Resistivity of Axoplasm

I. *Resistivity of Extruded Squid Axoplasm*

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ABSTRACT Six methods have given squid axoplasm resistivities of from 1.0 to 6.9 times seawater (× SW), so another was tried. A 100-μm platinized electrode was to be inserted from each end of an axon in iso-osmotic sucrose and impedance between them measured vs. separation. But observations that the resistance of axons in sucrose increased steadily ruled this out. Axoplasm from two or three axons was transferred to a glass capillary, 0.6 mm ID, and the 1-kHz series resistance and reactance were measured at electrode separations from 16 to 2 mm. The resistance was linear vs. distance, giving the resistivity, while the reactance was nearly constant, implying constant electrode contributions. Frequency runs from 10 Hz to 30 kHz at 10 mm gave electrode impedances of the form $(j\omega)^{-\alpha}$, allowing 1–2 % effects on the axoplasm resistivities. In nine experiments, one was discarded for cause, the range and average resistivities were, respectively, 1.2–1.6 and 1.4 times those of artificial seawater (19.7 Ωcm at 24.4°C). No single cause for the variability was apparent. These experiments essentially confirm the means and variations of two early experiments with intact axons and recent results with a single internal electrode to give overall resistivities of 1.4 ± 0.2 × SW.

The protoplasm resistivities of many cells and tissue shave been measured since Höber, 1910, 1912, and all but one remain unexplained. Measurements on a single preparation, even with the same technique, often vary widely, but, except for some freshwater forms, they are usually considerably higher than for the normal cell environments.

The first measurements on the giant axon of the squid *Loligo peali*, Curtis and Cole, 1938, were made in seawater with alternating current flow perpendicular to the axon. The data from 1 to 200 kHz were interpreted as a single membrane capacity dispersion which extrapolated to an infinite frequency resistance corresponding to an average axoplasm resistivity of 4.2 times seawater (× SW).[1] The beginning of another dispersion from 200

[1] It is convenient to express resistivities and conductivities relative to SW to avoid the confusions of various temperatures and salinities.

kHz up to the maximum frequency, 5 MHz, clearly showed another reactive component, such as had been seen, particularly in marine eggs, and largely ignored in the absence of an obvious explanation. Thus the extrapolation of the low frequency dispersion cannot be accepted as a valid measure of axoplasm resistivity. This is also the case for the 2.9 × SW of Cole and Curtis, 1939 (Table I).

The analysis of longitudinal measure of the direct current resistance vs. length of an axon between reversible electrodes to determine the membrane resistance (Cole and Hodgkin, 1939) gave an axoplasm resistivity of 1.4 × SW with no obvious artifacts. Longitudinal impedance data taken with G. Marmont in 1941 and extrapolated to zero and infinite frequencies later led, with several modifications, to a singularly simple analysis and an average resistivity of 1.6 × artificial seawater (ASW) (Cole, 1968). However, it has

TABLE I

SQUID AXOPLASM RESISTIVITY

Method	Reference	Resistivity to SW	Average	Temperature
Transverse impedance	Curtis and Cole (1938)	1.5–6.9 × SW	4.2 × SW	*RT*
	Cole and Curtis (1939)		2.9	2–4°C
Longitudinal resistance, impedance	Cole and Hodgkin (1939)	1.1–1.8	1.4	*RT*
	(Marmont) Cole (1968)	1.45–1.9	1.6	*RT*
Junction potential	Cole and Moore (1960)	1.1–1.24	1.2	20°C
Microelectrode	Carpenter et al. (1975)	0.95–2.0	1.6	*RT*
Extruded	(Taylor) Cole (1968)		1.0	*RT*
	Cole (1975)	1.2–1.6	1.4	*RT*

since become certain that the membrane frequency characteristics were abnormal and it seems highly probable that the extracellular electrolyte layer of the interpolar region exchanged with the axon.

The potentials observed across squid membranes by micropipets filled with different concentrations of KCl were calculated (Cole and Moore, 1960) in terms of a constant membrane potential and the liquid junction potentials at the SW-KCl and KCl-axoplasm interfaces. Reasonable agreements with the data were obtained using the Henderson equation (MacInnes, 1939) and axoplasm ion conductances which gave an average resistivity 1.2 × SW.

As a descendant of my suggestion to F. N. Wilson for measuring lung impedance *in situ* with a small electrode (Kaufman and Johnston, 1943), Carpenter, et al. (1973, 1975) have recently adapted a technique developed by Bak (1967) to measure intracellular resistivities by comparison of the

impedances of a microelectrode inside and outside a cell. The metal electrode was insulated by glass except for the tip, about 10 μm in diameter which was platinized. Among other cells measured, squid axoplasm gave, with considerable variation, an average resistivity of 1.6 $\times$ SW. Although measurements were made at 100 kHz to reduce electrode polarization impedance, there was evidence of a residual electrode component which was tacitly assumed proportional to the medium resistivity at the electrode.

In order to avoid this ambiguity and uncertainty it was initially planned to measure the resistance component of the impedance at various separations between two electrodes in axons in iso-osmotic sucrose. Only during preliminary experiments was it remembered that Julian et al. (1962) reported a steady rise of lobster axoplasm resistance under such conditions and J. W. Moore told me the same happens for squid. Since the plan depended upon a steady state, and no better external medium was known, this approach was abandoned. Instead I went back to the two similar experiments which R. E. Taylor and I did many years ago with extruded axoplasm in a glass tube. These were our only experiments and the axoplasm resistivity equal to that of SW, as mentioned by Cole (1968), was not to be taken seriously.

EXPERIMENTS

A simple Wheatstone bridge was assembled and used over the range 5 Hz–100 kHz. Axoplasm was rolled out of two or three cleaned axons and sucked into a 0.6-mm bore glass capillary. An insulated 100-μm platinum wire, scraped and platinized for 2 mm at the tip, was run into each end of the horizontal tube over an ASW pool and the whole covered to retard evaporation from the capillary. The 1-kHz parallel resistance and capacity were measured between the 100-μm wires with tip separations from 16 down to 2 mm and a frequency run was made from 10 Hz to 30 kHz at 10 mm. A similar run was then made with standard ASW[2] (with a resistivity of 19.7 Ωcm at 24.4°C). Four runs made with 0.5 M KCl checked the calibrations of the capillaries from dimensions. All measurements were made at room temperature.

Four volunteers in various combinations cleaned and rolled the axons for the nine completed experiments. The axons all appeared to be in good condition and the several tested were excitable. The few white spots were tied off and discarded. The axoplasm was very viscous, although not solid.

The data were all converted to series resistance, R, and reactance, X, with a pocket calculator and plotted against electrode separations (Fig. 1). The near linearity of R and near constancy of X support the assumption that the axoplasm is a pure resistance and that the impedance of the electrodes remained constant. In the temperature range 22.5–26°C they gave the following resistivities (in ohm centimeters) in the order in which they were done: 25, 30.4, 29.6, 24.7, 22.1, 24.5, 26.2, 25.0, 29.9. These are higher than ASW at the same temperatures by the following factors, in rank order: 1.12, 1.21, 1.24, 1.25, 1.25, 1.35, 1.52, 1.53, 1.60. The lowest value may be excused

[2] 430 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 10 mM TrisCl, pH 7.0 at 18°C.

but otherwise there are no explanations for the variability. The apparent bimodal distribution is striking but many more experiments would be necessary to establish its validity. Unfortunately, the present work was terminated by lack of axoplasm.

Electrode Errors

It can be shown that possible electrode errors are expected to be trivial at less than 2%. The frequency data were plotted on the R, X plane and extrapolated to R_∞ at infinite frequency which was subtracted to give electrode impedance, $Z_e = R - R_\infty + jX$. These were calculated and presented as Bode plots, log $|Z_e|$ and ϕ vs. log f (Fig. 2) to test the empirical relation $Z_e = |Z_1| (j\omega)^{-\alpha}$, where $|Z_1|$ is the absolute

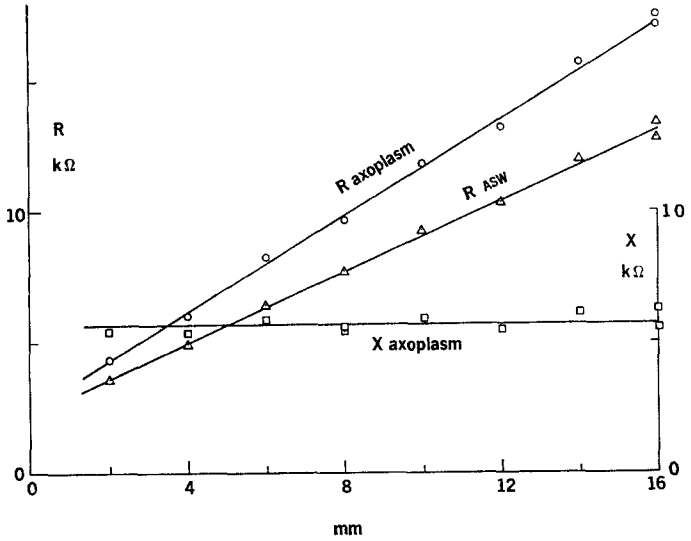

FIGURE 1. Plots of series resistances, R, for axoplasm and ASW and series reactance, X, for axoplasm vs. electrode separation at 1 kHz.

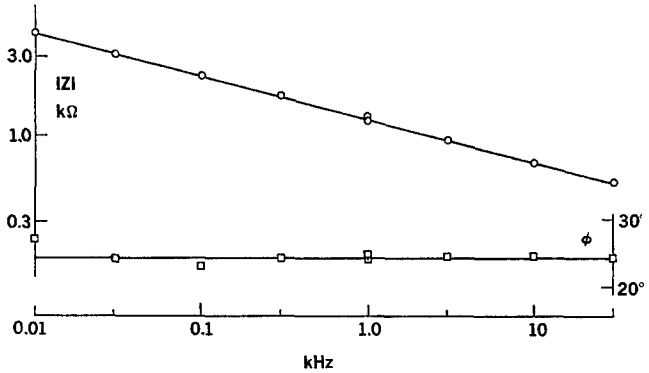

FIGURE 2. Plots of absolute value of electrode impedance $|Z|$ ($\alpha = 0.253$) log scale, and phase angle $\phi(\alpha = 0.270)$, linear scale, vs. frequency, log scale.

value at 1 kHz, $\omega = 2\pi f$, and $\phi = \alpha\pi/2$. This relation was reasonably well followed and remained essentially constant during a run although no special care was taken of the electrodes. Thus it is reasonable to assume that, insofar as X remained constant, neither $|Z_1|$ nor α changed significantly. In three axoplasm runs there were consistent downward trends of X as the electrodes came closer together. If these were decreases of $|Z_1|$, the resistance components would reduce the axoplasm resistivity by 1–2%. The intriguing possibility of reactive components in the axoplasm is too nebulous for present discussion.

However, for five electrodes and 21 frequency runs over a month values of α ranged from 0.19 to 0.53 with an average of 0.33 and $|Z_1|$ from 0.68 to 4.7 $k\Omega$, average 1.5 $k\Omega$, in an apparently chaotic manner. Although the values of both parameters were similar in axoplasm and ASW they were never the same, in six cases $|Z_1|$ was 10–30% higher in axoplasm. Thus calibrations in one medium may be somewhat proportional to those in another. Yet in the present arrangement the results would not have been qualitatively different even if the electrode impedances had not been largely eliminated by the procedure and analysis of the experiment.

DISCUSSION

The immediate incentive for the present work was to make a comparison with the recent squid axon experiments of Carpenter et al. (1973, 1975), using the Bak (1967) microelectrode technique. Their results from isolated Woods Hole axons, are calculated to give a range of 19–41 Ωcm with a mean of 31 Ωcm or 0.9–2.0 × SW and 1.55 × SW. The following differences should be considered:

Their results average, 31 Ωcm vs. 27 Ωcm, may be higher because of possible contamination of axoplasm with ASW in the extrusion process. The wider spread may be the result of the larger number of experiments, 15 vs. 9. Their measuring frequency was 100 vs. 1 kHz, which may not be significant. A significant temperature difference seems unlikely. The unknown residual electrode impedance may not have been proportional to the resistivity of the medium as they tacitly assumed. The present results are in this direction but differences in size and shape make it only possible to guess that they may not have been significant.

The only conclusion now possible is that there is no such thing as a standard value for the resistivity of extruded axoplasm, with 1.3–1.5 × SW as a best guess. However, L. J. Mullins (unpublished observations) has calculated from mobilities and concentrations a resistivity of 1.32 × SW and a 20% volume concentration of nonconducting mitochondria would increase this to 1.81 × SW. There is a strong inference that the same conclusion applies to the resistivity of the axoplasm of a dissected and cleaned axon. It seems possible that the deterioration of squid axons begins with any injury to the animal, as found for rest potentials and undershoot of action potentials. Although they may not be of major importance for most electrical conclusions,

at least such wide variations in the resistivity indicate the need for identification of the injurious stages of axon preparation and caution in the interpretation of chemical and biochemical data.

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