squid axon membrane in seawater at 20 °C

Frequency (kHz) [a]	Capacitance (μF)		Conductance (mmho)	
	Measured	Corrected	Measured	Corrected
0.5	0.2557	0.267	0.24	0.223
1.0	0.2610	0.279	0.48	0.377
2.0	0.2335	0.261	1.10	0.734
5.0	0.1715	0.225	3.31	1.93
10.0	0.1132	0.196	6.62	3.89
15.0	0.0832	0.183	9.12	5.24
20.0	0.062	0.168	10.87	6.87
30.0	0.0407	0.163	13.82	8.59
50.0	0.017	0.170	18.27	16.00
70.0	0.0059	0.200	20.92	56.40

The measured capacitance and conductance are corrected for series resistance (46.9 ohms).

[a] These are selected frequencies.

only a slight decrease (*see* curve 7 in Fig. 4). The limiting values of calculated membrane capacitance at various frequencies are shown in Fig. 5 (curve 1). This curve is somewhat different from the one obtained using the nominal value of series resistance (R_0 in Fig. 4). Usually the limiting capacitance values are not reached at the nominal value R_0 but at higher values as clearly shown in Fig. 4. For example, the limiting value at 50 kHz is reached at 38 ohms rather than 34 ohms (R_0). A question arises whether curve 1 or curve 2 represents the better approximation for the true membrane capacitance. If the value of series resistance 34 ohms is applied for measured conductance, we obtained a curve 4 in Fig. 5. Curve 3 represents the limiting conductance values which are the counterpart of curve 1. Obviously, the limiting conductance turns into negative values at high frequencies as a result of overcorrection and is unacceptable. In this work, therefore, it was decided to use the nominal values of series resistance rather than the limiting values.

The corrected capacitance and conductance at various frequencies are shown in Table 3. Fig. 6 shows the mean values of corrected membrane capacities and conductivities. Vertical bars represent standard deviations. From these curves it is obvious that the membrane capacity changes from the widely accepted value 1 μF/cm^2 to 0.55 μF/cm^2. This behavior is in contrast to artificial membranes where no dispersion of membrane capacity is observed. One can, thus, conclude that this apparent membrane capac-

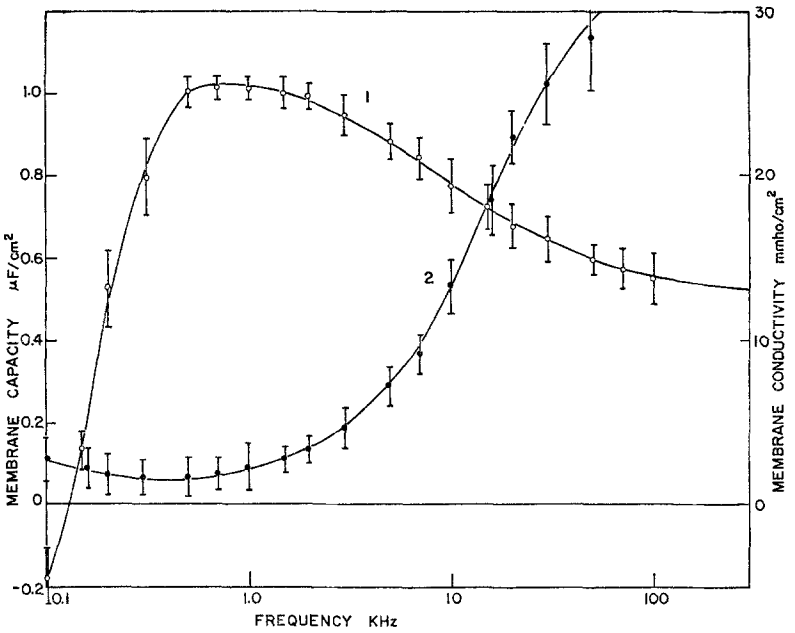

Fig. 6. Membrane capacity and conductivity of squid axon after correction for series resistance. Curve 1, capacity in μF/cm² and curve 2, conductivity in mmho/cm². Circles are mean values and vertical bars show the standard deviations. Averaged from 10 measurements

itance of squid axon consists of a frequency dependent as well as frequency independent parts. The frequency dependent part is about 40% of the total membrane capacity.

It is known that tetrodotoxin is a strong blocking agent of the early inward current and tetraethylammonium is an inhibitor of the late outward current seen in voltage clamp (Tasaki & Hagiwara, 1957; Mosher, Fuhrman, Buchwald & Fischer, 1964; Narahashi, Moore & Scott, 1964; Armstrong & Binstock, 1965; Nakamura, Nakajima & Grundfest, 1965; Moore, Blaustein, Anderson & Narahashi, 1967). To investigate the effect of these inhibitors on the frequency-dependent membrane admittance, the same bridge measurements were repeated in the presence of these toxins.

Tetrodotoxin was added in the external medium (approximately 10^{-6} moles) and after the disappearance of the action potential, the membrane admittance was measured in the same frequency range. Fig. 7 shows the membrane capacitance of axon without and with TTX. As obvious from this figure, TTX has very little effect on the dispersion of the membrane capacitance. However, the effect of TTX on the inductive element or nega-

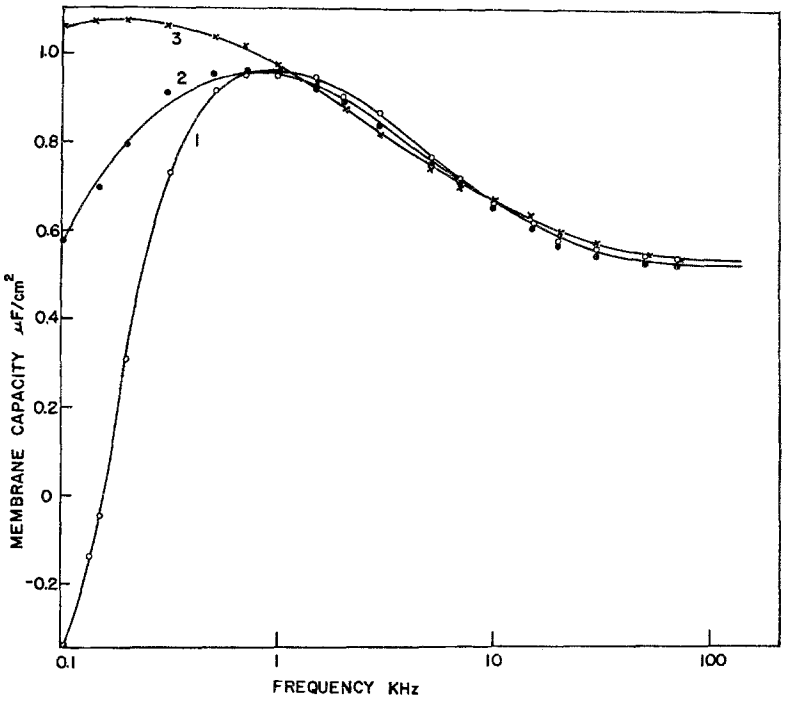

Fig. 7. Effect of tetrodotoxin (TTX) and tetraethylammonium (TEA) on membrane capacity. Curve 1, control; curve 2, with TTX (approximately 10^{-6} moles); curve 3, with TEA (approximately 100 mmoles)

tive capacitance is substantial and almost 80% of the negative capacitance disappears as measured at 100 Hz. Tetraethylammonium (TEA) was microinjected into the axon using a micropipette with a diameter of 100 μ. The duration of the action potential is between 40 and 100 msec. The height of the action potential is usually around 90 mV.

After the micropipette is pulled out, the axial electrode is inserted. Membrane capacitance and conductance with TEA are shown in Fig. 7. It is obvious that the negative capacitance at low frequencies is profoundly affected by TEA. Because of the elimination of the negative capacitance the membrane capacity increases beyond 1 μF/cm² and reaches 1.1 ~ 1.2 μF/cm².

Impedance loci of squid axon membrane are summarized in Fig. 8. The locus of control membrane and loci with TTX and TEA are shown in the same figure to facilitate the comparison between them. It should be noted that the impedance locus with TEA is drastically different from the other two.

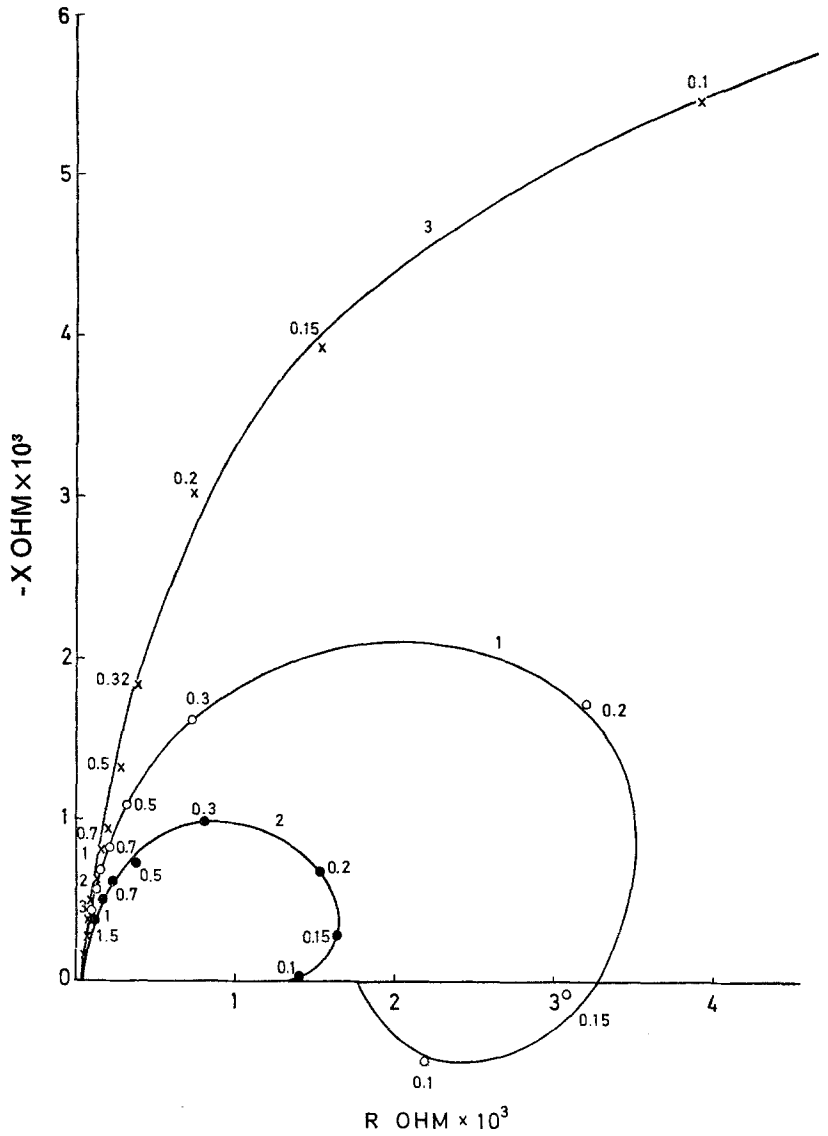

Fig. 8. Impedance loci of squid axon membrane. Curve 1, control; curve 2, with TTX; and curve 3, with TEA. Scales of reactances and resistance are in kΩ. Numbers on loci indicate the frequencies

Discussion

Membrane admittance of squid giant axon has been investigated by the use of an internal axial electrode combined with an external ground electrode. The experiments by Curtis and Cole (1938) and Cole and Curtis (1939)

were performed with external electrodes and in terms of a frequency-independent phase angle characteristic of the membrane. More recent measurements of the transmembrane impedance of squid axon have been carried out by Taylor (1965) with an arrangement similar to the present one. Taylor reported squid axon data between 10 and 70 kHz and suggested a dispersion with C_m limit values of 0.8 and 0.6 μF/cm^2 and a time constant of 6.5 μsec. However, the spread of the data and the high frequency range chosen did not permit accurate determination of the limit values of C_m at low and high frequencies and the time constant. On the other hand, Palti and Adelman (1969) investigated the squid axon membrane capacitance with a sinusoidal voltage clamp in the frequency range from 0.2 to 2 kHz and did not observe any frequency dependence. Clearly, the works of Taylor and of Palti and Adelman bracket our measurements and the results obtained by all are not in disagreement. As a matter of fact, the results of Taylor fit rather well with our data in the range from 15 to 70 kHz presented in Fig. 6. Adelman reports a small positive temperature coefficient of C_m of 1.4% per degree centigrade in the 3 to 21 °C range at 1 kHz. Such a temperature coefficient is anticipated for a dispersion behavior such as reported in this paper. The characteristic frequency of the dispersion should increase with temperature and, hence, for a temperature-independent static membrane capacitance C_{m0} a negative coefficient can be observed.

Some transmembrane admittance measurements are often impaired by insufficient analyses. Matsumoto, Inoue and Ohnishi (1967) and Matsumoto, Imai and Kishimoto (1970) performed transmembrane admittance measurements using an internal electrode. They state that the presence of a series resistance of 7 Ω cm^2 would not cause any measurable error in the measured membrane capacity. They appear to have concluded that the measured membrane capacitance was close enough to the true membrane capacitance. However, the present work clearly indicates that the presence of a series resistance of 7 to 10 Ω cm^2 is more than enough to cause considerable errors at high frequencies. Thus the sharp drop of the measured capacitances at high frequencies is partially or totally due to the presence of a series resistance even though its magnitude is much smaller than that of membrane resistance. For example, artificial lipid bilayer membranes display a distinct frequency-dependent capacitance and resistance. However, the capacitance and the conductance of the membrane *per se* are found frequency independent (Hanai, Haydon & Taylor, 1964; Schwan, Huang & Thompson, 1966; Takashima & Schwan, *to be published*). Therefore, to determine the membrane impedance or admittance, the measured impedance or admittance

must be carefully analyzed and proper corrections for the series resistance element must be applied.

The present work was aimed at the study of the frequency dependence of the membrane capacitance and conductance at relatively high frequencies using an a–c admittance measurement technique. The correction for the series resistance is theoretically feasible but the actual determination of the value of R_0 is difficult and relatively unreliable. The use of impedance plots facilitates the procedure of the determination of the series resistance. The results described above clearly indicate that membrane capacitance and conductance are partially frequency dependent. The calculated membrane capacity decreases from 1 $\mu F/cm^2$ to 0.6 $\mu F/cm^2$ between 1 kHz and 50 kHz which may be approximated by separate series or parallel dispersion elements or by a constant phase angle element. This result is in contrast to those obtained with artificial membranes where membrane capacity does not undergo a relaxation. The membrane capacity of artificial membranes arises either from hydrocarbon or from lipids. A recent attempt to measure the admittance of artificial membranes in the presence of an active element, alamethicin (Mueller & Rudin, 1964), reveals that the membrane admittance is still frequency independent (Takashima & Schwan, *to be published*). In natural membranes, proteins are present in addition to lipids. Therefore, some difference in the membrane admittance between natural and artificial membranes is not surprising.

It is still uncertain whether or not the frequency-dependent capacitance is characteristic of excitable membranes. The question can perhaps be formulated as follows: Is the frequency-dependent capacitance relevant to the excitability of axon membrane or does it merely represent the inactive bulk of the membrane? To investigate this problem, membrane admittance measurements were repeated with the addition of tetrodotoxin (TTX) and tetraethylammonium (TEA). These toxins are known to affect the excitation by blocking the early inward current and late outward current, respectively. As shown in Figs. 7, TTX partially eliminates the inductive element. However, as shown in Figs. 7 and 8, some inductive reactance contribution remains even in the presence of TTX without any action potential. However, it has only a minute effect on the dispersion of membrane capacity. The effect of TEA is in many ways similar to that of TTX in that it has only a small effect on the capacitive component; but the effect of TEA on the inductive reactance is more drastic than TTX. TEA virtually eliminates the inductive reactance.

The inductive elements in squid axon membrane are considered related in some way to the mechanism of excitation. Hodgkin and Huxley pointed

out (1952) that the n and h processes in the late and early channels would produce an inductive reactance. It is known that the linearization of the Hodgkin-Huxley equation leads, after Laplace transformation, to frequency domain terms which give rise to inductive reactance (Leibovic, 1972). Although it was not done in this work, the relative contribution of sodium and potassium terms can be evaluated by numerical calculations. Cole (1968) states "Cole and Baker (1941) and Hodgkin and Huxley (1952) concurred with the earlier conjecture in ascribing it (inductive reactance) primarily to potassium". Our observation that TTX eliminates the inductive reactance partially (perhaps through affecting the inactivation parameter h of the early channel) and that TEA eliminates it nearly completely (by affecting the activation parameter n) seems to support qualitatively those statements. The relative insensitivity of the capacity of the membrane at high frequencies to those toxins is somewhat disappointing and there are remote indications that the frequency-dependent capacitance may not be related directly with the excitability although it indeed suggests the presence of dipolar species in the membrane.

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