COMPENSATION FOR RESISTANCE IN SERIES WITH EXCITABLE MEMBRANES

JOHN W. MOORE, MICHAEL HINES, AND EDWARD M. HARRIS
Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710, and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT Extracellular resistance in series (R_s) with excitable membranes can give rise to significant voltage errors that distort the current records in voltage-clamped membranes. Electrical methods for measurement of and compensation for such resistances are described and evaluated. Measurement of R_s by the conventional voltage jump in response to a current step is accurate but the measurement of sine-wave admittance under voltage-clamp conditions is better, having about a fivefold improvement in resolution ($\pm$ 0.1 Ωcm^2) over the conventional method. Conventional feedback of the membrane current signal to correct the R_s error signal leads to instability of the voltage clamp when approximately two-thirds of the error is corrected. We describe an active electronic bridge circuit that subtracts membrane capacitance from the total membrane current and allows full, yet stable, compensation for the voltage error due to ionic currents. Furthermore, this method provides not only fast and accurate control of the membrane potential in response to a command step, but also fast recovery following an abrupt change in the membrane conductance. Marked changes in the kinetics and amplitude of ionic currents resulting from full compensation for R_s are shown for several typical potential patterns.

INTRODUCTION

The squid giant axon is sheathed by a layer of Schwann cells (Geren and Schmitt, 1953, 1954; Villegas and Villegas, 1960; Baker et al., 1962; Villegas, 1969; Adelman et al., 1977). Membrane currents in the axon traverse the clefts between the cells and produce a voltage drop across this resistance in series (R_s) with the membrane. This voltage drop plus others to be described causes the measured internal potential to deviate from that actually established across the active membrane. With a voltage clamp, this voltage error gives rise to changes in the shape of the ionic current (Taylor et al., 1960); these deviations preclude the development of a precise description of and model for the ionic conductance systems. For purposes of precision measurement of kinetics and distinguishing between models, it is essential that the R_s be fully compensated, and therefore, accurately known.

The major problem with previous methods of compensation for the R_s*I voltage error in voltage clamping is that oscillations begin to appear (or increase in amplitude) throughout the circuit as the error correcting signal is increased toward full compensation. In their pioneering work on the voltage clamp, Hodgkin, Huxley, and Katz (1952) noted this problem and usually settled for ~60% of full compensation, a compromise between clamp stability and the desired full compensation for their observed value of 7 Ωcm^2. With their peak sodium currents of ~1 mA/cm^2, this residual uncompensated R_s of 2.8 Ωcm^2 gave an error of ~3 mV maximum. However, for the much larger peak sodium currents (5–7 mA/cm^2) seen by Cole and Moore (1960), such an uncompensated residual R_s would have given an intolerable error of 15–20 mV. Noting the contributions of electrodes, axoplasm, and bathing solution to the total R_s, they placed microelectrodes just inside and outside the membrane to monitor its potential difference as closely as possible (see their Figs. 2 and 20). This put much of the R_s within the feedback loop and eliminated its effect. They measured a residual R_s of 2 Ωcm^2 (i.e., mainly Schwann cell layer) and were able to compensate for at least two-thirds of this, resulting in a net uncompensated R_s of ~0.5 Ωcm^2.

Nevertheless, today many investigators of squid axons employ a potential measurement system similar to that of Hodgkin, Huxley, and Katz and must deal with a residual R_s similar to theirs (~7 Ωcm^2) with intact axons. When axons are internally perfused with solutions of low ionic strength, considerably larger values of R_s are encountered (e.g., 20 Ωcm^2, Gillespie and Meves, 1980). To avoid large R_s errors, a number of investigators have reduced the sodium current by bathing the axon in a low sodium concentration but this may compromise the signal-to-noise ratio.

MEASUREMENT OF R_s

We have employed two techniques for the measurement of R_s. The first, following Moore and Cole (1963), measures the voltage jump in response to a current step. The requirements for precise measurement with this widely

used method have been carefully analyzed (Moore, J. W., unpublished results; Binstock et al., 1975). We found that each 1-μs lag (time constant) in the current reduced the measured value of the R_s by 1 Ωcm^2. Similar lags in the voltage measuring system gave rise to additional reductions in the observed value of R_s. Therefore we have made it standard practice to include a known R_s with our passive RC model for the membrane and to test the ability of the system to measure this resistance accurately. Using the circuit shown in Fig. 3 in a current clamp mode, our current step has a time constant of 0.5 μs or less and the membrane voltage monitor has a similar response time. The value of the R_s is determined by adjusting the potentiometer marked RSM to minimize the voltage jump seen at 10 V_m. Fig. 1 *A* shows the patterns (at 10 V_m) in response to a current step applied across the model membrane (R_mC_m) and R_s of 2 Ωcm^2 for RSM settings of 0, 2, and 4 Ωcm^2. We can determine the known resistance with a resolution of 1/2 Ωcm^2, establishing the validity of our R_s measurements on axons, which range from 1.0 to 3.0 and average 2.0 Ωcm^2. This is in good agreement with the values of 2 Ωcm^2 reported by Cole and Moore (1960) and of 3.78 ± 0.95 Ωcm^2 (total including Schwann cell layer and axoplasm; 2.57 ± 0.80 from Schwann cell layer alone) reported by Salzberg and Bezanilla (1983) who used optical methods.

Recently we have developed a second and more accurate sine-wave admittance technique to measure R_s under voltage-clamp conditions and, because of its improved resolution, it should become the method of choice. The RSM potentiometer dial is adjusted so that the component of membrane current, I_m, in phase with the measured membrane potential, V_m, is independent of frequency[1] over a wide range. The value of the R_s can then be read from the RSM potentiometer dial.

This method was also tested on the RC model membrane circuit with a 2 Ωcm^2 value for R_s. The two orthogonal components of admittance are determined by a computer program from digitized membrane potential and current data. Fig. 1 *B* shows that, when the RSC potentiometer value equals that in the model, the conductive component of admittance, Y_o, is flat up to 100 kHz. When there is too little compensation for R_s, the in-phase component veers up at high frequencies: for overcompensation, it veers down at high frequencies. With this method, we are able to measure R_m to better than 0.1 Ωcm^2. With a patch of squid axon membrane in a sucrose gap, we were able to measure R_s to nearly the same resolution.

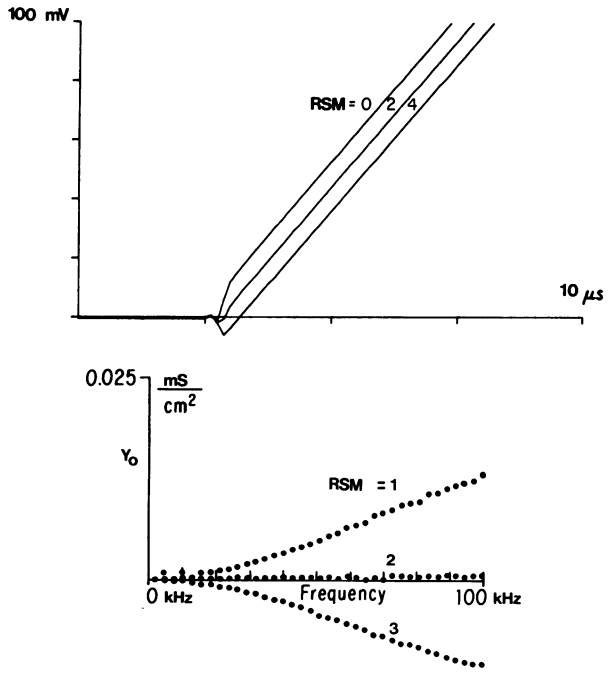

FIGURE 1 Measurement of a known 2 Ωcm^2 R_s by: (*A*) minimizing the voltage jump across a 2 Ωcm^2 RC model membrane in response to a current step at various settings of the RSM potentiometer (see Fig. 3) and by (*B*) finding the value of the R_s measurement (RSM potentiometer) for which the measured in-phase or conductive admittance, Y_o, is frequency independent. Each tick mark on the abscissa equals 10 μs/mark.

R_s Compensation

Our technique is based on the idea of separating the capacitive current from the ionic currents and feeding back only the latter signal to compensate for voltage errors. The small price we pay is to forego any compensation during the few microseconds required for the voltage across the membrane capacitance to be changed to a new value. With this technique we can achieve not only stable full compensation for ionic currents, but inadvertent overcompensation does not drive the system into oscillations.

The rationale for this technique can be viewed from two perspectives. First, using physical intuition, one can see that full (100%) compensation for the R_s means that (for a command step) the membrane capacitance must be charged in an infinitesimal time by an infinite current. This is not physically possible. Not only do practical amplifiers have limited bandwidth and current output capabilities, but the delay between the input and output[2] means that the compensating signal is delayed relative to the time at which it is needed. This delay in the feedback signal (to compensate for the voltage error in charging the capacitance) is the source of the oscillations in the control of the membrane voltage. That is to say, some R_s with the membrane capacitance is necessary (and often sufficient) for stability against oscillations!

The second perspective is to view the circuit by standard network analysis. For brevity we refer to Sigworth's (1980)

[1]In addition to measuring R_s, this frequency independence also establishes the upper bound on the degree to which the space clamp is violated. A manuscript is in preparation.

[2]This is especially true for the control amplifier where the delay is exacerbated by the capacitive load of the membrane on the amplifier's output resistance.

analysis of the general case of a voltage clamp with R_s compensation. He shows that the LaPlace transform of the membrane potential response is given by

$$V_m = \frac{Y_{CA} Z_m V_{CMD}}{1 + Y_{CA}(T_{VA} Z_m + T_{VA} R_s - Z_{SC})}, \quad (1)$$

where V_{CMD} is the transform of the command potential and Y_{CA}, Z_m, T_{VA}, and Z_{SC} are the transforms of the responses of the control amplifier (ideally infinite), membrane, voltage measuring circuitry (ideally unity), and series compensation network, respectively.

Because the response, T_{VA}, of the voltage measuring circuit falls off at high frequencies, it is necessary for Z_{SC} to fall off at least as rapidly to ensure that the denominator of Eq. 1 never vanishes (it is assumed that the clamp with $Z_{SC} = 0$ is stable). Sigworth met this criterion for the series compensation network by feeding back the membrane current through a low-pass filter. Levis (1971) matched the frequency response of Z_{SC} and T_{VA} by building special wideband amplifiers with internal networks that controlled the frequency response.

Our compensation method (see Fig. 3) employs operational amplifiers with network responses T_1, T_2, and T_3 (ideally unity) (*a*) to measure the membrane potential, $T_1 V_m$, (*b*) to form a capacitive current, $Y_C T_1 V_m$, that (*c*) then is subtracted from the measured membrane current, $T_2 I_m$, to generate a potential, $R_{SC} T_3 (T_2 I_m - Y_C T_1 V_m)$, as the compensation feedback signal to the control amplifier. After some algebraic manipulation of the equations given by Sigworth, we obtain the R_s compensation transform

$$Z_{SC} = R_{SC} T_3 (T_2 - Z_m Y_C T_1). \quad (2)$$

Since $Z_m = 1/(sC_m + G_m)$ and Y_C is manually set to closely match the membrane capacitive admittance, sC_m, then for frequencies where the responses are close to ideal, Eq. 2 simplifies to

$$Z_{SC} = R_{SC} \frac{G_m}{sC_m + G_m}. \quad (3)$$

The subtraction of capacitive current method is thus analagous to a low-pass filter with time constant C_m/G_m.

Notice that when the membrane current flows only through the membrane capacitance ($G_m = 0$), there is no compensation. Also, this filter is active in the sense that its time constant changes with the membrane conductance. At high frequencies the nonideal response of T_1 and T_2 invalidate the approximation in Eq. 3. In this domain, the response of T_3 is adjusted to fall off rapidly enough to ensure stability.

The above analysis is limited to the condition when the membrane conductance is constant. To predict the quality of voltage control (*a*) in response to a voltage step under the conditions of active (high) as well as resting (low) conductance and (*b*) in response to a step change in membrane conductance under the condition of a steady command potential, we have used computer simulations of the circuit. These simulations include realistic amplifiers[3] as well as important stray capacitances. Fig. 2 shows voltage records for a simple voltage clamp controlling an RC model membrane with 100% compensation (for an R_s of 2 Ωcm^2) in response to a voltage step at $t = 0$ for membrane conductances of 0.5 and 250 mS/cm, representing resting and active states, respectively. At 50 μs, the membrane conductance is changed abruptly from one of these values to the other.

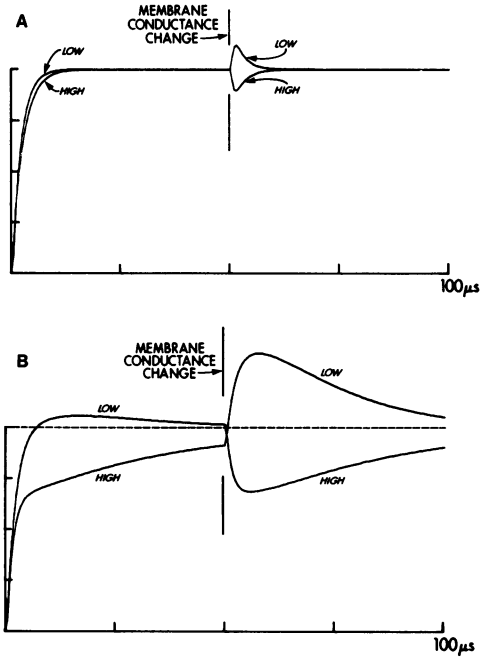

FIGURE 2 Calculated response of a voltage clamp of a 0.002 cm^2 patch of membrane with full compensation for the 2 Ωcm^2 of R_s. In each panel, the membrane potential is plotted in response to a command potential step at $t = 0$ and (at 50 μs) to a step change in membrane conductance. The conductance values chosen were 0.5 and 250 mS/cm^2 to represent resting (low) and active (high) conductances, respectively. The vertical scale is arbitrary because the circuits are linear. The *upper* panel (*A*) shows responses using the subtraction of capacitive current from the R_s compensation signal. The *lower* panel (*B*) shows the response using a two pole low-pass filter in the compensation network.

The performance of our method of subtraction of capacitive currents is shown in Fig. 2 *A*. For both low and high conductances, the clamp not only settles quickly and quietly following a command potential step, but it also recovers control of the membrane potential very quickly following a membrane conductance change. The time constant of the membrane potential control is, of course, the time constant in the absence of R_s compensation and is almost independent of the membrane conductance.

The response for a double pole low-pass filter used as the compensation network is shown in Fig. 2 *B*. The time

[3]We used the specified gain and two time constants for the model 48 operational amplifier (Analog Devices, Norwood, MA).

constants of the filter were chosen to be slightly underdamped.[4] Although this response is satisfactory for a step voltage applied to a resting (low conductance) membrane, it loses control of the voltage for a relatively long time when the conductance of the membrane is high. If the time constants are reduced in order to speed the sluggish response (and reduce the error) to the voltage step for the high membrane conductance condition, the clamp would ring (become more underdamped) for a voltage command step to a resting (low conductance) membrane. If, on the other hand, the time constants are made large enough, 250 μs to produce critical damping for a voltage step applied to a resting membrane, upon the change to high membrane conductance, the clamp would stay out of control for an even longer time.

Active Bridge and Voltage-Clamp Circuits

The method was convenient for us to implement because we normally use an active (electronic) bridge circuit to balance out the leakage current and multiple linear components of the capacitive currents. In squid axons, the latter contain not a single, but an infinite series of time constants (as shown by Cole and Cole, 1942). This infinite series can be represented and balanced out reasonably well by summing of three exponential decays of different time constants and amplitudes. Note that, for stability, only the main capacitive current needs to be subtracted from the feedback compensation current because the other capacitances in the equivalent circuit have large effective R_ss.

Our voltage-clamp and active bridge circuits are shown in the schematic diagram (Fig. 3). The controlled voltage, V_c, is that across the membrane plus R_s in series. The value of the R_s is measured, as noted earlier, by applying a fast current step at point C and adjusting the potentiometer marked RSM to minimize the voltage jump as observed at 10 V_m. Under this condition, we read the true voltage across the membrane, V_m, under any current patterns applied at point C, including those under voltage clamp.

A signal, I_m, proportional to the current density is obtained by automatically setting[5] the feedback resistance labeled AREA to be proportional to the area from which the current is measured. We apply the 10 V_m signal to the bridge circuit, which generates, and subtracts from I_m,

[4] 7 and 20 μs had the same ratio as chosen by Sigworth but were about twice as great in order to achieve a stability approaching critical damping for a resting membrane conductance.

[5] In our sucrose gap voltage clamp, the capacitive current of the artificial node is continuously monitored (except for the few milliseconds per second during which data is taken) with a 15-kHz sine wave applied to one of the voltage command inputs. A feedback circuit steps the resistance of a digital-to-analog multiplier (labeled AREA) so that the I_m signal remains proportional to current density in spite of any changes in the nodal area.

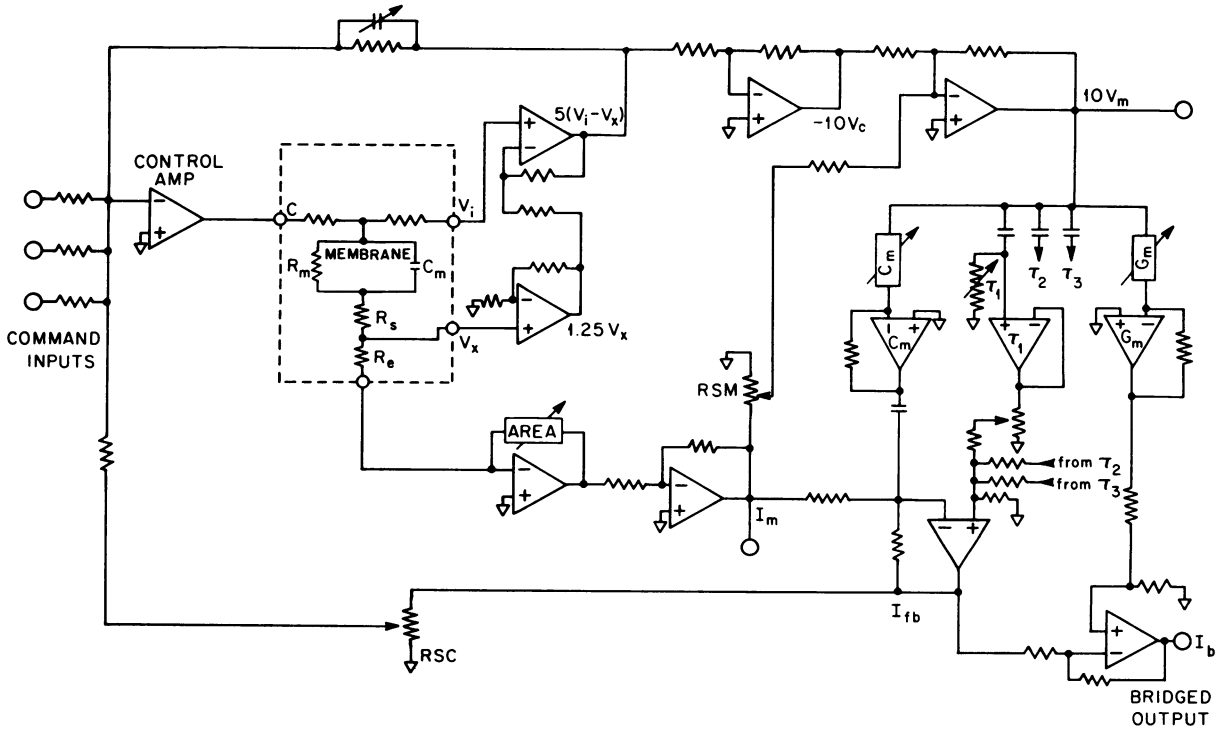

FIGURE 3 Schematic diagram of voltage-clamp circuit showing methods of measuring and full compensation for the R_s with the membrane. The active bridge circuit (*right*) develops signals to balance (subtract) the currents through normal 1 μF/cm^2 capacitance (C_m), the leakage resistance (G_m), and the nonideal Cole-Cole capacitance with three time constants. The amplifier circuits for τ_2 and τ_3 are identical to that for τ_1 and are not shown.

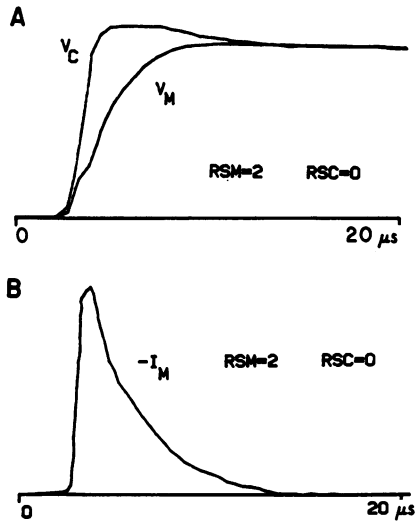

FIGURE 4 Test of voltage clamp and active bridge on a passive RC model. *A* shows that, without compensation for R_s, there is 2.5-μs lag of the voltage across the membrane, V_m, behind the control voltage, V_c. *B* shows that the membrane current density, I_m, decays with the time constant of V_m.

currents proportional to both the ideal and the Cole-Cole capacitors. This pattern, I_{fb}, (I_m minus the capacitative currents), is fed back through a compensation pot, RSC, set at the same value as RSM.[6] This provides full, yet quiet, compensation for the R_s throughout the time that ionic currents flow. This signal, I_{fb}, is then further processed to subtract the membrane leakage current proportional to G_m. This condition is achieved by balancing the residual steady current seen at I_b to 0 when a hyperpolarizing voltage pulse is applied to the membrane.

RESULTS

Performance of Clamp and Active Bridge

Passive Model. In tests of a passive RC model with an R_s of 2 Ωcm^2, the voltage control pattern, V_c, responds to a step command with a rise time of ~1μs, as can be seen in Fig. 4 *A*. However, the voltage across the membrane, V_m, actually lags behind V_c with a time constant equal to the product of the membrane capacity and the R_s (R_sC_m = 2 μs). The measured time constant for V_m was slightly greater, 2.5 μs, because V_c lags slightly behind the command step as noted above. The membrane's capacitive current decays (Fig. 4 *B*) with the time constant of V_m.

Squid Axon. When a squid axon membrane is voltage clamped, V_c and V_m patterns similar to those seen in Fig. 4 are seen. However, the decay of the membrane capacitive current is quite different. Fig. 5 shows that I_m, for a hyperpolarizing step to -180 mV, has not only the rapid decay of an ideal capacitance (e.g., as in Fig. 4 *B*), but also the long slow setting phase of the Cole-Cole nonideal capacitor, which outlasts the trace shown. With the active bridge one is able to achieve and, after a 25-μs transient, maintain a good balance (I_b in Fig. 5).

This balancing out of the linear capacitance allows the asymmetric nonlinear capacitance or gating current to be revealed for each depolarizing pulse, without averaging.

Effect of R_s Compensation on Ionic Currents

Having measured the value of the resistance in series, RSM, we can observe the deviation of the true membrane potential, V_m, from the controlled value, V_c. Fig. 6 *A* shows records from an experiment in which the membrane was held at −100 mV and pulsed to −25 mV near where the maximum peak sodium current flowed. The 11-mV peak error in V_m caused by a 4.3 mA/cm^2 peak in the inward sodium current can be seen in Fig. 6 *A* where no compensation has been introduced for the measured 2.5 Ωcm^2 of R_s. Full compensation for this resistance can be introduced (see Fig. 6 *B* where RSC = 2.5 Ωcm^2) without ringing, and the membrane potential follows the step command rather well. For this to occur, V_c, the voltage applied to the membrane and R_s together must deviate as shown. In this case the observed current density is increased by 16% to 5 mA/cm^2. Because this method of compensation for R_s is so stable (one can overcompensate by 75 to 100% before ringing becomes serious), one can set full compensation with complete confidence that the system will not ring.

Lack of compensation for R_s introduces both amplitude and dynamic distortions in the ionic current measurements, which depend on the membrane conductance, the ionic equilibrium potentials, and the command levels. Fig. 7 *A* shows patterns at three levels of depolarization selected

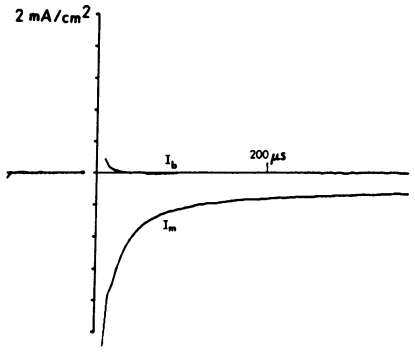

FIGURE 5 The membrane current density from a patch of squid axon membrane, I_m, for a hyperpolarizing voltage step of 80 mV. The output of the active capacitive subtraction bridge, I_b, is in excellent balance after 25 μs.

[6]The RSM and RSC pots are calibrated directly in ohms per centimeter squared because our current signals are proportional to the current density.

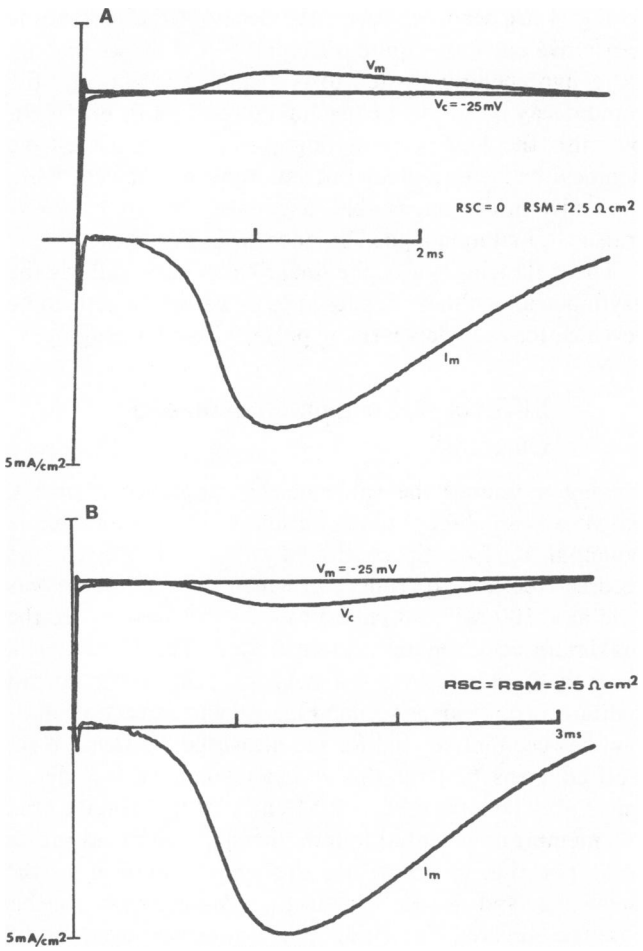

FIGURE 6 The effect of R_s compensation on the control voltage, V_c, the membrane voltage, V_m, and the early transient sodium current, I_m, for a 75-mV depolarization from a holding potential of 100 mV is shown.

to demonstrate this variability. At +70 mV (where the membrane is depolarized beyond E_{Na}), the presence of uncompensated resistance reduces the measured values of both outward sodium (early transient) and potassium (late steady) currents. At an intermediate depolarization (below E_{Na} but in the positive slope conductance region) to +10 mV, the presence of R_s also reduces the value observed for inward sodium currents and the time-to-peak. However, at a weak depolarization (in the region of negative slope conductance), the presence of R_s increased the peak current significantly and slightly changes the time-to-peak.

Compensation for R_s is perhaps most important when one is trying to discriminate between possible models on the basis of absolute conductances, kinetic patterns, and changes brought about by weak conditioning depolarizations. For example, when a brief strong depolarization is terminated near the peak of the inward sodium current, the slope of the tail current, which follows the repolarization, changes character in the presence of uncompensated R_s as can be seen in Fig. 7 *B*.

DISCUSSION

This paper details the need for careful measurement of and compensation for the R_s with excitable membranes in which voltage-clamp measurements are made. It describes a new method of subtracting of capacitive currents from the total membrane current to achieve full compensation for the R_s while maintaining fast, yet stable, control of the membrane potential. Independently, Oxford (1981) arrived at this method and was able to "achieve satisfactory compensation."

Both this method of subtracting the capacitive current (from the total membrane current) and the method of inserting a low-pass filter in the compensation feedback give similar voltage-clamp responses (at full compensations) to a command voltage step for a resting membrane. However, the method of subtraction of capacitive current gives superior accuracy and speed of control for high as well as low membrane conductances or changes in conductance. The changes in the ionic current patterns caused by uncompensated R_s, which we report here, are consistent

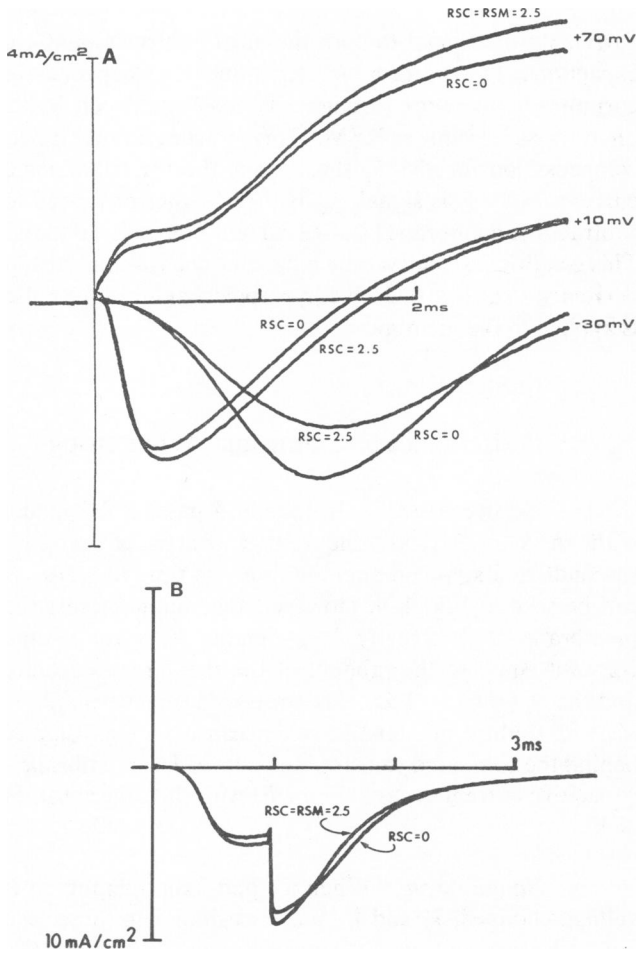

FIGURE 7 *A* shows the effect of R_s compensation on the membrane current density at three levels of potential. *B* shows kinetic changes in the sodium current tail with R_s compensation.

with previous observations (e.g., Taylor et al., 1960) and computer simulations (e.g., Ramon et al., 1975).

Reported Values of R_s

The 5 Ωcm^2 difference in the values of R_s reported (7 Ωcm^2 by Hodgkin et al. [1952] and the 2 Ωcm^2 reported here and by Cole and Moore [1960]) arises from differences in elements outside the voltage control feedback loop. For Hodgkin and co-workers, the resistance of the axoplasm from the axial electrode out to the membrane and part of the external sea water resistance were (as shown by Cole and Moore, 1960, Fig. 2) "outside the feedback loop." Cole and Moore (1960) having measured the value of these two elements in series as 3.6 Ωcm^2 (using axial electrodes that were carefully plated to minimize their surface resistances), put them "inside the feedback loop" and thus effectively eliminated their contribution. In the sucrose gap method of voltage clamp (Moore et al., 1964) employed in the present work, the electrode supplying current to the inside of the membrane is so large that its resistance is negligible and the longitudinal resistance through the axoplasm is ~4 kΩ. Again, because they are within the feedback loop, the resistance of these elements is also in effect eliminated in our sucrose gap voltage clamp.

Thus the value of 2.0 Ωcm^2 reported for the R_s in this paper and by Cole and Moore (1960) was dominated by the Schwann cell layer. It is in good agreement with the optical measurement of 2.6 Ωcm^2 by Salzberg and Bezanilla (1983). Other electrical measurements of R_s vary from 0.8 Ωcm^2 for the Schwann cell layer (Binstock et al., 1975) to 20 Ωcm^2, including axoplasmic resistance (Gillespie and Meves, 1980).

Problems Resulting from Lack of Compensation

The necessity for full compensation for R_s errors becomes obvious in experiments that employ the time course of ionic currents to choose between possible models for conductance and where the measured value of R_s is high (e.g., 20 Ωcm^2, Gillespie and Meves, 1980). Their statement "we think that at least 70% of that was compensated" implies a residual uncompensated resistance of 6 Ωcm^2. This, with a normal sodium current of ~5 mA/cm^2 (e.g., Cole and Moore, 1960, Hoyt and Adelman, 1970, and Fig. 6 *B* in this paper), would cause an error of ~30 mV. Reduction of the external sodium to one-third the normal concentration should reduce this error to ~10 mV but this is still unacceptable for purposes of discrimination between models. We are unable to understand why their (Gillespie and Meves, 1980) reported peak sodium currents are 10-fold smaller than the usual 5 mA/cm^2 for intact axons in a normal external sodium concentration.

Questions have been raised about whether or not several reports of experimental observations at variance with the Hodgkin and Huxley (HH) description might be caused by uncompensated R_s with the membrane. For example, Hoyt and Adelman (1970) reported a shift of the position of the h_∞ curve along the voltage axis when the test potential was changed.

Computer simulations of such voltage-clamp experiments using a patch of HH membrane with a R_s have been carried out. A shift of 4 mV was found for a R_s of 7 Ωcm^2 whereas a resistance of 14 Ωcm^2 gave a 10-mV shift. The HH membrane gives a maximum sodium current of ~2.5 $mA/\Omega cm^2$, only about one-half of that shown in Figs. 6 and 7 (and also in Hoyt and Adelman, 1970). Thus the lack of compensation for R_s may well be the cause of at least part of the observed shift.

"Gating" or asymmetric capacitive currents are small fractions of the large linear and symmetric capacitive currents that flow as a result of high rates of change in membrane potential in response to a command step (or a rapid conductance change). Such large currents flowing through any uncompensated R_s will introduce serious deviations of V_m from the controlled voltage, V_c, especially at early times following the change in command level. Thus accurate measurement of and compensation for R_s is necessary to achieve accurate gating current measurements.

Other Methods For R_s Measurement and Correction

Hodgkin et al. (1952) estimated R_s from the time constant of the decay in membrane's capacitative current following a step change in potential under voltage-clamp control. This is a reliable method but its resolution is limited by (*a*) the nonideality of the membrane capacitance; i.e., the phase angle is not 90° (Cole and Cole, 1942) and thus the decay of the capacitive current is not exponential (see Fig. 5), and (*b*) the asymmetric or gating currents; decays associated with hyperpolarizing pulses are relatively free of the extraneous gating current, which flows during a depolarizing pulse.

They (Hodgkin et al., 1952) also estimated R_s under current control from the voltage response to a current step, correcting for the limitations of bandwidth in both the current step and voltage measurement circuit. Their correction was obtained by fitting records to an equation involving the integral of the current. Cole and Lecar (1975) showed a graphic method of achieving this correction. Binstock et al. (1975) developed a simplified expression for the correction of bandwidth limitations by assuming a first-order lag in the current step, but they neglected any bandwidth limitation on the voltage measuring circuit. Simulations of realistic circuit responses (Moore, J. W., unpublished results) show that for each microsecond lag in the response of either the current drive or the potentiometric circuit, a 1 Ωcm^2 error (reduction in the measured value of R_s) is caused.

Eisenberg et al. (1982) have described how the R_s may be measured by integrating the current response to a voltage step. They also noticed a great improvement in the resolution of the R_s measurement when using a voltage clamp rather than a current clamp. With their method it is also possible to measure the R_s for other voltage waveforms. With on-line electronic integration, this might be a powerful and accurate method.

Compensation for R_s

As noted earlier, Sigworth (1980) used a low-pass filter with a sharp two-pole cutoff in the feedback signal to compensate for R_s in his voltage clamp of the node of *Ranvier*. Our analysis showed that, although this stabilized the voltage-clamp response, it introduced a loss of control at high membrane conductance and for a rapid change of membrane conductance as shown in Fig. 2 *B*. The time taken for the clamp to recover control is related to the time constants of the filter.

An off-line method for correction of current records at the data analysis stage has been described by Palti and Cohen-Aarmon (1982). This method requires many additional current records from closely spaced voltage steps about each level for which analysis correction is to be carried out. Not only is such an experimental protocol inconvenient, but also the time required to obtain the records will risk maintenance of stationarity in the biological preparation.

We appreciate the careful reading of early manuscripts and helpful comments of Brian Saltzberg and Gerry Oxford.

We gratefully acknowledge the support of the National Institutes of Health Grants NS-03437 and NS-11613.

Received for publication 14 June 1983 and in final form 17 May 1984.

REFERENCES

Adelman, W. J., Jr., J. Moses, and R. V. Rice. 1977. An anatomical basis for the resistance and capacitance in series with the excitable membrane of the squid giant axon. *J. Neurocytol.* 6:621–646.

Baker, P. F., A. L. Hodgkin, and T. I. Shaw. 1962. The effects of changes in internal ionic concentrations on the electrical properties of perfused giant squid axons. *J. Physiol. (Lond.).* 164:355–374.

Binstock, L., W. J. Adelman, Jr., J. P. Senft, and H. Lecar. 1975. Determination of the resistance in series with the membranes of giant axons. *J. Membr. Biol.* 21:25–47.

Cole , K. S., and R. S. Cole. 1942. Dispersion and absorption in dielectrics. II. Direct current characteristics. *J. Chem. Phys.* 10(2):98–105.

Cole, K. S., and J. W. Moore. 1960. Ionic current measurements in the squid axon membrane. *J. Gen. Physiol.* 44:123–167.

Eisenberg, R. S., R. T. Mathias, and J. L. Rae. 1982. Series resistance measured by integrals of transients. *Biophys. J.* 37 (2, Pt. 2): 63*a*. (Abstr.)

Geren, B. B., and F. O. Schmitt. 1953. The structure of the nerve sheath in relation to lipid and lipid-protein layers. *J. Appl. Phys.* 24:1421.

Geren, B. B., and F. O. Schmitt. 1954. The structure of the Schwann cell and its relation to the axon in certain invertebrate nerve fibers. *Proc. Natl. Acad. Sci. USA.* 40:863–871.

Gillespie, J. I., and H. Meves. 1980. The time course of sodium inactivation in squid giant axons. *J. Physiol. (Lond.).* 299:289–307.

Hodgkin, A. L., A. F. Huxley, and B. Katz. 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol. (Lond.).* 116:424–448.

Hoyt, R., and W. J. Adelman. 1970. Sodium inactivation. Experimental test of two models. *Biophys. J.* 10:610–617

Levis, R. 1971. Temporal control of potential in giant axon voltage clamp. *Biophys J.* 25(2, Pt. 2): 306*a* (Abstr.)

Moore, J. W., and K. S. Cole. 1963. Voltage clamp techniques. *In* Physical Techniques in Biological Research. Academic Press, Inc., New York. 6:263–321.

Moore, J. W., T. Narahashi, and W. Ulbricht. 1964. Sodium conductance shift in an axon internally perfused with a sucrose and low-potassium solution. *J. Physiol. (Lond.).* 172:163–173.

Palti, Y., and M. Cohen-Aarmon. 1982. Numerical method for correcting the R_s error in voltage clamp experiments. *Isr. J. Med. Sci.* 18:19–24.

Oxford, G. 1981. Some kinetic and steady-state properties of sodium channels after removal of inactivation. *J. Gen. Physiol.* 77:1–22.

Ramon, F., N. Anderson, R. W. Joyner, and J. W. Moore. 1975. Axon voltage-clamp simulations. IV. A multicellular preparation. *Biophys. J.* 15:55–69.

Salzberg, B. M., and F. Bezanilla. 1983. An optical determination of the series resistance of *Loligo*. *J. Gen. Physiol.* 82:807–817.

Sigworth, F. J. 1980. The variance of sodium current fluctuations at the node of *Ranvier*. *J. Physiol. (Lond.).* 307: 97–129.

Taylor, R. E., J. W. Moore, and K. S. Cole. 1960. Analysis of certain errors in squid axon voltage clamp measurements. *Biophys. J.* 1:161–202.

Villegas, G. M. 1969. Electron microscopic study of the giant squid, *Dosidicus gigas*. *J. Ultrastruct. Res.* 26:501–514.

Villegas, R., and G. M. Villegas. 1960. Characterization of the membranes in the giant nerve fiber of the squid. *J. Gen. Physiol.* 43:73–103.