ELECTRICAL PROPERTIES OF
THE SQUID AXON SHEATH

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ABSTRACT   The first impedance data on the squid giant axon gave a lossy membrane capacity of 1 $\mu F/cm^2$ from 1–200 kHz. The remaining observations of one experiment up to 5 MHz were not included in the simple conclusions and have now been ascribed to the mostly Schwann cell sheath. The results of these fragmentary data, 1.6 $\Omega \cdot cm^2$ and 12.5 membrane layers, are within the range of recent current clamp and electron microscope conclusions to give them independent confirmation and support.

INTRODUCTION

The impedance measurements on numerous cell suspensions at frequencies below about 1 MHz have been satisfactorily interpreted in terms of poorly conducting membranes with capacities of about 1 $\mu F/cm^2$ surrounding electrolytic interiors. However, as the data were extrapolated to infinite frequency, the cytoplasmic resistivities varied widely but were usually much higher than for the external media in which they could be expected to be in approximately osmotic equilibrium. With interest centering on the plasma membranes, only feeble attempts at explanation were made—organelles, nuclear membranes, proteins, and such. As measurements were extended to higher frequencies and/or larger cells, more and more distinct departures from the low frequency behavior appeared—particularly in some marine eggs. Some of these went far enough to indicate a second, high-frequency dispersion region (Cole, 1968).

It was not until the first squid axon current clamp measurements between internal axial and external electrodes (Marmont, 1949) that a possible explanation appeared. These gave zero time extrapolations of resistances far too large to be explained by the electrode geometry and polarization and the axoplasm resistivity of Cole and Hodgkin (1939). It was then realized that only a relatively small resistance of a few $\Omega \cdot cm^2$ in series and associated with the plasma membrane could explain these data (Cole, 1968). Similarly, earlier measurements on suspensions might be explained by an internal resistivity, r_2, and a membrane series resistivity, r_S, to give an equivalent resistivity

$$r_2' = r_2 + r_S/a$$

for the cell radius, a (Cole, 1928).

Hodgkin et al. (1952) investigated the axon resistivity in more detail and ascribed most of it to the external sheaths. They also suggested "further experiments—to determine whether the resistive layer has any measurable capacity." Only recently I noticed
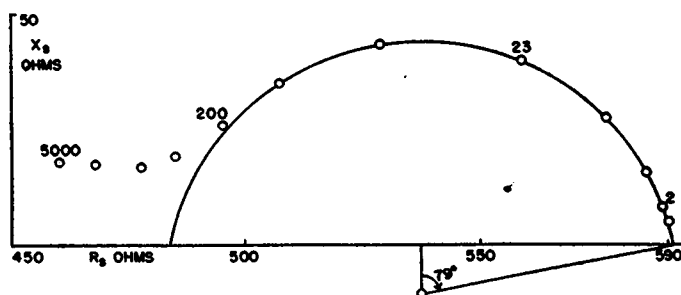
FIGURE 1 Complex transverse impedance locus for squid giant axon, series resistance, R vs. series reactance, X indicated frequencies are in kHz. Reproduced from Curtis and Cole, 1938.

and realized that our first squid axon paper (Curtis and Cole, 1938) on transverse impedance had given evidence, Fig. 1, which could at least answer their question and can now support the picture as it has been developed.

EQUIVALENT CIRCUIT, ANALYSIS, AND CALCULATIONS

The fundamental impedance analysis of a cylindrical cell in a square chamber between parallel electrodes gave a useful and convenient equivalent circuit (Cole and Curtis, 1936) which was not consistent with the axon properties of Fig. 1. However, Curtis and I used large electrodes, to reduce the effect of polarization impedance, with converging current paths in seawater to the effective axon chamber—as shown schematically in Fig. 2 A between the dashed lines. I have neither record nor recollection as to
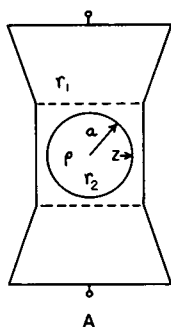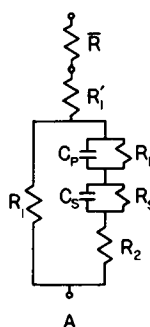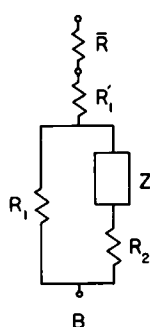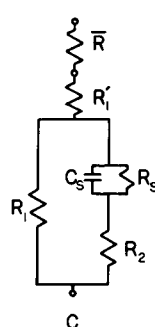

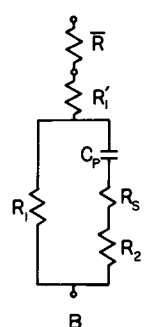FIGURE 2   FIGURE 3

FIGURE 2 (A) Schematic cross section of axon, radius a, internal resistivity r_2, surface impedance z, and volume concentration ρ, in hypothetical square chamber indicated by dashed lines in medium of resistivity r_1. (B) Equivalent circuit with $\bar{R}$ representing extraneous paths from chamber to distant electrodes, R_1 and R_1' are external resistances of current paths around and through axon, R_2 is internal resistance and Z is impedance corresponding to axon z.

FIGURE 3 (A) Equivalent circuit with C_P, R_P and C_S, R_S representing capacity and resistance of plasma membrane and sheath, respectively. (B) Approximate equivalent circuit for analysis of low frequency dispersion. (C) Approximate equivalent circuit for analysis of high frequency dispersion.

how we corrected for this added resistance, represented by $\bar{R}$ in Fig. 2 B, so the process is probably quite different. The circuit elements of Fig. 2 B could have been derived as had been done from the 1936 equation 9. But it was far simpler to calculate them as the equivalent circuit according to Zobel (1923). $\bar{R}$ is then a simple series element and the external, surface, and internal axon parameters are related to the remaining equivalent circuit elements ($r_1 \leftrightarrow R_1$, R_1'; $z \leftrightarrow Z$; $r_2 \leftrightarrow R_2$) by k, the chamber constant, the axon radius, a, and various functions of ρ, the hypothetical volume concentration of the axon in the hypothetical square chamber. Explicitly:

$$R_1 = \frac{4\rho}{1 - \rho^2} kr_1; \ R_1' = \frac{1 - \rho}{1 + \rho} kr_1; \ R_2 + Z = \frac{4\rho}{(1 + \rho)^2} k(r_2 + z/a).$$

The length of exposed axon was published, from which $k = 1.8 \ \text{cm}^{-1}$. From $R_0 - R_\infty$ and the assumptions $r_1 = 25 \ \Omega \cdot \text{cm}$ and $r_2 = 35 \ \Omega \cdot \text{cm}$ (Cole, 1975), the value $\rho = 58\%$ was obtained. However, the plasma membrane capacity, $C_p = 1.65 \ \mu\text{F}/\text{cm}^2$ was far from the published value, $0.97 \ \mu\text{F}/\text{cm}^2$, which is the important reference point. There is no reason, except convenience, to assume a square cross section for the chamber, and the 1938 "active" cell constant was not explained. So, the reasonable value $k = 1.42$ was chosen to give $\rho = 68.5\%$ and $C_P = 0.97 \ \mu\text{F}/\text{cm}^2$.

The present model, dissecting Z into plasma membrane and sheath components, C_P, R_P and C_S, R_S (Fig. 3 A), leads to such complicated analytical expressions as to require simplification. In the region 1–200 kHz, Fig. 1 of Curtis and Cole (1938) showed z was of the form $j^{-\alpha}$ representing a lossy capacitor of $0.97 \ \mu\text{F}/\text{cm}^2$ at 1 kHz and phase angle 79°. Also, R_P could not be detected, but must have been more than $3 \ \Omega \cdot \text{cm}^2$ and could be ignored. The evidence for a second dispersion appeared above
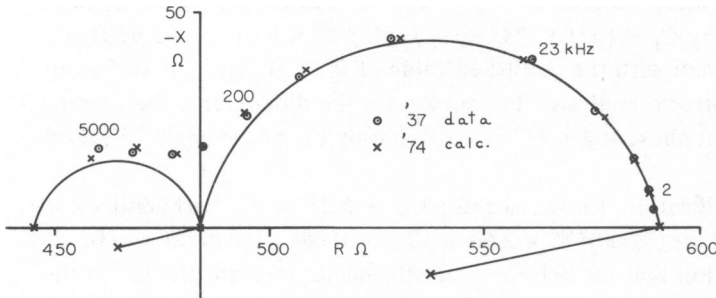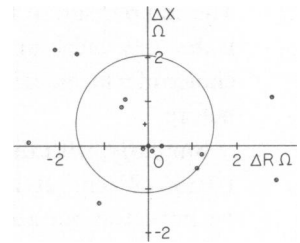

FIGURE 4   FIGURE 5

FIGURE 4   Complex transverse impedance loci for squid giant axon, resistance, R, vs. reactance, X, at frequencies shown in kilohertz. Larger and smaller circular arcs represent circuits of Figs. 3 B and 3 C respectively. Indicated data points are from Fig. 1 and calculated points are from Fig. 3 A.

FIGURE 5   Vector difference between data and calculated impedances at each frequency shown in terms of its resistance and reactance components, ΔR and ΔX. Circle is average vector difference with averages of ΔR and ΔX at center.

200 kHz with a characteristic frequency of about 3 MHz, so it will be assumed that C_S can be ignored at the lower frequencies to give Fig. 3 B. Here the zero frequency extrapolation, $R_0 = 591 \, \Omega$ with k and ρ gave $R_1 = 183 \, \Omega$, $R_1' = 6.6 \, \Omega$, and $\bar{R} = 400 \, \Omega$. The high frequency extrapolation, $R_X = 484 \, \Omega$, and gave $R_S + R_2 = 130 \, \Omega$.

The upper frequencies data were more difficult and uncertain—and not yet complete. The last two points are well fit by the low frequency phase angle of about 77° to give $R_\infty = 446 \, \Omega$. At such frequencies C_P is not effective and the equivalent circuit is given by Fig. 3 C, where $R_2 = 48 \, \Omega$ is calculated, and so $R_S = 82 \, \Omega$.

The capacity C_P is found from the time constant of the low frequency dispersion, Fig. 2 B, $\tau = (R_1 + R_2 + R_S) C_P = 1/2\pi\bar{f}$ where $\bar{f} = 42$ kHz is frequency of maximum reactance. This gives $C_P = 1.21 \times 10^{-2} \, \mu\text{F}$. Similarly for the upper dispersion, $\tau = [(R_1 + R_2)R_S/(R_1 + R_2 + R_S)]C_S$, $\bar{f} = 3$ MH and $C_S = 5.25 \times 10^{-4} \, \mu\text{F}$.

All elements of the equivalent circuit are now specified. As a test of its adequacy, the impedances have been calculated for $\alpha = 0.86$ (see below) as compared with the original data at the same frequencies on the complex impedance plane (Fig. 4). The average differences are $\Delta R = 0.12 \, \Omega$ and $\Delta X = 0.02 \, \Omega$, while the vector differences are scattered in direction and average 1.6 Ω in amplitude (Fig. 5).

MEMBRANE AND SHEATH PARAMETERS

The approximate experimental values of R_0, R_X, R_∞, and C_P and the assumptions for r_1 and r_2 have been used to obtain $\bar{R}$, R_1, R_1', and R_2, and so any considerations of these internal and external parameters are only arithmetic checks. This leaves the surface components, C_S and R_S, for analysis, involving the axon diameter. This was given only as between 500 and 600 μm and $a = 0.027$ cm was chosen. The plasma membrane capacity is now $c_P = 0.61 \, \mu\text{F/cm}^2$ at 42 kHz. However, it decreases with frequency, at a phase angle $\phi = 79°$ and an impedance change equivalent to 75° from the impedance locus, to give a mean value of $\alpha = \phi/90° = 0.86$. The capacitive component C_1 at $f_1 = 1$ kHz is given by $C_1 = (f/f_1)^{1-\alpha}C$, so $c_P = (42)^{0.144} \times 0.61 = 1.05 \, \mu\text{F/cm}^2$. The comparison of this result with the published value of 0.97 μF/cm^2 for this axon is the only check on the present analysis. The reason for the difference is the present choice of a mean equivalent phase angle, 77°, instead of only the phase angle 79°, used before.

Similarly, with this justification, for the sheath, $C_S = 5.25 \times 10^{-4} \, \mu\text{F}$ and $c_S = 0.0266 \, \mu\text{F/cm}^2$ at 3 MHz, or $(3,000)^{0.144} \times 2.66 \times 10^{-2} = 0.084 \, \mu\text{F/cm}^2$ at 1 kHz. If we persist in our assumption that the Schwann sheath membranes are similar to the plasma membrane, 12.5 of them in series would be required. This is comparable to other experimental values such as electron micrograph membrane counts across the Schwann layer with an average of seven membranes (Adelman, Moses, and Rice, in preparation). And from R_S, the series resistance from which this started, $r_S = 1.6 \, \Omega \cdot$ cm^2. This lies in the range of the recent estimates and measurements of Adelman et al., 1973, and Binstock et al., 1975.

Conversely, to the extent that these and other plasma membrane and Schwann layer descriptions support each other, the initial assumptions for external and internal

resistivities tend to be confirmed and agree with the results of other techniques (Cole, 1975).

LIMITATIONS

It should be pointed out that the 1937 data ought not to be considered more than preliminary. They are certainly fragmentary and may be stretched too far. The short length of exposed axon suggests the possibility of fringing current flow into the end troughs. This is difficult to calculate, but if it were appreciable, the effects are probably hidden in the calculated parameters. It will take more complete dispersions to support the assumed similarity of the plasma and the Schwann membranes, and constant phase angles. It seems necessary to repeat the work with better geometry to at least 50 MHz to get a new value for the internal resistivity, r_2, and to test the theory.

It has been tacitly assumed that the currents along channels and through the bounding membranes in the sheath were independent. An attempt was made to calculate the flows in and out of channels (Cole, 1968), but no analysis of the effects of folded membranes is known. The value of ρ is outside of the range of validity of the Rayleigh equation on which the analysis is based, but the theory of Cole et al. (1969), may apply for resistance of paths between cells.

COMMENTS

The many unexplained hints of high frequency dispersions have long been a source of curiosity, frustration, and perhaps importance in impedance measurements. The techniques are not easy, the possibilities are numerous, so definite objectives are probably needed. Since the anomalous resistance in the first axon current clamp measurements could not be located anywhere except in the immediate vicinity of the membrane, these impedance results should be valid. They may, however, be misleading. The ever-present problem of determining and compensating for r_S in voltage clamp experiments is essentially that of finding R_X, the high frequency terminus of the low frequency dispersion. Since this so overlaps the sheath dispersion, R_X cannot be found except by extrapolation from 220 kHz and less and shorter time transients would appear to be misleading. However, in the present experiments, the characteristic frequency $\bar{f}_P$ is largely determined by the shunt resistance, R_1, of the current flowing around the axon. With an internal electrode, this is eliminated, $\bar{f}_P = 1/2\pi c_P r_P = 160$ Hz at rest, and the valley between the low and the high dispersions approaches R_X and higher frequencies or shorter times may be extrapolated.

As clamp methods have struggled with measurements and their interpretations in terms of Schwann cell electron micrographs, I have been looking forward more and more eagerly to the time when impedance results would be available to correlate with them. So, it is ironic that Curtis and I had the data ten years before the current clamp located their origin and that they have not been interpreted for a quarter of a century. Yet, it is highly gratifying to have those results independently confirm and support the conclusions of the most modern and sophisticated equipment and analysis.

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