THEORY OF ALTERNATING CURRENT MEASUREMENTS IN BIOLOGY AND ITS APPLICATION TO THE INVESTIGATION OF THE BIOPHYSICAL PROPERTIES OF THE TROUT EGG

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(With Eight Text-figures)

Alternating current measurements in biology are concerned with the resistance and capacitance of the cell membrane, and the resistance of the cell interior. No systematic electrical measurements have so far been made on the cell nucleus.

Such expressions as resistance, capacitance and impedance are generally associated with wireless sets or wireless components. They have, however, a physico-chemical meaning. The resistance of the cell membrane is a measure of the ability of ions to pass through it, while the capacitance of the cell membrane gives an indication of its dielectric constant which in turn is an indication of chemical composition. The capacitance depends on the distribution of charge on, in, or round the membrane. Substitution or deformation of dipoles, or alteration of the forces controlling the relative positions of charged particles will make the capacitance vary. If the dielectric constant is known or guessed, measurement of the capacitance enables the thickness of the cell membrane to be computed. The equation for the capacitance of a parallel plate condenser summarizes these relationships:

\[ C = \frac{\epsilon A}{4\pi l} \]  

(1)

where \( \epsilon \) = dielectric constant, \( A \) = cross-sectional area, and \( l \) = distance between the 'plates' of the condenser.

The dielectric constant of a polar liquid or solution can be considered as a measure of the number of molecules oriented by an external field of unit strength. As the molecules are oriented by a torque which depends on the external field strength and the dipole moment of the molecular species concerned, and as the orientation is hindered by frictional forces in the solution which are proportional to the rate of orientation, it is evident that the dielectric constant may not be a constant but a variable depending on the frequency of the applied external field.

When the source of current in an electric circuit is direct, the relationship between voltage, current and resistance is given by Ohm's law:

\[ R = \frac{E}{I}, \]  

(2)
where $R =$ resistance, $E =$ voltage, and $I =$ current. If the source of electric current is alternating, of the form

$$I = I_0 \sin \omega t,$$

(3)

where $I_0 =$ amplitude, $\omega = 2\pi \times v$, and $v =$ frequency, Ohm’s law still holds good, but the system does not have a simple resistance $R$ but an impedance $Z$ which includes the resistance and also the reactance $X$ due to any capacitance the conductor may have. The reactance of a condenser is its ‘resistance’ to the passage of an alternating current. Unlike direct current, alternating current passes through an ideal condenser even though it has an infinite direct current resistance.

The reactance of a capacitative system is inversely proportional to the capacitance and the frequency: mathematically speaking, $X = \frac{1}{\omega C}$. It is this fact which often makes measurements with alternating current (a.c.) more practical than those with direct current (d.c.).* Suppose, for example, we wish to investigate the resistance of the cytoplasm in a spherical cell. Measurements would be almost insuperably difficult by d.c. methods. The resistance of the cell membrane is usually very high compared with that of the cytoplasm. If measurements were made by means of electrodes outside the cell, the membrane resistance would swamp the cytoplasm resistance. The only alternative method would be to insert two electrodes into the cytoplasm and to flow current through the cytoplasm between them. This procedure is technically too difficult to be successful. The cell membrane has a significant capacitance. If a.c. is used, the impedance of the cell membrane can be varied at will by varying the frequency of the applied current. If we wish to measure the resistance of the cytoplasm, all that is necessary to do, in principle, is to increase the frequency of the applied current until the membrane impedance is small compared with the cytoplasm resistance. The capacitance of the cell membrane enables the high resistance of the cell membrane to be ‘shorted’, and the difficulties which are inherent in the d.c. method are obviated. The a.c. technique also obviates difficulties due to the medium surrounding the cell. If this medium has a high conductivity and current has to pass through the cell, the frequency of the applied current is increased until the impedance of the cell membrane is low compared with that of the medium. At high frequencies most of the current passes through the capacitance of the cell membrane; at low frequencies, where the capacitative reactance is greater, more of the current flows through the resistance of the cell membrane, until in the limiting case at zero frequency or with d.c., all the current passes through the membrane resistance. It has previously been stated that $X = \frac{1}{\omega C}$. If $v = 0$, $X = \frac{1}{\infty} = 0$, and no current passes through the capacitance. At the other end of the frequency spectrum, $v = \infty$ and $X = \frac{1}{\infty} = 0$. All the current passes through the capacitance of the cell membrane which offers no resistance to the passage of current.

The cell membrane may for the moment be represented electrically as a resistance and a capacitance in parallel (Fig. 1a). When a.c. is applied across the membrane, the current flow will be distributed between the two branches of the network.

* d.c. pulses are considered as a special case of a.c.
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According to their respective impedances at the particular frequency used. The impedance of a resistance and capacitance in parallel is given by the expression

\[ Z_p = \sqrt{\frac{R_p^2}{1 + \omega^2 C_p^2 R_p^2}} \]  \hspace{1cm} (4)

and, in complex notation,

\[ Z_p = \frac{R_p}{1 + j\omega C_p R_p} \]  \hspace{1cm} (4’1)

where \( j = \sqrt{-1} \). At a particular frequency \( \nu_j \), a network consisting of a resistance and a capacitance in parallel is indistinguishable from a resistance and a capacitance in series (see Fig. 1b). The impedance of the latter is given by the expression

\[ Z_s = \sqrt{\left(R_s^2 + \frac{1}{\omega^2 C_s^2}\right)} \]  \hspace{1cm} (5)

and

\[ Z_s = R_s + \frac{j}{\omega C_s} \]  \hspace{1cm} (5’1)

At the particular frequency \( \nu_j \),

\[ R_s = \frac{R_p}{1 + \omega^2 C_p^2 R_p^2} \]  \hspace{1cm} (6)

and

\[ C_s = C_p \left(1 + \frac{1}{\omega^2 C_p^2 R_p^2}\right) \]  \hspace{1cm} (6’1)

The two networks are indistinguishable and equivalent at a particular frequency.

Electrical networks have a function \( \phi \), the phase angle, which is an index of the difference between the time when a sinusoidally varying current flowing into the terminals of the network reaches its maximum or any other specified value, and the time when the voltage across the terminals of the network reaches its maximum or any other specified value. In the parallel network

\[ \phi = \tan^{-1} \omega C_p R_p. \]  \hspace{1cm} (7)

In the series network

\[ \phi_s = \tan^{-1} \frac{1}{\omega C_s R_s}. \]  \hspace{1cm} (8)

If at a particular frequency, \( Z_p = Z_s \),

\[ \phi_p = \phi_s. \]  \hspace{1cm} (9)

In any electrical network which is not purely resistive, the voltage across the terminals of the network and the current through the terminals are not in phase.

* If \( 1/\omega C_s \) is written as \( X_s \) (the reactance), \( Z_s = R_s + X_s/j \) or \( R_s - jX_s \). This expression will be used later.
The value of $\phi$ varies with the frequency except in the special case of a network consisting of a pure condenser and other cases referred to later in this paper. The phase angle is constant at all frequencies and equals $90^\circ$ across the terminals of a pure condenser.

When considering the cell as an electrical network, it is not sufficient to consider the cell membrane alone; the resistance of the cytoplasm and of the external medium must be included. In general these have negligibly small capacitances. An electrical network which is equivalent to a cell or suspension of cells in a conducting medium is shown in Fig. 2. This network is over-simplified in various ways, particularly in that the cell membrane resistance in parallel with the capacitance is omitted. Other omissions will be considered in due course.

![Fig. 2. A simplified electrical network which is equivalent to a spherical cell, or suspension of spherical cells, in a conducting medium. $R_1$, resistance of conducting medium; $R_2$, resistance of cytoplasm or cell interior; $C_3$, capacitance of cell membrane.]

This is not the only equivalent electrical network. Others could be invented. Its impedance is given by the expression

$$Z = R_1 \frac{1 + \omega^2 C_3^2 R_2 (R_2 + R_1) - j \omega C_3 R_1}{1 + \omega^2 C_3^2 (R_2 + R_1)^2}. \tag{10}$$

At a particular frequency, this network is equivalent to a simple parallel network. If it is balanced in an a.c. bridge by a resistance and a condenser in parallel,

$$C_n = \frac{C_3}{1 + \omega^2 R_2^2 C_3^2}, \tag{11}$$

and

$$R_n = \frac{R_1 (1 + \omega^2 R_2^2 C_3^2)}{1 + \omega^2 R_2 (R_1 + R_2) C_3^2}. \tag{11.1}$$

In biological a.c. measurements, the material (a cell, suspension of cells, a membrane, etc.) is placed in the unknown arm of an a.c. bridge, balance being effected by a resistance and capacitance in parallel in the standard arm (Fig. 3). The frequency is then varied over as wide a range as possible. From the observed values of resistance and capacitance in the standard arm and from a knowledge of the electrolytic cell constant and the ratio of biological cell volume to volume of external medium plus cell, the various electrical parameters in the biological material can be determined. These parameters are: the capacitance of the cell membrane $C_3$, 

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the resistance of the cell membrane \( R_1 \), and the resistance of the cytoplasm or cell interior \( R_2 \).

How can the observed values of the parallel resistance and capacitance in the standard arm, \( R_p \) and \( C_p \), be manipulated to reveal the values of the membrane resistance, the membrane capacitance, and the resistance of the cell interior? A convenient starting-point for the mathematical theory of the conversion is Maxwell's equation for the resistance of a random suspension of spherically packed homogeneous spheres (Maxwell, 1873):

\[
\frac{1 - r_1/r}{2 + r_1/r} = \frac{1 - r_1/r}{2 + r_1/r} = \frac{1 - r_1/r}{2 + r_1/r},
\]

where \( r \) = specific resistance of the whole suspension, \( r_1 \) = specific resistance of the suspending medium, \( r_2 \) = specific resistance of the suspended spheres, and \( \rho \) = the volume concentration of the spheres. If the volume of the spheres is \( x \) and that of the medium is \( y \), \( \rho = x/x + y \).

The biological material is placed in an electrolytic cell in the unknown arm of the a.c. bridge. This electrolytic cell has a cell constant \( k \). The specific resistance of the medium in this cell, \( r_1 \), is equal to the observed resistance, \( R_x \), + \( k \). In the following pages lower-case letters represent specific values, and upper-case letters observed values, with one exception which will be discussed in due course.

Maxwell's equation was derived for a suspension of spheres.* We are concerned here with a single sphere. Before proceeding to the development of the equations for biological analysis, it is therefore necessary to establish that the relationship holds good for a single sphere, and under what conditions. Experiments have been done with this end in view. The sphere selected was a glass marble, which may be considered to be homogeneous and as having an infinite resistance \( r_2 \); the suspending medium \( r_1 \) was tap water.

If \( r_2 = \infty \), equation (12) becomes

\[
\frac{1 - r_1/r}{2 + r_1/r} = \frac{1 - r_1/\infty}{2 + r_1/\infty},
\]

* A similar expression can be derived for a suspension of cylinders. In this case equation (12) becomes

\[
\frac{1 - r_1/r}{1 + r_1/r} = \frac{1 - r_1/r}{1 + r_1/r}.
\]
Solving this equation for $\rho$,

$$\rho = 2 \frac{1 - r_1/r}{2 + r_1/r}.$$  \hspace{1cm} (14)

In experiments with a marble, $\rho$ can be calculated when $r_1$ and $r$ have been evaluated. (These are measured at one particular frequency only. As no capacitances are involved, they remain approximately constant over a wide frequency range.) $\rho$ can also be calculated by direct observation, as the volume of the marble can be measured and the volume of tap water put into the cell is known. Comparison of the observed and calculated values of $\rho$ is a convenient method of finding out if Maxwell's equation holds good for single spheres and, if so, up to what volume concentrations.

Some results of measurements and calculations of this sort are shown in Table 1. The electrolytic cell used was cubical and made of paraffin wax. Two opposite sides were completely covered by platinized platinum plates, the electrodes. The top of the cell was covered with a piece of ground glass, the whole being filled with tap water. At the frequencies employed, no electrical conduction takes place through the glass or paraffin wax. Therefore the operative volume of the electrolytic cell is the volume bounded by the platinum electrodes. Four sizes of marbles or glass balls were used.

It is clear from this table that Maxwell's equation holds good up to about 5% for single spheres as well as for suspensions of spheres, and up to high volume concentrations, at any rate when $r_2 = \infty$. The highest volume concentration which is possible, i.e. when the inserted sphere touches all sides of the electrolytic cell internally, is 0.523, which may be compared with Exp. 1 in the above table.

Solving Maxwell's equation (12) for $r$, the specific resistance of the suspension,

$$r = r_1 \frac{r_2 (1 - \rho) + \bar{r}_2 (2 + \rho)}{r_1 (1 + 2\rho) + 2\bar{r}_2 (1 - \rho)}.$$  \hspace{1cm} (15)

This equation assumes that the sphere is homogeneous. A trout egg is not. Nor, for that matter, are any other biological cells. A trout egg is a biphase sphere whose internal resistance is low compared with that of the cell membrane and where a capacitance is present, situated mainly in the cell membrane. In these circumstances we must substitute for $\bar{r}_2$,

$$z_2 = r_2 + z_3/a,$$  \hspace{1cm} (16)
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where $z_2$ = complex impedance of the cell (instead of the resistance $r_2$), $z_3$ = complex impedance of a unit area of cell membrane, $a$ = radius of the cell in cm., and $r_2$ = specific resistance of the cell interior. The substitution of $z_2 = r_2 + z_3/a$ for $r_2$ in Maxwell’s original equation depends on the following assumptions (Cole, 1928):

(a) that the thickness of the cell membrane is so small that higher powers than the first can be neglected;
(b) that the ratio $r_2/r_4$ is small compared with unity and can be neglected;
(c) that current flow in the surface layer of the cell is radial;
(d) that the potential gradient inside the cell is uniform.

In (b) above, $r_4$ is the specific resistance of the cell membrane: that is, the resistance of a centimetre cube of the membrane. It is measured in ohm-cm. When, however, $r_4$ occurs in other parts of this paper, or in others dealing with similar subjects, it refers to the resistance of a square centimetre of membrane and is therefore expressed in ohm-cm$^2$. Capacitances are, however, expressed in $\mu$F-cm$^2$. These differences are intrinsically somewhat confusing, and not made less so by occasional printed references to membrane resistances in ohm/cm$^2$ which are wrong. Furthermore, different authors use different conventions as regards the meanings of upper- and lower-case letters. At present it seems impossible to resolve these differences.

The trout egg differs from many cells in that the protoplasmic or ‘living’ part of it, the vitelline membrane, is restricted to a thin shell only a few microns thick, surrounding a yolky globulin-containing fluid whose electrical conductivity is said to be equivalent to that of a $0.125 M$ solution of NaCl (Gray, 1932).

If it is assumed that the vitelline membrane has no resistance in parallel with its capacitance, i.e. that it is non-conducting,

$$z_3 = \frac{1}{j\omega c_3},$$

(17)

where $c_3$ = capacitance per unit area of cell membrane. If $c_3$ has a resistance in parallel with it,

$$z_3 = \frac{r_4}{1 + j\omega c_3 r_4},$$

(18)

where $r_4$ = resistance of a unit area of cell membrane. Calculations are much simplified by assuming that the cell membrane is non-conducting, though this assumption would not be justified in a more refined analysis. If $r_2 + z_3/a$ is substituted for $r_2$ in equation (15), the resistance $r$ of the suspension becomes the complex impedance $z$. Then

$$z = \frac{r_2(1 - \rho) + (2 + \rho)(r_2 + z_3/a)}{r_2(1 + 2\rho) + 2(1 - \rho)(r_2 + z_3/a)},$$

(19)

where $z = r + jx$, $r$ = the resistive part of the total impedance, and $x$ = the reactive part of the total impedance. The resolution of $z$ into the components $r$ and $jx$ is discussed in greater detail on p. 85.
Solving equation (19) for $r_2 + z_3/a$ and substituting $r+jx$ for $z$,

$$r_2 + z_3/a = r_1 \frac{(1-\rho)-r(1+2\rho)-jx(1+2\rho)}{2r(1-\rho)-r_1(2+\rho)+2jx(1-\rho)},$$

(20)

As it is assumed that the membrane resistance $r_4$ is infinite, $z_3/a = 1/j\omega c_3$. Substituting this in equation (20) and separating into real and imaginary parts:

$$r_2 = r_1 \frac{mn-qx(1+2\rho)}{n^2+q^2},$$

(21)

and

$$c_3 = \frac{1}{2\omega r_2^2} \frac{n^2+q^2}{n(1+2\rho)+2m(1-\rho)},$$

(21.1)

where $m = r_1(1-\rho)-r(1+2\rho)$, $n = 2r(1-\rho)-r_1(2+\rho)$, and $q = 2x(1-\rho)$.

Equation (21.1) can be simplified to

$$c_3 = \frac{1}{2\omega r_2^2} \frac{n^2+q^2}{9\rho}.$$  

(21.2)

In equations (21) all quantities are observable and the observed values can be converted to specific values where necessary by dividing the observed value by the electrolytic cell constant.* $r_1$ can be observed in a blank run, i.e. with no cell present, while $\rho$ can be measured directly. $r$ and $x$ are the equivalent series resistance and reactance of the whole suspension, and can be calculated from the measured values of the parallel resistance and capacitance in the a.c. bridge standard arm by means of equations (6) and (6.1).

There is a simpler method of calculating the specific resistance of the cell interior than by equation (21) which does not involve certain difficulties inherent in this equation. At infinite frequency the capacitative part of the cell membrane has zero impedance. In these circumstances a.c. measurements are only concerned with the internal resistance $r_2$, as the spherical cell becomes a homogeneous sphere of specific resistance $r_2$. Solving equation (12), for $r_2$ at infinite frequency,

$$r_2 = r_1 \frac{(1-\rho)-r_\infty(1+2\rho)}{2r_\infty(1-\rho)-r_1(2+\rho)},$$

(22)

where $r_\infty =$ specific resistance of the suspension at $\nu = \infty$.

It is not always easy, and sometimes even impossible, to measure $\rho$. But at zero frequency $\rho$ can be expressed in terms of observable quantities. Returning to equation (12),

$$\frac{1-r_1/\tau}{2+r_1/\tau} = \rho \frac{1-r_1/\bar{r}_2}{2+r_1/\bar{r}_2}.$$  

At zero frequency this equation becomes

$$\frac{1-r_1/\tau_0}{2+r_1/\tau_0} = \rho \frac{1-r_1/\infty}{2+r_1/\infty},$$

(23)

* $r_2 = R_d/k$. Therefore $R_d/k$ is substituted for $r_1$ in each case. Equation (21.2) must be multiplied by a factor to correct for $c_3$ being of the 'polarization' type. This is discussed in detail later.
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where \( r_0 \) = specific resistance of the suspension at \( \nu = 0 \). \( r_2 = \infty \) because \( x_3 = \frac{1}{\omega \cdot c_3} = \infty \).

Solving equation (23) for \( \rho \),

\[
\rho = \frac{2 \cdot \frac{1 - r_1/r_0}{2 + r_1/r_0}}
\]

(24)

\( \rho \) is now in terms of observable quantities as \( r_0 \) can be measured by a method similar to \( r_\infty \).

Any electrical network consisting of any combination of resistances and condensers can be expressed at a particular frequency in terms of a single resistance and a single condenser in series. The impedance of the latter may be expressed in the form

\[
Z = R_s - jX_s,
\]

(25)

where \( X_s = 1/\omega C_s \) (see footnote on p. 79), \( R_s \) is the resistance and \( X_s \) the capacitative reactance of the network. Multiplying by \( j \) implies multiplication by \( \sqrt{-1} \); it also involves the rotation of a line through 90°. In a.c. measurements the unknown is balanced by a resistance and condenser in parallel. At any particular frequency, the electrical network or system in the unknown arm is equivalent to a resistance and condenser in series, and therefore can be expressed in the form

\[
Z = R_s - jX_s.
\]

If the observed standard arm parallel resistance and condenser values are converted to the form \( R_s - jX_s \) and the values of \( R_s \) so obtained are plotted along the \( x \)-axis and the values of \( X_s \) along the \( -jx \) (\( x \) rotated through 90°) or \(-y\)-axis, the points so obtained describe a semicircle with the centre of the circle on the \( x \)- or resistance-axis (see Fig. 4). Each co-ordinate \( R_s, -jX_s \) is the terminal of the impedance vector at that frequency. Therefore if any network consisting of any combination of resistances and one condenser\( \dagger \) is expressed in the equivalent form \( R_s - jX_s \), the locus of the terminals of the impedance vectors at various frequencies is a semicircle with its centre on the resistance axis.

With certain reservations discussed later, a trout egg in tap water is a combination of resistances and a capacitance. If the values of \( R_p \) and \( C_p \) which balance it at various frequencies are expressed in the equivalent form \( R_s - jX_s \), and the values so obtained plotted in the way described above, the points at the ends of the semicircle, where it cuts the \( R_s \)-axis, will be \( R_\infty \) and \( R_0 \), \( R_\infty \) being the one nearer the origin. \( R_0 \) and \( R_\infty \) are obtained by extrapolation.

In certain physical systems, in the trout egg, and in many other biological cells, the \( R_s - jX_s \) plot does not give a semicircle with its centre on the resistance axis, but an arc of a circle, cut by the resistance axis, with its centre above it (see Fig. 5). The half-angle formed by the radii from \( R_0 \) and \( R_\infty \) to the centre of the circle is the phase angle between current and voltage of the capacitative element in the network.

\* The standard arm could equally well contain a resistance and a condenser in series. The advantage of the parallel arrangement is that with it, one terminal of the resistance and of the condenser can be at earth potential, thus eliminating hand capacitances, etc.

\( \dagger \) If the network contains more than one condenser, this analysis is incorrect.
Fig. 4. The unknown arm of the a.c. bridge contains the network shown in Fig. 2. This is balanced at various frequencies $A$, $B$, $C$, $D$ and $E$ by a resistance $R_p$ and a condenser $C_p$ in parallel in the standard arm. The values of $R_p$ and $C_p$ so obtained are converted into the equivalent form $R_s - jX_s$, and the values of $R_s$ and $jX_s$ are plotted on the $x$- and $y$-axis respectively. The values at $A$, $B$, $C$, $D$ and $E$ are the terminals of the impedance vectors, $OA$, $OB$, etc. The magnitude of the impedance vector $OC$ is $\sqrt{(R_s^2 + X_s^2)}$, which is the modulus of $R_s - jX_s$. The centre of the semicircle lies on the resistance axis at $C'$. As the angle $OC'R_0$ is $180^\circ$, the phase angle between current and voltage across the terminals of the ideal condenser in the unknown arm is $90^\circ$. $R_0$ and $R_\infty$ are obtained by extrapolating the semicircle until it cuts the resistance axis (dotted lines).

Fig. 5. $R_s - jX_s$ plot for a network containing one polarization capacitance. Note centre of semicircle above the resistance axis.
in the unknown arm. Alternatively, the angle formed by the tangent to the semi-circle at \( R_0 \) or \( R_\infty \) and the resistance axis is the phase angle. Suppose that there is an ordinary electric network consisting of a condenser and various resistances in the unknown arm and \( R_s - jX_s \) is plotted in the usual way at various frequencies. The centre of the resultant semicircle will be on the resistance axis; the half-angle subtended by the radii from \( R_0 \) and \( R_\infty \) at the centre is \( 90^\circ \), which is the phase difference between current and voltage at all frequencies across the terminals of an ideal condenser.* (In an ideal condenser, \( \phi = K = \frac{1}{2} \pi \).) By means of the \( R_s - jX_s \) plot and from the position of the centre of the resulting circle, certain information is extracted about the capacitative element in any network consisting of any combination of resistances and a capacitative element. That information is either (1) that the capacitative element is an ordinary condenser where \( \phi = K = \frac{1}{2} \pi \), (2) that the capacitative element is not an ordinary condenser but is one of a special type in which the phase angle is constant at all frequencies but is less than \( \frac{1}{2} \pi \), or (3) that the capacitative element is an ordinary condenser but that it is in some way indistinguishably associated with a resistance whose value varies in such a way that the phase angle of the associated condenser and resistance remains constant at all frequencies and less than \( \frac{1}{2} \pi \). Alternatively, both the condenser and resistance could vary with frequency, but a variation in capacitance alone will not produce the effect. This capacitative system, where \( \phi = K = < \frac{1}{2} \pi \), has been called a variable impedance element, a dielectric impedance element, or a polarization capacitance. No very satisfactory physico-chemical model has so far been devised to reproduce the behaviour of variable impedance elements with constant phase angles.

If the cell membrane is permeable to ions, one cannot assume that the resistance \( r_4 \), shunting its capacitance, is infinitely high. The evaluation of the membrane resistance in terms of observable quantities involves measurements at zero and infinite frequency, and measurements of the volume concentration. At zero frequency, the membrane impedance for a square centimetre of membrane, \( z_0 \), becomes a pure resistance \( r_4 \), because at zero frequency, the impedance of the membrane capacitance is infinite. In these circumstances, Maxwell's equation (equation (12)) becomes

\[
\frac{1-r_4/r_0}{2+r_4/r_0} = \rho \frac{1-r_1/(r_4 + r_4/a)}{2 + r_1/(r_4 + r_4/a)},
\]

(26)

where \( r_4 \) = membrane resistance of a square centimetre of membrane. The other parameters in this equation have their usual significance. Solving this equation for \( r_4 \),

\[
r_4 = a \left[ \frac{r_2^2 (1 - \rho) - r_0 r_1 (1 + 2 \rho)}{2r_0 (1 - \rho) - r_1 (2 + \rho)} - r_2 \right],
\]

(27)

or in observable quantities

\[
r_4 = \frac{a}{\bar{k}} \left[ \frac{R_2^2 (1 - \rho) - R_0 R_1 (1 + 2 \rho)}{2R_0 (1 - \rho) - R_1 (2 + \rho)} - R_2 \right].
\]

(27.1)

* In a capacitative circuit where \( E = E_0 \sin \omega t \), the charge \( Q \) on the condenser is \( CE = CE_0 \sin \omega t \); \( I = dQ/dt \); therefore \( I = \omega CE_0 \cos \omega t = I_0 \sin (\omega t + \frac{1}{2} \pi) \). The current leads the voltage by \( 90^\circ \).
From a consideration of equation (22), equation (27·1) can clearly be written

\[ r_4 = \frac{a}{k} \left( \frac{\lambda R_1^2 - \mu R_0 R_1}{2 \lambda R_0 - \sigma R_1} - \frac{\lambda R_1^2 - \mu R_{oo} R_1}{2 \lambda R_{oo} - \mu R_1} \right), \]  

(27·2)

where \( \lambda = 1 - \rho \), \( \mu = 1 + 2\rho \), and \( \sigma = 2 + \rho \).

Cole (1937) has given a simpler expression for \( r_4 \), based on the assumptions that \( r_4 \gg r_1 a \) and \( r_4 \gg r_2 a \). The expression is

\[ \frac{\rho - \bar{\rho}}{\rho} = \frac{3r_1 a}{r_4}, \]

(28)

where \( \bar{\rho} \) = volume concentration if \( r_4 = \infty \). \( \bar{\rho} \) is sometimes known as the 'non-conducting volume concentration', and from equation (24),

\[ \bar{\rho} = 2(1 - r_1/r_0)/(2 + r_1/r_0). \]

Substituting this value in equation (28) and solving for \( r_4 \),

\[ r_4 = \frac{3r_1 a \rho (2r_0 + r_1)}{\rho (2r_0 + r_1) - 2(r_0 - r_1)}. \]

(29)

Experimental conditions may, however, be such as to make the assumptions unwarrantable, in which case equations (27·1) and (27·2) must be used for determining \( r_4 \).

Membrane resistances are so high that their measurement presents considerable difficulties. Cells such as Nitella, which are normally in fresh water, may have membrane resistances of 250,000 ohm-cm\(^2\) or more (Blinks, 1930), while the longitudinal resistance of the squid giant axon, after being immersed in sea water, is about 1000 ohm-cm\(^2\) (Cole & Hodgkin, 1939). Cole pointed out (1937) that if the resistance of Hipponoe egg-cell membranes is as high as that of Nitella, the difference between \( \rho \) and \( \bar{\rho} \) in equation (28) would be about 0·002%, which means that extremely accurate measurements of volume concentration are necessary to distinguish between a cell membrane which is assumed to have an infinite d.c. resistance and one which has a finite but very high d.c. resistance.

When such very high resistances are involved, a small error in measurement may lead to highly improbable results, such as the resistance of an egg in tap water being higher than the resistance of a glass sphere, of equal volume, in the same tap water. A convenient way of checking if measurements are of the right order and in the right sense is by comparing \( \rho \) measured, and \( \bar{\rho} \) calculated on the assumption that \( r_4 = \infty \), in which case \( \bar{\rho} = 2(1 - r_1/r_0)/(2 + r_1/r_0) \). \( \rho \) can be measured volumetrically or by indirect methods described by Cole (1937). The larger \( r_4 \) is, the nearer \( \rho \) approaches \( \bar{\rho} \). The value of \( \rho \) is bounded below by the value of \( \bar{\rho} \). This means that unless the cell is actively extracting ions from the surrounding medium, a somewhat improbable situation, \( \rho \) will always be greater than \( \bar{\rho} \), though, as mentioned above, the difference may be very small if \( r_4 \) is large. It automatically follows that unless the membrane resistance is so great as to be experimentally indistinguishable from a membrane with an infinite d.c. resistance, the measured volume concentration
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should always be greater than the volume concentration calculated on the assumption that $r_4 = \infty$.

If the membrane resistance is finite, the previous equations for the capacitance of the membrane, in which it was assumed that $r_4 = \infty$, need modification. Instead of equation (20) being solved for $z_3 = \frac{1}{j\omega c_3}$, it must be solved for $z_3 = \frac{r_4}{1 + j\omega c_3 r_4}$.

By simple algebraic rearrangement the capacitance per unit area is found to be

$$c_3 = \frac{-r_4 \pm \sqrt{(r_4^2 - 4aI^2)}}{2a\omega r_4 I},$$

where $r_4$ has the value given in equation (27.1) and

$$I = \frac{g r_1^2 \cos}{[2r(1 - \rho) - r_1(2 + \rho)]^2 + [2x(1 - \rho)]^2}.$$

![Diagram](image)

Fig. 6. a, spherical cell between electrodes. b, equivalent electrical circuit assuming $r_4 = \infty$. $R_1$, resistance of tap water; $R_2$, resistance of cell interior; $R_3$, resistance of medium in path of current flow between electrodes and cell surface (this is small enough to be ignored); $z_3$, polarization capacitance of cell surface.

This more refined method of analysis has not yet been attempted with the trout egg, though it is proposed to do it in the future.

In previous sections $c_3$ has been calculated from the original Maxwell equation. The cell or cells have not been considered in terms of equivalent electrical circuits and the relationships which have been derived hold good for any frequency. But it is sometimes convenient to use a different method of deriving $c_3$. This method is a combination of a pure electrostatic and an equivalent electrical circuit method. A spherical cell in its electrolytic cell is shown diagrammatically in Fig. 6a. The various cell parameters have already been defined. $z_3$ is the impedance of the cell membrane. It is a polarization impedance or capacitance. $r_4$ is assumed to be
infinite. Fig. 6b shows an electrical network which is equivalent to Fig. 6a. By rearranging equation (19) it can be shown to be

\[ Z_a = \frac{2 + \rho}{2(1 - \rho)} \frac{\frac{1 - \rho}{2 + \rho} r_1 + r_2 + z_3/a}{\frac{1 + 2 \rho}{2(1 - \rho)} r_1 + r_2 + z_3/a}. \] (31)

The impedance of system b is

\[ Z_b = R_1 + \frac{R_2 + Z_3}{R_1 + R_2 + Z_3}. \] (32)

As b is equivalent to a,

\[ Z_a = Z_b. \] (33)

At \( \nu = 0 \), \( z_3 = \infty \) and \( Z_a = \infty \). Therefore

\[ R_1 = \frac{2 + \rho}{2(1 - \rho)} r_1. \] (34)

At \( \nu = \infty \), \( z_3 = 0 \) and \( Z_a = 0 \). Therefore

\[ \frac{R_2}{R_1 + R_2} = \frac{\frac{1 - \rho}{2 + \rho} r_1 + r_2}{\frac{1 + 2 \rho}{2(1 - \rho)} r_1 + r_2}. \] (35)

Equating the numerators and denominators of each side of this equation

\[ R_2 = \gamma \left[ \frac{\frac{1 - \rho}{2 + \rho} r_1 + r_2}{\frac{1 + 2 \rho}{2(1 - \rho)} r_1 + r_2} \right], \] and

\[ R_1 + R_2 = \gamma \left[ \frac{\frac{1 + 2 \rho}{2(1 - \rho)} r_1 + r_2}{\frac{1 - \rho}{2 + \rho} r_1 + r_2} \right], \] (36)

where \( \gamma = \frac{2(2 + \rho)^2}{9\rho} \). By algebraical rearrangement it can be shown that

\[ Z_3 = \frac{\gamma z_3 a}{a}. \] (37)

Therefore

\[ z_3 = \frac{9\rho}{(2 + \rho)^2} z_3 a. \] (38)

If \( r_4 \) is assumed to be infinitely great, then, as before,

\[ \rho = 2 \frac{1 - r_1/r_0}{2 + r_1/r_0}, \] therefore

\[ \gamma = \frac{2}{(2 + r_1/r_0)(1 - r_1/r_0)} \] (39)

and

\[ z_3 = \frac{2}{(2 + r_1/r_0)(1 - r_1/r_0)} Z_3 a. \] (40)

As

\[ Z_b = R_1 + \frac{R_2 + Z_3}{R_1 + R_2 + Z_3} \text{ (equation (32))}, \]

\[ Z_3 = (R_1 + R_2) \frac{Z_0 - R_1 R_2}{R_1 - Z_0}. \] (41)
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At \( v = 0 \), \( Z_b \) becomes \( R_0 \) and \( Z_3 \) becomes infinity. Substituting these values in equation (32)

\[
R_0 = R_1. \tag{42}
\]

At \( v = \infty \), \( Z_b \) becomes \( R_\infty \) and \( Z_3 \) becomes zero. Therefore

\[
R_\infty = \frac{R_1 R_2}{R_1 + R_2}. \tag{43}
\]

Solving equation (43) for \( R_2 \) and substituting \( R_0 \) for \( R_1 \),

\[
R_2 = \frac{R_0 R_\infty}{R_0 - R_\infty}. \tag{44}
\]

Substituting this value of \( R_2 \), and \( R_0 \) for \( R_1 \) in equation (41),

\[
Z_3 = \frac{R_0^2}{R_0 - R_\infty} \frac{Z_b - R_\infty}{R_0 - Z_b}; \tag{45}
\]

At any particular frequency, the electrical network \( b \) is equivalent to \( R_s - jX_s \). \( R_s - jX_s \) is plotted in Fig. 7. In this diagram

\[
u = Z_b - R_\infty \quad \text{and} \quad \nu = R_0 - Z_b. \tag{46}
\]

The subtractions are not scalar but vectorial. Returning to equation (45),

\[
Z_3 = \frac{R_0^2}{R_0 - R_\infty} u; \tag{47}
\]

At a particular frequency, \( X_s \) is a minimum and \( u = -j\nu.\) This is the ‘characteristic frequency’ \( \omega_c. \) At \( v = \omega_c/2\pi \),

\[
\bar{Z}_3 = -j \frac{R_0^2}{R_0 - R_\infty}, \tag{48}
\]

where \( \bar{Z}_3 = Z_3 \) at \( \omega_c. \) Substituting equation (48) in equation (40),

\[
\bar{Z}_3 = -j \frac{2 + r_1/r_0}{2(r_0 - r_\infty)}; \tag{49}
\]

where \( \bar{Z}_3 = Z_3 \) at \( \omega_c. \)

As \( \bar{Z}_3 = 1/j\\omega_c \bar{Z}_3 \),

\[
\bar{Z}_3 = \frac{2(r_0 - r_\infty)}{a\omega_c^2 (2 + r_1/r_0) (1 - r_1/r_0)}; \tag{49.1}
\]

Equation (49.1) assumes that the membrane capacitance is ‘pure’, i.e. that it acts like a good wireless condenser. If, however, the capacitance is of the polarization type, equation (49) must be modified. At the characteristic frequency \( \omega_c \), equation (45) can be written

\[
Z_3 = \frac{R_0^2}{R_0 - R_\infty} e^{-j\phi}; \tag{50}
\]

\[
= \frac{R_0^2}{R_0 - R_\infty} (\cos \phi - j \sin \phi). \tag{50.1}
\]

* This is only true when the \( R_s - jX_s \) plot is a semicircle. The more general case, when the plot is an arc of a circle, as in Fig. 7, is discussed below.
The capacitative part of this expression is

\[ \frac{R_0^2}{R_0 - R_\infty} \sin \phi, \]  

(50.2)

and the polarization capacitance will therefore be \( \frac{1}{\sin \phi} \) of the 'pure' capacitance value. Equation (49.1) will become

\[ \tilde{\varepsilon}_3 = \frac{1}{a \omega r_0^2 (z + r_1/r_0)(1 - r_1/r_0) \sin \phi} \frac{2(r_0 - r_\infty)}{3} \]  

(51)

When the capacitance is 'pure', \( \phi = 90^\circ \), \( \sin \phi = 1 \), and equation (51) is identical with equation (49.1).

The characteristic frequency \( \omega \) of cells or suspensions of cells varies over wide limits according to the type of cell and the electrical properties of the surrounding medium. In those cases where the capacitance is of the polarization type, the value at \( \omega \) may not be as suitable for comparing different cells as the value at a standard frequency, for example, 1 kcyc.
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Equation (51) can be converted to the capacitance at the standard frequency by means of an equation due to Cole:

\[ c_{31} = \frac{1}{a \omega_1} \frac{2(r_0 - r_\infty)}{(2 + r_1/r_0) (1 - r_1/r_0) r_0^2 \sin \phi} \left( \frac{\omega_1}{\omega} \right)^2, \]

where \( c_{31} \) = capacitance per unit area of membrane at 1 kcyc., \( \omega_1 = 2\pi \cdot 1000 \), and \( \alpha = 2\phi/\pi \).

THE TROUT EGG

The trout egg is well suited to a.c. measurements. As tap water, with its relatively high resistance, is the external medium, high frequencies are not necessary to pass current through the egg surface; moreover, the egg is large, being about 5 mm. in diameter. This might give the impression that measurements of egg volumes are easy to make, but unfortunately this is not the case as will be seen later in the section entitled Sources of Error.

MATERIAL

The eggs of *Salmo irrideus* Gibbons and *Salmo fario* were used. Experiments were started about 3 hr. after the eggs had been placed in tap water. To obtain fertilized eggs, a ripe female was stripped into a bowl, and sperm, which had previously been tested for activity after dilution with water, were added to the dry eggs. Tap water was then added. A high percentage of fertilized eggs is usually obtained by this method.

METHOD

Measurements were made with an a.c. bridge which has been fully described elsewhere (Hubbard & Rothschild, 1939). The electrolytic cells were approximately cubical and made of paraffin wax, two opposite faces being platinized platinum electrodes. A glass plate was placed on top of the cell after the egg and tap water had been inserted. There was no air between the glass plate and the surface of the tap water. The dimensions of the electrolytic cells were about 7.5 mm. (length) \times 7.2 mm. (width) \times 7.2 mm. (height), giving a volume concentration between 0.1 and 0.2 and a cell constant of 1.08. Larger electrolytic cells, with correspondingly lower volume concentrations, were occasionally used, but as the sensitivity of this method of analysis is directly proportional to the volume concentration, the dimensions of the cell given above represent a compromise between sensitivity and the inapplicability of Maxwell's equation at high volume concentrations.

A run involves the following measurements and calculations:

1. Determination of the cell constant \( k \). This need not be done for each run, as it depends entirely on the geometrical characteristics of the cell.

2. Blank run with tap water. This is to determine the specific resistance of the tap water, \( r_1 \), the resistance of the electrodes, and the capacitance of the electrodes. The values of the parallel resistance \( R'_p \), and of the parallel capacitance \( C'_p \),

* Private communication.
necessary to balance the electrolytic cell filled with tap water, are determined at frequencies \( \nu = 0.1 - 50 \) kcyc.

(3) Egg run. This determines the values of the parallel resistance \( \bar{R}_p \), and of the parallel capacitance \( \bar{C}_p \), necessary to balance the electrolytic cell when it contains an egg and tap water, at frequencies \( \nu = 0.1 - 50 \) kcyc.

(4) Volumetric determination of the apparent egg volume.

(5) Calculation of the resistance \( R_p \), and capacitance \( C_p \), due to the egg in tap water, and not including the resistance and capacitance of the electrodes, at each frequency. This is done by the relationships (Cole & Cole, 1936):

\[
\bar{R}_p = R_p, \quad \bar{C}_p = C_p = \left( \frac{R_p^2}{\bar{R}_p} \right) C_p', \quad R_1 = R_p' \text{ at } 50 \text{ kcyc. (53)}
\]

(6) Calculation of the series resistance \( R_s \) and series reactance \( X_s \), equivalent at each frequency to \( R_p \) and \( C_p \), by means of the relationships

\[
R_s = R_p/(1 + \omega^2 C_p^2 R_p^2), \quad X_s = \omega C_p R_p^2/(1 + \omega^2 C_p^2 R_p^2).
\]

(7) Construction of circle diagram or \( R_s - jX_s \) plot, and determination of \( R_0 \), \( R_\infty \), the characteristic frequency \( \bar{\nu} \), and the phase angle \( \phi \).

(8) Calculation of \( c_{31} \), the capacitance per unit area at 1 kcyc., by means of the equations

\[
c_{31} = \frac{1}{aw_1 r_1^2 x} \frac{[2r'(1 - \rho) - r_1(2 + \rho)]^2 + [2x(1 - \rho)]^2}{9 \rho \sin \phi}, \quad (A)
\]

\[
c_{31} = \frac{1}{aw_1 (2 + r_1/r_0)(1 - {r_1/r_0}) r_0^2 \sin \phi} \left( \frac{\omega_1}{\omega} \right)^x \quad (B)
\]

In all calculations in this paper, the d.c. resistance of the cell membrane is assumed to be infinite; according to some calculations of Cole & Guttmann (1942), Holzer (1933) obtained a value of 5000 ohm-cm\(^2\) for the cell membrane.

A characteristic egg circle diagram is shown in Fig. 8. The phase angle between current and voltage across the membrane capacity is 82.1°. The characteristic
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frequency, at which $X_s$ is a minimum, is 0.905 kcyc. The terminals of the impedance vectors at each frequency fall accurately on an arc of a circle with centre below the $R_s$ axis, showing that the membrane capacitance is of the polarization type.

The $R_s - jX_s$ plot for a dead egg does not give a semicircular arc because a dead egg has no capacitance and no significant resistance. The chorion is permeable to water and ions while little or nothing remains of the vitelline membrane. This fact casts some doubt on Holzer's results (referred to above) on the membrane resistance, as to measure it he cut the egg in half. This kills the egg and destroys the resistive properties of the cell membrane. In Table 2 certain egg characteristics or parameters have been calculated or measured by various alternative methods.

These experiments were originally done to see if fertilization caused any change in the cell surface, as evidenced by changes in its resistance or capacitance. Similar
experiments on other eggs have not indicated that fertilization is systematically associated with changes in capacitance, while, except in the frog's egg (Cole & Guttman, 1942), the membrane resistance of eggs seems to be almost too high to be measured. Table 3 gives the results of experiments on a batch of unfertilized and fertilized eggs. This batch was selected out of a number of other batches because of better temperature conditions. Measurements were made alternately on fertilized and unfertilized eggs to reduce the effect of variation in temperature. It will be seen that there is no significant change in capacitance after fertilization.

**DISCUSSION**

The main sources of error in the experiments described in this paper are:

1. **Volume concentration.** A small error in the measurement of egg volume, and subsequent calculation of the actual egg volume causes a relatively large error in the value of a cell parameter. Suppose for example that the egg volume is 70 mm., and the electrolytic cell volume is 0.5 c.c.; therefore \( \rho = 0.14 \). Let the cell constant \( k \), \( R_1 \) and \( R_\infty \), respectively have the values 1.5, 2400 and 1700. Substituting these values in equation (22)

\[
\rho = \frac{R_1 R_1(1-\rho) - R_\infty(1+2\rho)}{k2R_\infty(1-\rho) - R_1(2+\rho)}, \quad r_2 = 81 \text{ ohm-cm.}
\]

If an error of 2% is made in egg volume, \( r_2 \) becomes 93 ohm-cm., an error of 15%.

Although superficially the measurement of the trout-egg volume presents no difficulties, this is not in fact the case. The protoplasmic membrane is surrounded by the chorion which according to Bogucki (1930) is about 80 \( \mu \) thick. Within this chorion there is a perivitelline space in which the egg proper can rotate according to its centre of gravity. The thickness of the perivitelline space is difficult to measure in living eggs and virtually impossible to measure in dead eggs. As the chorion is freely permeable to small molecules and ions, the perivitelline space must normally have the same composition as the external medium. Measurements on dead eggs show that the resistance and capacitance of the chorion is negligible if one assumes, as is usually the case, that the chorion is an 'inanimate' membrane and does not change when the egg dies. Neither the chorion nor the perivitelline space should therefore contribute to the egg volume proper, and their volumes must be subtracted from that of the total egg volume. A 100 \( \mu \) shell must therefore be subtracted from the total, or apparent egg volume, but for obvious reasons a small error in shell width will cause a large error in shell volume. Equation (26) in the theoretical part of this paper shows that a small error in egg volume also has a large effect on the value of the membrane resistance.

2. **\( R_0 \).** The resistance of the egg and its surrounding medium at zero frequency depends on an extrapolation. The terminals of the low-frequency impedance vectors may show deviations from the circular arc locus which is theoretically required. These deviations are probably due to electrode polarization, although a correction is included in the calculations for this effect. The value of \( R_0 \) could be checked by substituting non-polarizable silver-silver chloride electrodes for the platinized
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platinum ones and by using d.c. instead of a.c. A.c. at zero frequency is of course
d.c. A silver-silver chloride electrode requires a significant Cl\(^{-}\) concentration in the
outside medium to be non-polarizable, which should therefore be changed to a
solution such as a mixture of NaCl and CaCl\(_2\). Although this experiment should be
done, it has not been found practicable to do it this year.

(3) Temperature. Conductivity measurements are well known to be very
sensitive to temperature changes. Thermostatting presented considerable dif-
ficulties in these experiments and was not attempted, though the room temperature
was maintained as constant as possible at 17 ± 1° C. The frequency of the periodic
impedance changes that occur in fertilized and unfertilized trout eggs after about
8 hr. in tap water is, however, very sensitive to temperature changes, and quanti-
tative experiments on them would require rigorous temperature control.

(4) Geometrical and chemical heterogeneity of the egg. The trout egg vitelline
membrane is not a perfect spherical shell as it is thickened at the top to form the
blastodisc. Furthermore, oily globules are distributed throughout the vitelline
membrane.

(5) Ratio of egg volume to electrolytic cell volume. Table 1 on p. 82 shows that with
glass spheres, Maxwell’s equation (12) is inaccurate at high volume concentrations,
as Maxwell indicated on theoretical grounds. At low volume concentrations, how-
ever, other errors creep in; a compromise is therefore necessary.

Comparisons between fertilized and unfertilized eggs would be more satisfactory
if the capacitance of one egg could be measured before and after fertilization. This is
impossible, as the act of measuring the capacitance of an unfertilized egg precludes
the possibility of subsequent fertilization. Before measurements begin, the egg must
have been in tap water for about 2 hr., to get osmotic equilibrium between the egg
and its environment. But after being in tap water for 5 min., the chorion of the egg
hardens, the micropyle, through which the spermatozoon fertilizes the egg, is
occluded, and the egg is unfertilizable.

The capacitance of the trout-egg membrane is similar to that of other cell mem-
branes. The fact that living cell membranes always seem to have a capacitance of
about 1 \(\mu\) F.cm\(^{-2}\) strongly suggests that there is some structural similarity between
such widely different membranes as those of the Squid axon, 1·1 \(\mu\) F.cm\(^{-2}\) (Cole &
Curtis, 1938), unfertilized Arbacia eggs, 0·73 \(\mu\) F.cm\(^{-2}\) (Cole & Cole, 1936), red blood
cells, 0·81 \(\mu\) F.cm\(^{-2}\) (Fricke & Curtis, 1934), and the trout egg. At present, all that can
be said from the chemical point of view about cell membranes is that they are probably
protein-lipoid complexes. Given a value for the capacitance, the membrane di-
electric constant can be calculated if the thickness of the membrane is known; or,
the thickness of the membrane can be calculated if the dielectric constant is known.
As mentioned earlier in this paper, the dielectric constants of polar media depend on
the polarizability of the molecules in the alternating electric field. We do not know
what dipole moments the molecules in the cell membrane have, and therefore to
make a guess at the membrane dielectric constant, as has sometimes been done, may
result in serious errors, particularly as the tendency for non-conductors to have low
dielectric constants might be counteracted by the high dielectric constants that such
compounds as glycine are known to have. Similar arguments apply with regard to
the membrane resistance, when it can be measured. If the chemical composition,
and therefore the specific resistance of the membrane, were known, its thickness
could be calculated. If its thickness were known, its specific resistance could be
calculated. In the case of the membrane resistance there are other difficulties. The
membrane has holes in it which seem to permit K+ ions to pass through more easily
than Na+ ions in the case of nerve, though this difference is less marked in the case
of the trout-egg membrane (Pumphrey, 1931). What effect this heterogeneity may
have on the general resistivity, or even the dielectric constant of the membrane, is
difficult to estimate.

As will be seen from Table 2, there is a discrepancy between the resistance of the
inside of the trout egg when measured by a.c. and when measured by Gray’s more
direct conductivity method. It is by no means impossible that squeezing the con-
tents of the egg out and then measuring the conductivity might destroy or break
down some colloidal structure, which would result in an increased conductivity,
though some rough experiments done by Gray’s method confirmed the values re-
ported in this paper. It is very difficult to make accurate measurements of the real
volume of the egg, which are required for calculation of the internal conductivity
by a.c. If this difficulty is considered insurmountable, it is necessary to fall back on
the assumption that the membrane has an infinite d.c. resistance. At the same time,
the discrepancy would be greater if the cell membrane had a significant conductance.
The resolution of this difficulty involves removing the chorion without damaging
the vitelline membrane, an exceedingly delicate operation in newly laid eggs. The
average resistance of the egg interior was 206 ohm-cm. (32 eggs), though low values
were occasionally noted. There is not sufficient evidence to say whether the
difference between the internal resistance of fertilized and unfertilized eggs is
significant or not.

No physico-chemical explanation of a polarization capacitance has yet been found.
A number of complicated suggestions have been made and are summarized by Cole
& Cole (1941), but none are entirely successful in explaining this phenomenon,
which also occurs in non-living material. Until it is better understood in relatively
simple non-living systems, there is little point in speculating about its meaning in
highly complex systems such as cell membranes.

**SUMMARY**

1. The theory of alternating current measurements as applied to biological
systems is discussed and the equations for determining the resistance and capaci-
tance of the cell membrane, and the resistance of the cell interior are deduced.

2. Maxwell’s equation for the specific resistance of a random suspension of
spherically packed homogeneous spheres is involved in these equations, and its
applicability to single glass spheres of various diameters is established.

3. This method of analysis is applied to the egg of the trout, to examine the
capacitance of its cell membrane before and after fertilization. If the membrane
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resistance is assumed to be infinite, the capacitance of the cell membrane, scaled to a standard frequency of 1 kcy., is approximately 0.57 μF.cm⁻² in unfertilized eggs and 0.58 μF.cm⁻² in fertilized eggs. The difference is not statistically significant.

4. The capacitative element of the cell membrane has a constant phase angle of about 83° in fertilized and unfertilized eggs, indicating dielectric loss in the membrane.

5. The characteristic frequency of fertilized and unfertilized eggs was between 0.8 and 0.9 kcy.

6. The internal resistance of the egg varied in different batches but in general was higher than that found by Gray (90 ohm-cm.). The average value for 32 eggs was 206 ohm-cm.

7. The experiments described in this paper are neither connected with, nor affected by, the periodic impedance changes which occur at a somewhat later stage in fertilized and unfertilized trout eggs.

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