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Membrane Capacity of Squid Giant Axon during Hyper- and Depolarizations

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Summary. The change in membrane capacitance and conductance of squid giant axons during hyper- and depolarizations was investigated. The measurements of capacitance and conductance were performed using an admittance bridge with resting, hyperpolarized and depolarized membranes. The duration of DC pulses is 20–40 msec and is long enough to permit the admittance measurements between 1 and 50 kHz. The amplitudes of DC pulses were varied between 0 and 40 mV for both depolarization and hyperpolarization. Within these limited experimental conditions, we found a substantial increase in membrane capacitance with depolarization and a decrease with hyperpolarization. Our results indicate that the change in membrane capacitance will increase further if low frequencies are used with larger depolarizing pulses. The change in membrane capacitance is frequency dependent and it increases with decreasing frequencies. The analyses based on an equivalent circuit (vide infra) gives rise to a time constant of active membrane capacitance close to that of sodium currents. This result indicates that the observed capacitance changes may arise from sodium channels. A brief discussion is given on the nature of frequency-dependent membrane capacitance of nerve axons.

Years ago, Cole and Curtis (1938, 1939) investigated the change in membrane capacitance and conductance during nerve impulses using squid giant axons. They found that while membrane conductance underwent an enormous increase, membrane capacitance virtually remained constant. The consequence of this observation was far reaching. The results obtained by Cole and Curtis became the basis of the hypothesis that membrane capacitance represents only the inactive bulk of excitable membranes and has no bearing on the mechanism of nerve excitation. In voltage-clamp experiments, under normal conditions, the membrane capacitance manifests itself as an early spike current and provides no information of physiological importance. Under these circumstances, it is not surprising that the study of membrane capacitance played the less important role in the development of axon physiology in recent

years compared to the voltage-clamp technique (Moore & Cole, 1963; Cole, 1968).

A few years ago, however, several reports were published by Armstrong and Bezanilla (1973), Bezanilla and Armstrong (1974) and Keynes and Rojas (1974). These authors report observations of gating currents. This current apparently had been hidden behind overwhelmingly large ionic currents under the normal voltage-clamp conditions. In the experiments, ionic currents are blocked by appropriate pharmacological agents. Therefore, when depolarizing or hyperpolarizing pulses are applied, only capacitive currents flow through the membrane. The spike current which is produced by the frequency-independent capacity is carefully subtracted. The remaining current after this subtraction represents a time-dependent displacement current and is called by these authors the gating current or asymmetry current. If these displacement currents are indeed due to the opening and closing of ionic gates, the observation of gating currents indicates that the nerve impulse would involve yet unknown polar molecules and the action potential would be accompanied by a change in the membrane capacity. Moreover, the change in the membrane capacity must be frequency dependent if, as claimed by these authors, the gating current is a time-dependent displacement current.

These recent developments pose a question why no capacity change was observed by Cole and Curtis during the action potential. There may be a few possible explanations. First of all, it is quite possible that the so-called gating current is merely due to a dipole orientation of some polar species in the membrane. These polar molecules may not be located in or around ionic channels, and, if so, the displacement current may not have any relevance to the gating mechanism. Actually, the waveform of the gating current shown by these authors is typical of the displacement current due to dipole orientation. Therefore, as long as there are some polar molecules in the membrane, they would give rise to a time-dependent capacitive current. The asymmetry of gating current with regard to the membrane potential can perhaps be explained in terms of the uneven internal field due to asymmetric distribution of ionic species. Under these circumstances, we do not have to invoke ionic channels to explain the asymmetric gating current.

On the other hand, however, a study by Meves (1974) provides some evidence that the asymmetry current is somehow related to the gating mechanism. Under these circumstances, the asymmetry current may not be totally unrelated to the gating mechanism and we have to find some other explanation. The amplitude of the gating currents is exceedingly small.

Therefore, it is possible that the capacity change was too small to be detected with the transient technique using an AC bridge. Secondly, the time constant of gating currents is about 0.4–0.8 msec. If this is converted to frequency, it would be about 400 Hz or less. Therefore, in order to observe the capacity change, the measurements have to be done at much lower frequencies than those used by Cole and Curtis. The lowest frequency used by them was 5 kHz and mostly 20 kHz was used for their measurements. The duration of the action potential is only 1 msec and the use of frequencies below 5 kHz for admittance measurements during the action potential is very difficult. These may be the possible reasons why Cole and Curtis did not observe any change in the membrane capacity during the action potential. A recent study by Feldman and Guttman (1975) on the membrane impedance of squid axons during hyper- and depolarizations indicates that the changes in the reactance as well as resistance components are much more pronounced at low frequencies. This result indicates the importance of using low frequencies for detecting capacitance changes during hyper- and depolarizations.

The concept that the opening of gates may be a response of dipoles to an electrical field appears already in the paper by Hodgkin and Huxley (1952). Subsequently, Schwan (1957) points out the possible existence of gating currents and further discusses that the conductance change observed by Cole and Curtis must be accompanied by a capacity change (Schwan, 1966). We carried out measurements of membrane capacity and conductivity during depolarization as well as hyperpolarization using a long pulse duration which enabled us to make transient admittance measurements down to 1 kHz. Although this frequency is not sufficiently low, we nevertheless observed changes in membrane capacitance in addition to the conductivity change.

Materials and Methods

All experiments were performed using squid *Loligo pealeii* available at the Marine Biological Laboratory, Woods Hole, Mass. Nerve axons were dissected for a length of 4–5 cm. After moderate cleaning, they were mounted in an axon chamber. During all measurements, cold seawater was circulated and the temperature was maintained at 6–8 °C. In these experiments, three internal electrodes were used. A Pt-Ir wire with a diameter of 75 μ and a length of 16 mm coated with platinum black was used for admittance measurements. The second wire which was also Pt-Ir wire with a diameter of 25 μ coated with teflon was used to apply square pulses. Finally, a microglass pipette filled with 3 mol KCl-agar was used to monitor the membrane potential. DC pulses were generated with a Grass Stimulator S-88 and applied to the membrane through a 500 kΩ resistor. The amplitudes of pulses were adjusted empirically

until the glass electrode recorded the desired membrane potential. Although we used square pulses with a rise-time of about 1 μsec, the actual rise-time of the membrane potential is much slower than the rise-time of the applied pulses. Usually, we observed the rise-time of membrane potential to be of the order of 1–2 msec. According to Cole and Baker (1941), the membrane admittance with depolarization or hyperpolarization does not depend upon the speed with which the desired membrane potential is attained. If depolarizing pulses exceed

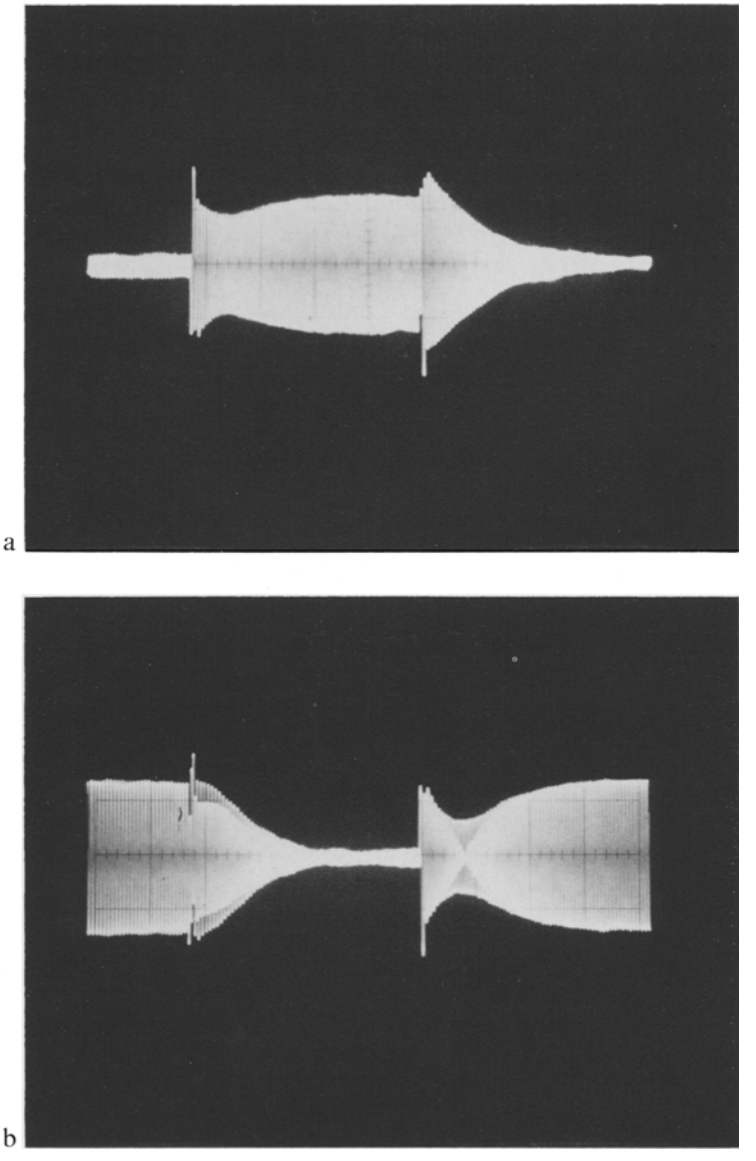

Fig. 1. (*a*) AC current flowing through the bridge arm during a depolarizing pulse. The bridge unbalance is caused by the change in the membrane capacitance as well as conductance with depolarization. (*b*) Rebalancing of the bridge with the depolarized membrane

20 mV, an action potential is elicited. However, the firing of action potential can be avoided by using a ramp voltage pulse instead of square waves. The presence and absence of an action potential at the beginning of long pulses apparently does not change the final membrane admittance as long as the pulse amplitudes are the same.

The measurements of membrane capacitance and conductance were carried out with a Wayne-Kerr admittance bridge B-221. The bridge is capable of measuring capacitance and conductance between 100 Hz and 50 kHz. The bridge is first balanced to the resting membrane, and capacitance and conductance are recorded. When hyper- or depolarizing pulses are applied, the admittance of the membrane changes and the bridge becomes unbalanced. The AC current flowing through the bridge arms is monitored on an oscilloscope. The capacitance and conductance dials are carefully adjusted until the bridge becomes rebalanced for hyper- or depolarized membranes. These procedures are essentially the same as those used by Cole and Curtis (1938) and by Cole and Baker (1941) except for the use of internal electrodes in the present experiment. Fig. 1 illustrates an example of these measurements. Diagram (*a*) shows the AC current after it became unbalanced by a depolarizing pulse and diagram (*b*) shows the rebalancing of the bridge toward the end of the pulse. The transient admittance measurements were carried out between 1 and 50 kHz and the amplitude of DC pulses were varied between 0 and 40 mV for both depolarization and hyperpolarization. As shown in Fig. 3, the change in membrane capacity is nonlinear with the DC potential. For the measurement of membrane admittance using AC fields, the amplitude of AC potential must be kept as low as possible in order to avoid the shift of membrane potential by the AC field. In these measurements, AC fields with an amplitude of 3–4 mV were used for all frequencies. In order to establish the linearity of measured capacitance and conductance, some of the measurements were repeated by varying the voltage of applied AC fields. This procedure was used previously by Takashima and Schwan (1974) for resting membranes. They observed that the measured capacitance and conductance did not depend on the amplitude of AC fields within 2–8 mV peak-to-peak and thus concluded that the applied voltage of 3–4 mV of AC fields is sufficiently low to secure the linearity of the measured admittances. However, the transient measurements of membrane capacitance and conductance during hyper- and depolarizing pulses involve a greater error than those with resting membranes. In spite of this, the membrane capacitance and conductance did not depend on the voltage of applied AC fields between 2–8 mV within the experimental error. Having obtained these results, we concluded that the shift in the membrane potential by the applied AC field is minimal.

As mentioned in the previous publication (Takashima & Schwan, 1974), measured capacitance and conductance were corrected for the series resistance. The values of series resistance were determined by using impedance loci (Fig. 2). Usually, the value of the series resistance does not change either with hypolarization or depolarization and this indicates that the admittance change during pulses is only due to the change in the membrane. Therefore, if the value of series resistance is determined with resting membranes, the same value can be used for depolarized and hyperpolarized membranes. As will be discussed later, the correction for the series resistance is crucial for the correct determination of membrane capacities during pulses and has a far more important consequence on the final result than we originally expected.

To estimate the contribution of electrode polarization to the capacitance of depolarized membranes, we used the method discussed by Schwan (1963). In general, measured capacitance consists of capacitance of the sample and capacitance due to electrode polarization as shown by Eq. (1).

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

where C_t and C_s are total measured capacitance and the capacitance of the sample. G is the conductance measured at angular frequency $\omega(=2\pi f)$ and C_p is electrode polarization capacitance. It is well established that if electrode polarization is predominant, the total measured capacitance will be a straight line with a slope of -1.5 when C_t is plotted against

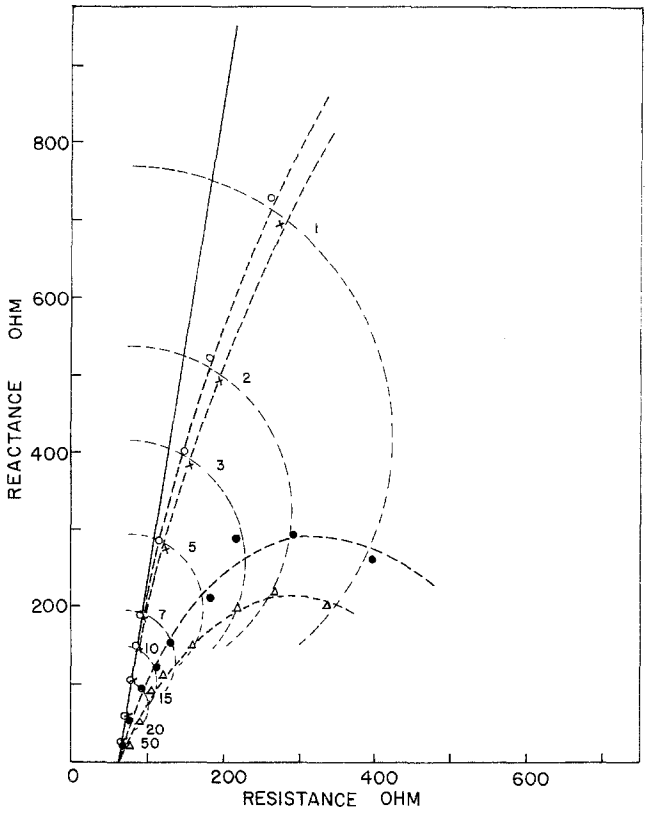

Fig. 2. Impedance loci of squid axon membrane. Crosses show the impedance of resting membrane (−60 mV), open circles, hyperpolarized membrane at −100 mV, and filled circles and triangles, depolarized membranes at −40 and −30 mV. Circular trajectories connect points obtained at the same frequencies. The numbers are frequencies in kilohertz. Reactances and resistances are not corrected for area. The straight line is the limiting slope of the constant phase angle

frequency in double logarithmic scale. The double logarithmic plot was attempted for resting, hyperpolarized and depolarized membranes. However, we observed, in no case, the sign of −1.5 slope and we concluded that the interference of electrode polarization is negligible even with depolarized membranes where the membrane conductance increases considerably.

Results

Measured membrane capacitance and conductance were transformed into impedance quantitites and the resistance and reactance are presented in a complex plane. The locus obtained with a resting membrane is shown in Fig. 2 by curve 1. As shown, the impedance locus is an arc with the high frequency limiting slope having an angel of 82°. The resistance value at

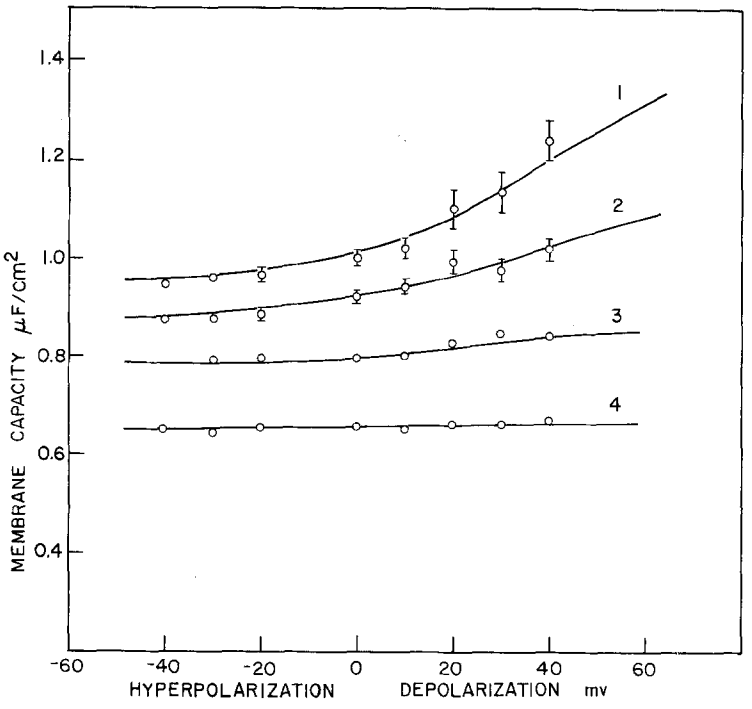

Fig. 3. Changes in measured membrane capacity at different frequencies. Curves 1, 2, 3 and 4 are obtained at 1, 2, 5 and 20 kHz. Vertical lines are standard deviation. The absence of the bar indicates sufficiently small errors. The center of the abscissa is taken to be the resting potential, i.e., −60 mV

the intersection between the arc and the resistance axis is the approximate value of series resistance (R_0). In this particular instance, we find a value of 60 ohms for R_0 and this value is used to correct the measured capacitance at each frequency and for hyper- and depolarizations. The capacity of the resting membrane after the correction for the series resistance is shown in Fig. 5 by curve 1.

Hyperpolarization

In order to investigate the electrical characteristics of axon membrane during excitation, the properties of passive membrane must be established. However, it is well known that axon membranes at the resting potential are not necessarily passive membranes. Ionic channels are partially open at a membrane potential of −60 mV. In particular, about 30 percent of potassium channels are still open at the resting potential. In this regard,

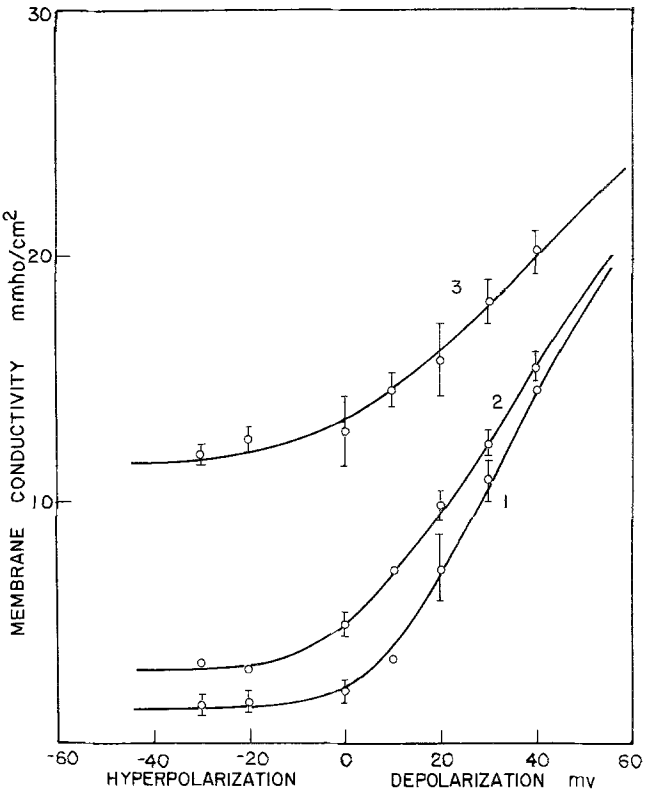

Fig. 4. Changes in the measured membrane conductivity at different frequencies. Curves 1, 2 and 3 are determined at 1,5 and 20 kHz. Vertical bars are standard deviation

the resting membrane contains certain active elements and cannot be considered a passive membrane. The passive membrane can be obtained by hyperpolarizing the resting membrane by at least 40 to 60 mV. At these potentials, the m and n parameters defined by Hodgkin and Huxley (1952) reduce to zeo and the h parameter to unity. Only under these conditions, can the membrane be considered passive.

Capacitance and conductance of axon membranes during the hyperpolarization at various amplitudes are shown in Figs. 3 and 4. These Figures show the mean values obtained with several axons and the standard deviations are indicated by vertical bars. The impedance locus of a hyperpolarized membrane at -100 mV is shown in Fig. 2. It should be noted that the locus approaches the limiting slope of a constant phase angle (Cole, 1932). The membrane capacity of hyperpolarized membrane is shown in Fig. 3. Note the decrease in the membrane capacitance at all frequencies. The conductances of hyperpolarized membranes at several

frequencies are shown in Fig. 4. Capacitances and conductances of hyperpolarized membranes are replotted against frequencies and shown in Figs. 5 and 6 (curve 2). These two curves may be regarded as the capacity and conductivity of the passive membrane. The electrical properties of depolarized or active membranes must be compared with these two curves rather than those of the resting membrane.

Depolarization

Capacitances and conductances of several axons with depolarizing pulses are shown in Figs. 3 and 4 for various pulse amplitudes. By contrast with hyperpolarization, depolarization causes an extensive increase in the membrane capacity in addition to the increase in membrane conductivity. For example, with a 40 mV depolarizing pulse, the membrane capacity increases from 1 $\mu F/cm^2$ to 1.2 $\mu F/cm^2$ and the curve has not reached saturation at this potential. This is an indication that further depolarization would increase the membrane capacity even more. Unfortunately, during the larger depolarization, the membrane tends to deteriorate and the results become progressively inconsistent. It should be pointed out that the increase in the membrane capacity is nonlinear with the amplitude of depolarizing pulses. The change in the membrane conductivity with depolarizing pulses are shown in Fig. 4 at several frequencies. While membrane capacity increases by at most 20–30 %, the membrane conductance increase is easily several times that of the resting membrane. In particular, the increase at low frequencies is pronounced. At high frequencies however, the increase gradually diminishes and finally at 50 kHz, no conductance change can be detected even with the largest depolarizing pulse used in this experiment.

The membrane capacity and conductivity with a 40-mV depolarization are shown in Figs. 5 and 6 at various frequencies. As shown, the increase in membrane capacity becomes progressively large as the frequency decreases. The increase at 1 kHz is about 25 %. However, the curve indicates a further increase at frequencies below 1 kHz. In order to observe the full extent of the capacity change, therefore, the measurement must be performed at frequencies below 1 kHz and at the same time, with larger depolarizations. It is unfortunate that these conditions are difficult to obtain with the present experimental set-up. The duration of depolarizing pulses used in these experiments is 20–40 msec. In order to use low frequencies for the transient admittance measurement, a much longer pulse

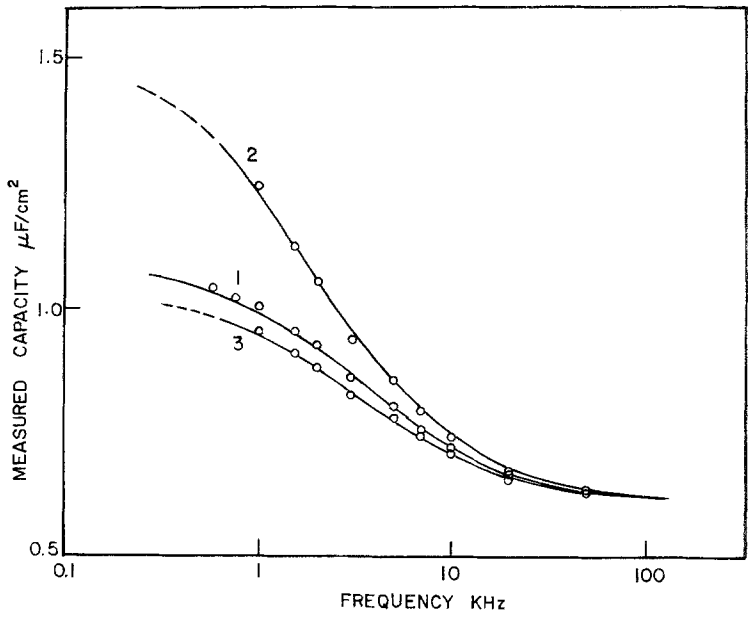

Fig. 5. Measured capacities of resting (curve 1), depolarized (curve 2) and hyperpolarized (curve 3) membranes. Membrane potentials of hyper- and depolarized membranes are −100 and −20 mV, respectively

duration may be required. However, these long pulses cannot be used because of the reasons mentioned above. The membrane conductance with 40 mV depolarization is shown in Fig. 6 at various frequencies. Obviously, the conductance change is pronounced at low frequencies and gradually diminishes as the frequency increases.

The capacitance changes described above are analyzed using the following equivalent circuit shown in Fig. 7. This is the diagram having the Hodgkin-Huxley Na branch added to the membrane capacitance C_0 (Cole, 1932). In this diagram, C_0 is the membrane capacitance at resting state and R_k is the lumped potassium and leakage conductances. R_1, R_2 and C_1 represent the sodium branch. Diagram (*a*) can be reduced to (*b*) by lumping R_k and R_2 and renamed as R_∞. Diagram (*b*) can further be represented by a lumped circuit (*c*) having a parallel capacitance C and conductance G. The capacitance C and conductance G are given by Eqs. (2) and (3):

$$C = C_0 + C_1/(1+\omega^2\tau^2) \tag{2}$$

$$G = G_\infty + \frac{\omega^2\tau^2}{1+\omega^2\tau^2}\frac{1}{R_1} \tag{3}$$

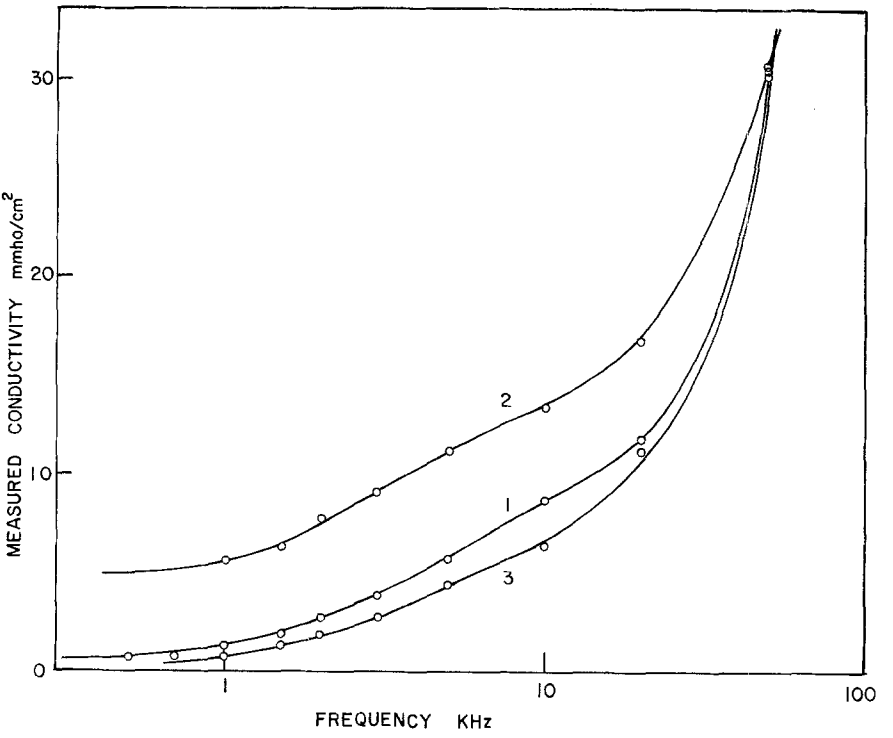

Fig. 6. Conductivities of resting (curve 1), depolarized (curve 2) and hyperpolarized (curve 3) membranes. Curves 2 and 3 are obtained at the membrane potentials −100 and −20 mV, respectively

where $G_\infty = 1/R_\infty$ and $\tau = C_1 R_1$. Eliminating ω from Eqs. (2) and (3), we obtain

$$C = (C_0 + C_1) + \tau(G_\infty - G). \tag{4}$$

Eq. (4) indicates that if measured capacitance C is plotted against $(G_\infty - G)$, a straight line should result if the circuit shown in Fig. 7 is a correct presentation of an active membrane. The slope of this plot is the time constant τ and the intersection between this plot and ordinate is the sum of capacitances of resting and active membranes, i.e., $(C_0 + C_1)$. One of the results of these analyses with a 40-mV depolarization is shown in Fig. 8. As shown in this Figure, the plot of C *vs.* $(G_\infty - G)$ is a straight line within the experimental error indicating that the equivalent circuit (*a*) in Fig. 7 indeed represents the active membrane rather correctly. From the slope of this plot, we obtain a time constant of 0.59 msec. This value should be compared with the time constant of sodium currents with the same depolarization (40 mV) calculated with the Hodgkin-Huxley (1952) equation,

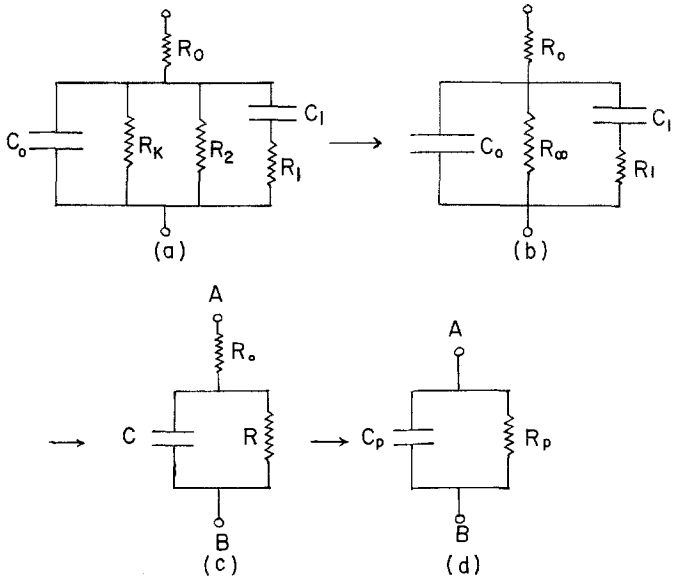

Fig. 7. (*a*) Equivalent circuit of the Hodgkin-Huxley membrane having the sodium branch replaced with the constant phase angle elements, R_1, R_2 and C_1. C_0 is the resting membrane capacity, R_K is the lumped potassium and leakage conductances and R_0 is series resistance. (*b*) Potassium and leakage conductances are lumped together and renamed to be R_∞. (*c*) Represents the lumped circuit of the membrane (*a*). (*d*) A lumped membrane equivalent circuit including series resistance R_0. C and R are the quantities experimentally measured

i.e., 0.33 msec. The observed time constant is sufficiently close to the calculated value. This result indicates that the capacitance changes observed by us may indeed arise from the activation of sodium channels.

The method described above is also an excellent analytical tool to determine the low frequency limit of the capacitance change. As shown in Fig. 6, the capacitance change has not reached the limiting value even at the lowest frequency used in these measurements. In order to determine the full extent of capacitance change, we must further extend the measurement to lower frequencies. As mentioned before, this is not feasible with the present experimental technique. As seen from Fig. 8, the plot of C *vs.* $(G_\infty - G)$ is linear except at the high frequency end. The linearity of the plot makes the interpolation relatively easy and less arbitrary. The intersection between the ordinate and the interpolated line yields the sum C_0 and C_1 and this, by definition, is the full amplitude of the capacitance change for this depolarization. From Fig. 8, we obtain the sum $C_0 + C_1$ to be 1.32 $\mu F/cm^2$. This value is consistent with the value shown in Fig. 3 (the membrane capacity with a 40-mV depolarization is about 1.2 $\mu F/cm^2$).

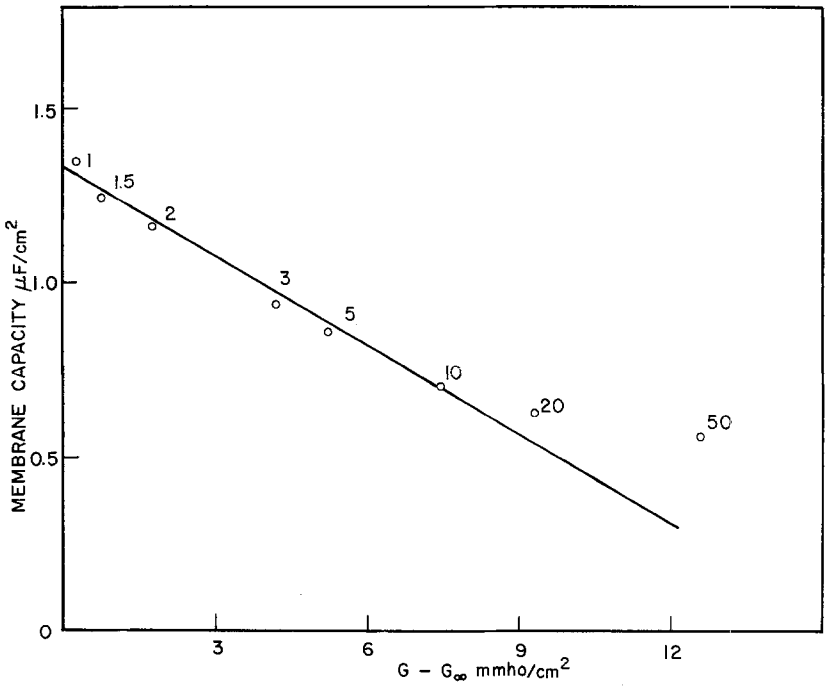

Fig. 8. The plot of the capacity C against $-(G_\infty - G)$. G_∞ is equal to $1/R_\infty$ and can be determined as the low frequency limit of G measured at various frequencies during depolarizing pulses. Numbers at each experimental point indicate the frequency in kHz

In addition to these, the close fit of experimental points on a straight line indicates the absence of electrode polarization in spite of the considerable increase in the membrane conductance with depolarizations. If electrode polarization is present, this would cause an upswing of the plot at low frequencies and cause a considerable systematic deviation from the linearity. Combining this observation with the discussion presented above, we can conclude that the possible error due to electrode polarization is perhaps minimal.

Discussion

The results described above indicate that the membrane capacity of squid axon changes substantially with depolarization. Furthermore, the capacity change is frequency dependent, i.e., the amplitude of the capacity change increases with the decreasing frequency. Although we were unable to observe the full extent of the capacitance increase, the

amplitude of the capacity change is more than 25 % of the total membrane capacity with moderate depolarizing pulses. Our results indicate that the full amplitude of the capacity change may reach as high as 30 % or more if measured at sufficiently low frequencies with larger depolarizing pulses.

As has been discussed frequently, the electrolyte solution between electrodes and the membrane constitutes a series resistance to the membrane admittance. The equivalent circuit of the membrane with a series resistance is presented in Fig. 7. The capacitance measured between points A and B in diagram (c) can be easily calculated and is given by Eq. (5):

$$C_p = \frac{C R^2/(R+R_0)^2}{1+\omega^2 \left(\frac{R C R_0}{R_0+R}\right)^2}. \tag{5}$$

At sufficiently low frequencies, the measured capacitance reduces to

$$C_p = \frac{C}{(1+R_0/R)^2}. \tag{6}$$

In passive membranes, the membrane resistance is much larger than the series resistance and, therefore, the membrane capacitance becomes virtually equal to the measured capacitance, i.e., $C \approx C_p$. However, in depolarized membranes, the resistance R decreases substantially and the relation $R \gg R_0$ no longer holds. This, in turn, indicates that measured capacitances are considerably smaller than the true membrane capacitance. Therefore, the correction of the measured capacitance for the series resistance becomes increasingly important as the depolarization progresses. Normally for resting and hyperpolarized membranes, the correction for the series resistance is small at low frequencies. However, for depolarized membranes, the correction for the series resistance becomes significant even at low frequencies. This is simply due to the increase in the relative magnitude of the series resistance compared to that of the membrane resistance. Table 1 summarizes the membrane capacitance at 1 kHz before and after the correction. This Table clearly demonstrates that without the correction for the series resistance, we would not have observed the increase in the membrane capacity. In most cases, the apparent membrane capacitance decreases slightly with depolarizing pulses. This can be easily explained if the increase in C [*see* Eq. (6)] is relatively small compared with the increase in the denominator.

As discussed above, the correction for the series resistance is critically important to correctly determine the capacitance change during depolar-

Table 1. Membrane conductance and capacitance with depolarizing pulses with and without correction for series resistance (Frequency: 1 kHz)

G_m (mmho)	C_m (without) (μF)	C_m (with) (μF)	Depol (mv)
0.74	0.234	0.252	0 (resting)
0.98	0.232	0.256	10
1.44	0.231	0.268	20

izing pulses. Moreover, as has been emphasized repeatedly, the capacitance change becomes apparent only below 5 kHz. To observe the full extent of these changes, frequencies below 1 kHz and, at the same time, larger pulse amplitudes will be necessary. Although these conditions are difficult to realize, the present investigation demonstrates that membrane capacitance increases with depolarizing pulses. This conclusion is contrary to the common belief that membrane capacitance is invariant during depolarization and hyperpolarization.

In the presence of the time-dependent displacement current, the total membrane current during depolarization is given by Eq. (7):

$$I = C_0 \frac{dV(t)}{dt} + C_1 \int \frac{dV(t-u)}{dt} \varphi(u) + \sum_j I_j \tag{7}$$

where the first and second terms on the right-hand side represent the time-independent and -dependent capacitive currents. C_0 is the membrane capacity due to the inactive bulk and really represents the frequency-independent capacity (*see* Fig. 7*a*). C_1 represents the frequency-dependent membrane capacity, perhaps due to the Na branch (Fig. 7*a*). The variable u is time after the pulse is turned on and $\varphi(u)$ is a transfer function which dictates the decay of the displacement current. At this stage, we do not have to specify the decay function but later on an exponential function will replace the general formulation. The third term is a collective expression for ionic currents. The difference between this equation and the Hodgkin-Huxley (1952) equation is in the presence of the time-dependent displacement current and the introduction of C_1.

It is well known that the linearization of the Hodgkin-Huxley equation gives rise to the following expression in the frequency domain (Chandler, Fitzhugh & Cole, 1962):

$$Y(p) = p\,C + g_\infty + \frac{g_n}{1+p\tau_n} + \frac{g_m}{1+p\tau_m} + \frac{g_h}{1+p\tau_h} \tag{8}$$

where $p=j\omega$

$$g_\infty = \bar{g}_k n^4 + \bar{g}_{Na} m_\infty^3 h_\infty + \bar{g}_L \tag{9}$$

$$g_n = 4\bar{g}_K n_\infty^3 \tau_n (V-E_k)[\dot{\alpha}_n - n_\infty(\dot{\alpha}_n+\dot{\beta}_n)] \tag{10}$$

$$g_m = 3\bar{g}_{Na} m_\infty^2 h_\infty \tau_m (V-E_{Na})[\dot{\alpha}_m - m_\infty(\dot{\alpha}_m+\dot{\beta}_m)] \tag{11}$$

$$g_h = \bar{g}_{Na} m_\infty^3 \tau_h (V-E_{Na})[\dot{\alpha}_h - h_\infty(\dot{\alpha}_h+\dot{\beta}_h)]. \tag{12}$$

After rearrangement of Eq. (8) and collecting only the capacitive terms, we obtain

$$Y(\mathrm{Im}) = j\omega\left[C + \frac{\tau_n g_n}{1+(\omega\tau_n)^2} + \frac{\tau_m g_m}{1+(\omega\tau_m)^2} + \frac{\tau_h g_h}{1+(\omega\tau_h)^2}\right]. \tag{13}$$

Eq. (13) indicates that the time-dependent Na and K currents have a reactive component and give rise to frequency-dependent capacitances. Thus, it is quite possible that the third term of the right-hand side of Eq. (7) instead of the second term may be the cause of the capacity change.

The numerical calculation of capacitances due to time-dependent ionic currents was performed by Majer (1973) and repeated by Takashima *(unpublished results)*. Both calculations indicate that, of these terms due *m*, *h* and *n* parameters, terms due to *n* and *h* give rise to large negative capacitances even with moderate depolarizations. Furthermore, the time constants of these terms are large indicating characteristic frequencies far below the frequency range of the present experiment. Therefore, it is quite unlikely that these terms are responsible for the membrane capacitance changes observed during depolarizations. The only term which gives rise to a positive capacitance is the one due to the *m* parameter. Also the time constant is such that it would produce a capacitance in the kilohertz region. This term is a well-behaved function for hyperpolarization and for small depolarization. However, even this term turns into a negative value above the membrane potential of -30 mV. The value of capacitance reaches as large as $-10\ \mu F/cm^2$ at a membrane potential of -37 mV. Thus the membrane capacitance which arises from the sodium activation deviates considerably from the measured values even for relatively small depolarizations. Eq. (8) was derived using the Taylor expansion deleting all nonlinear terms and thus valid only for very small depolarizations. A membrane potential -40 mV is already well in the nonlinear region and this may be the reason why Eq. (13) begins to be ill-behaved at moderate depolarizations. At any rate, the large deviation between the observed capacitance change and the calculated value suggests that the capacitance

change with depolarization and hyperpolarization is due to the change in membrane permittivity, i.e., the second term in Eq. (7) rather than the reactive component of ionic currents.

The presence of a time-dependent displacement current in Eq. (7) modifies the term C in Eq. (8) and Eq. (13) into two components, namely [*see* Eq. (2)]

$$C = C_0 + \frac{C_1}{1 + (\omega \tau_0)^2} \tag{14}$$

where τ_0 is the time constant due to dipole or counterion relaxations. It may be indirectly related to other time constants, i.e., τ_m, τ_n and τ_h but not necessarily identical with any one of them. The second term on the right-hand side is the frequency-dependent capacity which arises from the time-dependent capacitive current. This term can be obtained by introducing an exponential function for $\varphi(u)$ in Eq. (7) and performing the Fourier transformation. Thus the time-dependent capacitive current or gating current adds an additional term in the Hodgkin-Huxley equation and gives rise to a frequency-dependent membrane capacity.

The frequency characteristics of membrane capacitance of biological cells has been discussed by several investigators. The frequency dependence of membrane capacitance may be due to the presence of a constant phase angle element proposed by Cole (1932) or due to the dielectric relaxation of membrane components (Schwan, 1957, 1965). The latter may arise from the orientation of dipolar species (*see* Takashima, 1969), fluctuation of counterions (Schwarz, 1962; and *see* Takashima & Minakata, 1975) or electrophoretic mobility of particles (Schwan, Schwarz, Maczuk & Pauly, 1962). Even chemical reactions may be responsible for dielectric relaxation in the membrane (Hodgkin & Huxley, 1952; Bergman, Eigen & DeMayer, 1963; Schwarz, 1967). The origin of the time-dependent displacement current or frequency-dependent membrane capacity in the nerve is yet to be investigated. The present study and the studies by others on the gating current do not provide any information as to the nature of the dipolar species. The electrophoretic movement of Ca ions may play an important role for the opening and closing of ionic channels but this alone would not give rise to the time-dependent displacement current or the frequency-dependent capacity change. Molecular species which produce a displacement current with a time constant of 0.4–0.8 msec or a frequency-dependent capacitance change with a center frequency less than 1 kHz must be a molecule with a large dimension or a large aggregate of relatively small molecules.

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References

Armstrong, C. M., Bezanilla, F. 1973. Currents related to movement of the gating particles of the sodium channels. *Nature* **242**:459

Bergman, K., Eigen, M., De Mayer, L. 1963. Dielektrische Absorption als Folge Chemischer Relaxation. *Ber. Bunsenges. Phys. Chem.* **67**:819

Bezanilla, F., Armstrong, C. M. 1974. Gating currents of the sodium channel: Three ways to block them. *Science* **183**:753

Chandler, W. K., Fitzhugh, R., Cole, K. S. 1962. Theoretical stability properties of a space-clamped axon. *Biophys. J.* **2**:105

Cole, K. S. 1932. Electric phase angle of cell membranes. *J. Gen. Physiol.* **15**:641

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

Cole, K. S., Baker, R. F. 1941. Transverse impedance of the squid axon during current flow. *J. Gen. Physiol.* **24**:535

Cole, K. S., Curtis, H. J. 1938. Electrical impedance of nerve during activity. *Nature* **142**:209

Cole, K. S., Curtis, H. J. 1939. Electrical impedance of the squid giant axon during activity. *J. Gen. Physiol.* **22**:649

Feldman, L., Guttman, R. 1975. Effect of low Ca and polarization upon impedance of space clamped squid axons stimulated by white noise as derived from input-out cross correlations. Annual Meeting of Biophysical Society, Philadelphia, Pa. (Abstract F-AM-b2)

Hodgkin, A. L., Huxley, A. F. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**:500

Keynes, R. D., Rojas, E. 1974. Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axons. *J. Physiol.* **239**:393

Majer, B. A. 1973. Analysis of the linearized ionic current contribution to the membrane properties in the Hodgkin-Huxley membrane. Master's Thesis, University of Pennsylvania, Philadelphia, Pa.

Meves, H. 1974. The effect of holding potential on the asymmetry currents in squid giant axons. *J. Physiol.* **243**:847

Moore, J. W., Cole, K. S. 1963. Voltage clamp techniques. *In:* Physical Techniques in Biological Research. W. L. Nastuk, Editor. Vol. VI, chapter 5. Academic Press Inc., New York

Schwan, H. P. 1957. Electrical properties of tissue and cell suspension. *In:* Advances in Biological and Medical Physics. J. H. Lawrence and C. A. Tobias, Editors. Vol. V, p. 147. Academic Press Inc., New York

Schwan, H. P. 1963. Determination of biological impedance. *In:* Physical Techniques in Biological Research. W. L. Nastuk, Editor Vol. VI, chapter 6. Academic Press Inc., New York

Schwan, H. P. 1965. Biological impedance determinations, *J. Cell. Comp. Physiol.* **66**:5

Schwan, H. P. 1966. Membrane properties-AC state studies. *Second International Biophysics Congress*, Vienna (Paper Sy-1A)

Schwan, H. P., Schwarz, G., Maczuk, J. Pauly, H. 1962. On the low frequency dielectric dispersion of colloidal particles in electrolyte solution, *J. Phys. Chem.* **66**:2626

Schwarz, G. 1962. A theory of the low frequency dielectric dispersion of colloidal particles in electrolyte solution. *J. Physic. Chem.* **66**:2636

Schwarz, G. 1967. On dielectric relaxation due to chemical rate processes. *J. Phys. Chem.* **71**:4021

Takashima, S. 1969. Dielectric properties of proteins. I. Dielectric relaxation. *In:* Physical Principles and Techniques of Protein Chemistry. S. J. Leach, Editor. p. 291. Academic Press, Inc., New York

Takashima, S., Minakata, A. 1975. Dielectric behavior of biological macromolecules. *In:* Digest of Dielectric Literature. W. Vaughn, Editor. National Research Council, National Academy of Sciences *(In press)*

Takashima, S., Schwan, H. P. 1974. Passive electrical properties of squid axon membrane. *J. Membrane Biol.* **17**:51