TRANSMEMBRANE IMPEDANCE OF THE *CHARA* CELL

Uichiro KISHIMOTO

*Laboratory of Biology, College of General Education,
Osaka University, Toyonaka, Osaka*

Summary The voltage clamp method was used for determining transmembrane impedance of the internodal cell of *Chara*. Between 1 Hz and 1 kHz the impedance locus on a complex plane was generally not a circular arc, but could be successfully simulated by equivalent circuit models having two time constants.

The impedance of living cells and tissues were measured extensively by many investigators (for references and analyses see COLE, 1968). The frequency characteristic of impedance was generally shown to be expressed by a circular arc on the complex impedance plane (*i.e.*, Cole-Cole plot, COLE and COLE, 1941). In general the center of the circle lies below the abscissa (the resistance axis). Many later observations also showed these types of the impedance characteristics in a variety of cells and tissues. Such a circular arc impedance locus is explained by assuming that the membrane impedance has a constant phase angle (FRICKE, 1932; COLE, 1928; COLE and CURTIS, 1938, 1939). This is similar to the impedance for electrode polarization. Unfortunately, however, the physical model for explaining the constant phase angle impedance is lacking at present.

Almost all measurements have been carried out so far with a pair of parallel external electrodes. In such a situation the direction of electric current was not necessarily perpendicular to the membrane surface. By using a bridge method TAYLOR and CHANDLER (1962) and TAYLOR (1965) measured the transmembrane impedance of the squid giant axon between an internal axial wire and an external platinum electrode. In this case the electric current was expected to flow almost perpendicularly to the axon membrane. Under these experimental conditions the impedance locus was not a circular arc, but could be explained reasonably well by assuming a membrane model with either a constant phase angle or a bounded distribution of time constants. A similar method was applied recently to the squid axon membrane by MATSUMOTO *et al.* (1970). By employing the range of frequency between 50 Hz and 1 MHz they obtained an impedance locus which could be simulated by a model with two relaxation time constants, but not by a

Received for publication March 18, 1974

岸本卯一郎

model with a single time constant.

In the present experiments a voltage clamp method is used for determining the transmembrane impedance of the *Chara* internode.

MATERIAL AND METHOD

The *Chara australis* was collected at Ikeda Lake in southern Kyushu. The mature internode, 3–7 cm in length and 0.4–0.6 mm in diameter, was isolated from adjacent cells and kept in a Petri dish containing artificial pond water (APW) for at least three days before being used for experiments. The APW composition was as follows: 0.05 mM KCl, 0.05 mM NaH_2PO_4 , 0.2 mM NaCl, 0.5 mM $\text{Ca}(\text{NO}_3)_2$ and 0.1 mM MgSO_4 ; pH of the solution was 6.5.

An internode was placed in a leucite cell shown in Fig. 1. A partition was

Fig. 1. Scheme of the method for controlling the membrane potential of the *Chara* cell. The membrane potential V_m between an internal micropipette electrode and an external reference electrode was measured. AC command signal V_c for the impedance measurement was applied to the summing junction Σ . The summed potential was amplified by a control amplifier having both large gain ($>2,000$) and large output voltage swing (± 130 V). The amount of current was measured as the potential drop across a $1,000 \Omega$ resistor. See text for further details.

made across a part of the internode in the groove (about 0.8 mm wide) with white vaseline. This sealing was needed to hold the internode stable in position and to establish a good electrical insulation between the pools of the leucite cell. A sharpened straight tungsten wire electrode, 0.2 mm diameter, was introduced into the axis of the internode from the right-hand end toward the measuring area of the cell, which was 7 mm in length. The tip of the wire was sharpened elec-

trollytically in an 1 N NaOH solution. The diameter of the tip was less than $5\ \mu$. The surface of the wire was coated with platinum black to produce a low electrode impedance, *i.e.*, $15\ \text{k}\Omega$ with DC and less than $1\ \text{k}\Omega$ at 1 kHz in 100 mM KCl. A pair of external current electrodes was made of a chlorinated silver plate, the surface of which was plated with platinum black for both stability of the electrode potential and a low electrode impedance (COLE and KISHIMOTO, 1962). The impedance of such a hybrid electrode in APW was about $1\ \text{k}\Omega$ with DC and less than $100\ \Omega$ at 1 kHz.

The membrane potential of the *Chara* internode was measured with a pair of conventional glass capillary microelectrodes. The tip of the internal microelectrode (generally in the vacuole of the internode) was about $1\ \mu$ in diameter, and the inside was filled with 3 M KCl. The resistance of this microelectrode was about $5\ \text{M}\Omega$. A similar external reference electrode had a large opening at the tip, *i.e.*, about $50\ \mu$ in diameter, and was plugged with 50 mM KCl-agar. The resistance of the reference electrode was about $10\ \text{k}\Omega$ in APW.

The external solution was introduced into the measuring pool and was sucked up at a constant rate (3–5 ml per minute) by a suction pump from another pool on the left side. The temperature of the solution was between 16° and 19°C .

The membrane potential was amplified with a differential DC amplifier of unity gain, which was made up of two operational amplifiers (1009, Teledyne Philbrick) of high-input impedance ($10^{11}\ \Omega$) and a subtractor amplifier (1009). The output of the preamplifier was led through a buffer amplifier (1009) to the summing point (Σ in Fig. 1). A sinusoidal voltage change was supplied to the summing point by a function generator (FG-1100, NF) through a constant resistor. The summed voltage was amplified with another operational amplifier, *i.e.*, control amplifier (1022, Teledyne Philbrick), which had a large output voltage swing (± 130 volts) and a variable gain between 1 and 2,000. Whenever the gain of this negative feedback control amplifier was at its maximum, the membrane potential followed any shape of the voltage which was supplied to the summing point. The current which flowed across the *Chara* membrane under the voltage clamp was monitored with a calibrated resistor and the signal was amplified with a low-noise parametric amplifier (P2A, Teledyne Philbrick). The current signal, Δi , was fed to the Y-axis, and the voltage signal, Δv , to the X-axis of an oscilloscope (VP-549A, Matsushita). There was practically no phase difference between the X-axis and the Y-axis amplifiers of the oscilloscope at low frequency ranges and less than 3° at 2 MHz. When both signals were fed to the oscilloscope, the Δi - Δv curve appeared as an ellipse with its axis oblique to the Δv axis (Fig. 2). The absolute value of the impedance is equal to $\Delta v/\Delta i$. On the other hand the phase angle (θ) of the impedance is related to M ($=\Delta v$) and N in Fig. 2 by an equation: $\sin \theta = N/M$. With the voltage clamp Δv is constant. N is independent of the change in amplification of Δi . Taking photographs of one series of Δi - Δv ellipse from 1 kHz to 1 Hz could be achieved within 2 min. Under the voltage clamp not only could

Fig. 2. Oscilloscope records of Δi - Δv ellipse at 600, 80 and 8 Hz. Amplification of Δi at less than 10 Hz was made twice as large as that at higher frequency in order to get better information on the phase angle, though the curve became broader. Lower right: Δv or M is kept constant by the voltage clamp method. N remains constant for different amplification of Δi at each frequency. The phase difference between Δi and Δv is equal to $\sin^{-1}(N/M)$.

the amplitude of Δv be kept constant but also the phase of Δv was locked by the externally supplied command voltage.

The output of the control amplifier is equal to $-\mu(V_m + V_c)$, where μ is the gain of the control amplifier, V_m the potential difference between μ -tip and Ref.,

and V_e the external command voltage in Fig. 1. If R_1 denotes the resistance of the internal axial wire, Z_m the impedance of the membrane, R_2 the resistance of the external current electrode and R_3 the resistance of the resistor for the current measurement (Fig. 3), then, the following relation is obtained between the potentials and the current I which flows through this circuit.

Fig. 3. A dummy circuit for the *Chara* membrane. Z_m is the impedance of the membrane. R_1 , R_2 and R_3 are resistances of the internal axial wire, the external current electrode and the constant resistor for current reading, respectively. The resistance of the internal micro-pipette electrode is about 5 MΩ and that of the reference electrode is about 10 kΩ.

$$-\mu(V_m + V_e) = I(R_1 + R_2 + R_3 + Z_m) \quad (1)$$

Since the input impedance of the preamplifier is far larger than the impedance of the membrane, all the current flows across the membrane. Thus,

$$V_m = IZ_m \quad (2)$$

From these two equations the expression for I can be written as follows:

$$I = -\mu V_e / (R_1 + R_2 + R_3 + Z_m + \mu Z_m) \quad (3)$$

As mentioned before $R_1 < 1$ kΩ, $R_2 < 100$ Ω and Z_m of the *Chara* membrane in APW is about 10 kΩ (≈ 1 kΩ cm²) for the high frequency extreme, while $R_1 \approx 15$ kΩ, $R_2 = 1$ kΩ and Z_m is larger than 100 kΩ for the low frequency extreme, and R_3 is 1 kΩ. Accordingly, I is almost equal to V_e/Z_m so long as μ is larger than 2,000, which is realized under the voltage clamp. The error is estimated to be less than 1 percent by calculation. However, another larger error (about 10 percent) was

encountered in the determination of phase difference between I and V_e in the very low frequency region. That is, since the voltage drop across a constant resistor $R_s (=1 \text{ k}\Omega)$ which was used for current reading was as small as $20 \mu\text{V}$, the curve of the Lissajous ellipse became fairly broad owing to a superposition of noise.

The reliability of the whole measuring system was tested by applying the voltage clamp to a dummy circuit such as shown in Fig. 3. First, capacitance C_m in Fig. 3 was removed. In such a case the Lissajous figure should be a straight line with a constant slope to the X-axis of the oscilloscope. To achieve this it was necessary to adjust each stage of the operational amplifier carefully with a negative capacitance feedback. Without such a shaping at each stage the phase

Fig. 4. a: The absolute value of the measured impedance Z_m and its phase angle are plotted against the logarithm of frequency. The amplitude of the sinusoidal potential change (Δv) was 20 mV in this series of experiment. The phase angle is maximum at around 70 Hz. There were 10 min waiting periods between each series of measurements. —●— is the first series, —×— the second, —○— the third and —▲— the fourth. b: All the measured points are plotted together on the same plane. An interrupted line is a semi-

shift between the input and the output of each amplifier was not at all negligibly small even at a low frequency like 100 Hz. After this adjustment the frequency dependence of the impedance of the dummy circuit with the capacitance was tested. As expected the impedance locus for the circuit having a single relaxation time constant such as shown in Fig. 3 was a semi-circle, the center of which lay on the real axis. Thus, the impedance measurement using the voltage clamp technique proved to be reliable at least for a frequency region between 1 Hz and 2 kHz.

As will be described later the impedance loci of the *Chara* membrane were not a circular arc. Two representative models having two relaxation time constants were adopted to simulate the loci. The resistances and capacitances in these models were determined with a desk microcomputer (Sobax ICC-2700,

circle, the center of which lies on the abscissa. c: The impedance locus for the first series. d: The locus for the second series. The larger roman numerals are frequency in Hz. Smaller italic numerals correspond to frequencies in the calculation by series or parallel model. These two loci are neither a semi-circle nor a circular arc, but could be simulated satisfactorily with a series circuit model and a parallel circuit one shown in Fig. 5. The resistances and capacitances are calculated and listed in Table I.

Sony).

RESULTS

The frequency dependence of the transmembrane impedance of the *Chara* internode in artificial pond water was studied. The amplitude of the voltage change (Δv) using the voltage clamp was 20 mV. This was sufficiently small in comparison with the threshold potential change for excitation of the *Chara* internode in artificial pond water (APW). The steady-state current-voltage relation was linear for such a small potential change. Generally, there were 5 min waiting periods between each series of measurements from 1 kHz to 1 Hz. One series of measurements lasted less than 2 min. Four successive series of measurements are shown in Fig. 4. The absolute value of the impedance $|Z_m|$ stays almost constant for less than 1 Hz, decreases with the increase of frequency and reaches another constant low value for higher than 500 Hz (Fig. 4a). The phase difference between Δi and Δv is maximum at a round 70 Hz. By calculating the real and imaginary parts of the impedance from these data the Cole-Cole plot of the impedance locus is obtained (Fig. 4b). A glance at all the data shows the locus to be apparently fairly close to a semi-circle. However, the impedance locus for each of the series of measurements obviously deviates from a semi-circle (Fig. 4c and 4d)

This type of deformed impedance locus cannot be simulated either by a constant phase angle model or by a single relaxation time model. However, it was found by calculation that each impedance locus could be simulated reasonably well with an equivalent circuit having two relaxation time constants. Although it is possible to imagine several other circuits having two time constants, two representative models having three resistances and two capacitances are shown in Fig. 5a (series model) and 5b (parallel model). It is worth noting that the present imped-

Fig. 5. Two representative circuit models for simulating non-circular impedance loci in the *Chara* membrane. The series model (a) and the parallel model (b) consist of three resistances and two capacitances. These values can be determined by the successful simulation of the impedance locus.

ance loci could be simulated well with both of these models. Three resistances and two capacitances of series and parallel models which could simulate the measured impedance loci were calculated and are listed in Table 1. At present, there seems to be no good reason to choose one model from the two. A similar series of data is shown in Fig. 6 (a, b, and c). In this experiment Δv was purposely increased to 70 mV. Half of this amplitude (35 mV) was still smaller than the threshold (depolarization by 45–60 mV) for excitation of the *Chara* internode which was used in the present experiments. However, it is likely that the sub-threshold activation of Cl^- and/or K^+ permeation processes might occur. As shown in Figs. 6b and 6c deviations from a semi-circle are more obvious than those in Fig. 4. The shape of the whole impedance locus looks more or less like a skewed arc. In this case too the whole impedance locus was found to be simulated satisfactorily with the equivalent circuit having two relaxation time constants such as shown in Fig. 5. Resistances and capacitances in series and parallel models were calculated and are listed in Table 1.

Table 1. Numerals in brackets stand for time constants in msec., *i. e.*, $c \times r$ in the series model and $C \times R$ in the parallel model. R 's and C 's of the parallel model in the last two lines (Fig. 7; 10 mM KCl) were calculated from r 's and c 's of the corresponding series model.

	Series model					Parallel model				
	r_s ($\text{k}\Omega \cdot \text{cm}^2$)	r_1 ($\text{k}\Omega \cdot \text{cm}^2$)	c_1 ($\mu\text{F} \cdot \text{cm}^{-2}$)	r_2 ($\text{k}\Omega \cdot \text{cm}^2$)	c_2 ($\mu\text{F} \cdot \text{cm}^{-2}$)	R_m ($\text{k}\Omega \cdot \text{cm}^2$)	R_1 ($\text{k}\Omega \cdot \text{cm}^2$)	C_1 ($\mu\text{F} \cdot \text{cm}^{-2}$)	R_2 ($\text{k}\Omega \cdot \text{cm}^2$)	C_2 ($\mu\text{F} \cdot \text{cm}^{-2}$)
Fig. 4c (APW)	1.45	8.18	0.81 (6.58)	3.43	5.65 (19.4)	13.1	47.9	0.174 (8.35)	1.68	0.485 (0.82)
Fig. 4d (APW)	1.45	6.40	1.04 (6.65)	4.85	2.47 (12.0)	12.8	31.9	0.079 (2.50)	1.72	0.460 (0.79)
Fig. 6b (APW)	1.30	2.88	1.15 (3.31)	19.8	0.902 (17.8)	24.0	18.9	0.255 (4.82)	1.48	0.372 (0.55)
Fig. 6c (APW)	1.30	3.27	1.25 (4.09)	18.1	0.972 (17.6)	22.7	14.5	0.298 (4.32)	1.52	0.340 (0.52)
Fig. 7 (APW)	3.38	13.5	0.398 (5.38)	85.3	0.228 (19.4)	101	135	0.028 (3.76)	3.58	0.108 (0.38)
(10mM KCl)	1.68	18.3	0.208 (3.81)	26.4	0.675 (17.8)	46.4	90.1	0.108 (9.75)	1.76	0.142 (0.25)
(10mM KCl)	1.68	13.1	0.171 (2.24)	19.3	0.575 (11.1)	34.1	61.56	0.097 (5.98)	1.81	0.113 (0.20)

When the external APW was switched to 10 mM KCl, the *Chara* membrane was depolarized by about 60 mV with a marked decrease in the membrane resistance. In Fig. 7 the impedance locus of the cell in 10 mM KCl is compared with that in APW. The impedance locus of the cell in APW was again simulated

satisfactorily with the series and the parallel models. When APW was exchanged for 10 mM KCl, the locus shifted to the left and became smaller. Unfortunately, the impedance characteristic of the cell in 10 mM KCl was not stable enough but changed slowly with time. Thus, it was not possible to reliably calculate the r 's and c 's with either of the series and the parallel model. In other words, although the shape of the locus could be simulated with these models, the position of the corresponding frequencies on the locus was different by a factor of two between the calculated values and the measured ones. Anyway, it is worth noting that r_s , the frequency for high frequency extreme or the resistance in series with the membrane, decreased to some extent in 10 mM KCl in addition to the decrease of the membrane resistance.

DISCUSSION

Electrode impedance is not necessarily negligible in impedance measurement carried out with bridge balancing. Generally it was necessary to correct this amount in order to discuss membrane impedance itself. Since electrode impedance becomes fairly large at a frequency such as 1 Hz, a careful correction is inevitable to obtain a reliable impedance value. On the other hand, it is preferable to measure the transmembrane impedance directly between an internal and an external electrode in order to eliminate the effect of tangential flow of electric current along the surface of the membrane. Such a tangential current flow was suspected to be one of the causes for deviation of the impedance locus in a sense that it is another relaxation phenomenon (TAYLOR, 1965; COLE, 1968). One merit of using the voltage control method, which is adopted in the present report, is that impedances of the internal axial wire and the external current electrode can be safely neglected so long as the gain of the negative feedback amplifier is large enough. To achieve such an impedance measurement it was necessary to eliminate in advance the possible phase shift between the input and the output of each operational amplifier stage for the frequency change from 1 Hz to 1 kHz. Practically, this kind of shaping was performed by the proper negative capacitance

Fig. 6. Almost the same record as in Fig. 4 except that a larger sinusoidal potential change (70 mV) was applied here on purpose. Half of this amplitude is still less than the threshold of the present *Chara* cell. a: The absolute value of the measured impedance and its phase angle are plotted against the logarithm of frequency. The maximum of the phase angle is around 70 Hz. There were 10 min waiting periods between each series of measurements. —○— is the first series, —●— the second, —▲— the third and —□— the fourth. b: The impedance locus for the first series. Larger roman numerals are frequency in Hz. Smaller italic numerals correspond to frequencies in the calculations by series and parallel models. The deviation of the impedance locus from a circular arc is more marked here than in Fig. 4 and the locus looks more or less like a skewed arc. Impedance loci in b and c could be simulated satisfactorily with the series and the parallel models shown in Fig. 5. The resistances and capacitances in these circuit models are listed in Table 1.

Fig. 7. Changes of the impedance locus after replacing the external APW with 10 mM KCl. The impedance locus of the *Chara* cell in APW (full line) is satisfactorily simulated by the series and the parallel models shown in Fig. 5. Roman numerals are frequencies in Hz. Two impedance loci for the cell in 10 mM KCl (interrupted and dotted lines) are more or less flattened. Smaller numerals in italic are the frequencies in Hz. The shape of the locus could be simulated by the series and the parallel models. However, the corresponding frequency differed from the measured one by a factor of two especially in the low frequency region. Some change in the membrane property was suspected to have progressed during one series of measurement. In addition to the decrease of the resistance at the low frequency extreme the resistance at the high frequency extreme also decreased. This corresponds to the decrease of the series resistance r_s in 10 mM KCl beside the decrease of the resistance of the *Chara* membrane.

feedback.

The simplest circuit model for the living membrane is the one such as a resistance being in parallel with a capacitance. According to BODE (1945) the admittance of any two-terminal resistance (r)-inductance (l)-capacitance (c) network can be expressed with a following equation:

$$Y = \frac{4}{V^2} [F + j\omega(U - T)]$$

where $F = \sum_i r_i i_i^2$ is the average rate of energy dissipation in the entire network, $U = (1/2) \sum_j c_j v_j^2$ the average potential energy, $T = (1/2) \sum_k l_k i_k^2$ the average kinetic energy and V the average voltage applied to the network. So long as $(U - T)$ is a positive value the network can be expressed with a circuit $R (= V^2/4F)$ and $C (= 4(U - T)/V^2)$ being in parallel. Then, $RC = (U - T)/F$. In this sense R and C in this simplest parallel circuit model cannot be independent. In other words, C does not necessarily correspond to the electrostatic capacitance of the lipid component of the membrane. If the impedance locus were a semi-circle, R and C

being constant, then the ionic process for the applied frequency range should be regarded as a single relaxation phenomenon. On the other hand, if the impedance locus is not a semi-circle, R and C in this simplest model should be quantities which are frequency dependent.

By adopting a mobile site membrane model, SANDBLOM *et al.* (1972) said that the impedance locus exhibited a 45° phase angle at the high frequency end (*i.e.*, a skewed arc type). However, the impedance loci in the present *Chara* cell and in the squid giant axon (MATSUMOTO *et al.*, 1970) made an angle larger than 45° to the R -axis at the high frequency extreme.

The impedance locus of *Chara* internodes obtained with the voltage control technique was not a circular arc (Figs. 4c, 4d, 6b, 6c and 7), but could be simulated with the equivalent circuit model having two time constants (Fig. 5). Similar results were obtained with a bridge balancing method in the transmembrane impedance of the squid giant axon in the sea water and in the varied K-sea water (MATSUMOTO *et al.*, 1970) and also in the internally perfused giant axon of the squid (Inoue, unpublished). Deviations from a circular arc were larger in Fig. 6 where the amplitude of AC voltage was 70 mV than in Fig. 4 where it was 20 mV. Both voltages were smaller than the threshold for firing the *Chara* membrane in the present experiments. Subthreshold activation and inactivation of ionic processes might play an important role. HODGKIN and HUXLEY (1952) analysed the ionic process of the squid axon membrane in terms of activation and inactivation of sodium ion permeation and activation of potassium permeation. GRUNDFEST (1966) proposed an additional potassium inactivation process beside these three in order to explain the anomalous rectification, hyperpolarizing response and so on of various excitable membranes. In this sense it is also probable that one can obtain better approximation by assuming the existence of more than three relaxation processes instead of two which are adopted in the present report.

FALK and FATT (1964) measured the impedance of the frog sartorius muscle with two internal microelectrodes, the one for sending sinusoidal current across the membrane and the other for receiving the corresponding potential change. With a proper correction due to the cable properties of the muscle fiber they showed that their impedance loci could be explained with an equivalent circuit having two time constants. A similar impedance locus was obtained on the same cell (FATT, 1964) also with a conventional transverse impedance measurements between a pair of parallel electrodes. They attributed the origin of the two time constants to the impedances of the sarcolemma and the sarcoplasmic reticulum respectively, both being in parallel. However, such an impedance locus having more than two time constants has been observed in the single membrane of the squid giant axon (TAYLOR, 1965; MATSUMOTO *et al.*, 1970), where the impedance was measured between an internal and an external electrode.

The two time constants model may be a first approximation for describing the impedance locus of the excitable membrane as mentioned before. However,

one merit of adopting such a model is that each of five elements (*i.e.*, three resistances and two capacitances) remains constant for the change in frequency. This is reasonable in a low frequency region such as between 1 Hz and 1 kHz. Although this is a functional model, each r and c should correspond to some physical properties of the membrane. In structure there are the cell wall, the plasmalemma, the cytoplasm, and the tonoplast in series between the external and the internal electrodes. The result showing the transmembrane impedance of the *Chara* cell to have two time constants may prompt one to suppose that one is for the plasmalemma and another for the tonoplast, if one adopt the series model. It has been tacitly assumed that the resistance of the tonoplast may not change appreciably for the change in the external salt concentration in spite of the large change in the resistance of the plasmalemma, though there was no direct measurement. However, in the present experiments not only r_s , r_1 and c_1 but also r_2 and c_2 changed soon after the increase of the external K^+ concentration (Table 1). On the other hand, if one adopts the parallel model, the plasmalemma itself may be supposed to be mosaic in its structure. For instance, there may be at least two types of capacitance in a single membrane; one is due to the electrostatic capacitance, probably, of lipid bilayer component of the membrane, the other being caused by the phase retardation due to the time needed for ions to be redistributed in the charged membrane moiety.

At present it seems better not try to identify the location of structures having two time constants. One reason is the difficulty in separating the impedance of the plasmalemma or the tonoplast from the total impedance. Another reason comes from the fact that the impedance locus could be simulated with the series model as satisfactorily as with the parallel model. Actually these two representative models are interchangeable. In other words, if one chooses the series model and determines r 's and c 's in Fig. 5a, five elements of the parallel model in Fig. 5b can be calculated, both showing the same impedance characteristic for the change in frequency. Moreover, it is also possible to devise several other equivalent circuits (see COLE, 1968, p. 81) having more than two time constants to simulate the measured impedance locus as satisfactorily as these two representative ones. In this sense it is safer here only to conclude that there are ionic processes having more than two different time constants. There are too many factors to be decided before being able to discuss about the morphological correspondence of r 's and c 's.

This research was aided by a grant from the Ministry of Education of Japan.

REFERENCES

- BODE, H. W. (1945) Network Analysis and Feedback Amplifier Design. N. Y. Van Nostrand, pp. 151-157.
COLE, K. S. (1928) Electric impedance of suspensions of spheres. *J. Gen. Physiol.*, **12**: 29-36.
COLE, K. S. (1968) Membranes, Ions and Impulses. University of California Press.

- COLE, K. S. and COLE, R. H. (1941) Dispersion and absorption in dielectrics. I. Alternating current characteristics. *J. Chem. Phys.*, **9**: 341-351.
- COLE, K. S. and CURTIS, H. J. (1938) Electric impedance of *Nitella* during activity. *J. Gen. Physiol.*, **22**: 37-64.
- COLE, K. S. and CURTIS, H. J. (1939) Electric impedance of squid giant axon during activity. *J. Gen. Physiol.*, **22**: 649-670.
- COLE, K. S. and KISHIMOTO, U. (1962) Platinized silver chloride electrode. *Science*, **136**: 381-382.
- FALK, G. and FATT, P. (1964) Linear electrical properties of striated muscle fibres observed with intracellular electrodes. *Proc. Roy. Soc. B. (London)*, **160**: 69-123.
- FATT, P. (1964) An analysis of the transverse electrical impedance of striated muscle. *Proc. Roy. Soc. B. (London)*, **159**: 606-651.
- FRICKE, H. (1932) The theory of electrolytic polarization. *Phil. Mag. (7)*, **14**: 310-318.
- GRUNDFEST, H. (1966) Heterogeneity of excitable membrane: Electrophysiological and pharmacological evidence and some consequences. *Ann. N. Y. Acad. Sci.*, **137**, Art. 2: 901-949.
- HODGKIN, A. L. and HUXLEY, A. F. (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.*, **117**: 500-544.
- MATSUMOTO, N., INOUE, I., and KISHIMOTO, U. (1970) The electric impedance of the squid axon membrane measured between internal and external electrodes. *Jap. J. Physiol.*, **20**: 516-526.
- SANDBLOM, J., WALKER, J. L., Jr., and EISENMAN, G. (1972) The transient response and impedance locus of a mobile site membrane. *Biophys. J.*, **12**: 587-596.
- TAYLOR, R. E. (1965) Impedance of the squid axon membrane. *J. Cell. Comp. Physiol.*, Suppl. 2, **66**: 21-26.
- TAYLOR, R. E. and CHANDLER, W. K. (1962) Effect of temperature on squid axon membrane capacity. *Biophys. Soc. (Abstr.)* TDI.