If the same standard state is assumed for the solution in both phases $S$ would be unity, but it is more convenient to assume different standard states so that as long as the properties of the two phases remain constant $S$ will be constant but as a rule its value will not be unity.

The partition coefficient includes only the ions $K^+$ and $Cl^-$. It would not be constant if it included the undissociated $KCl$ in $X$ (but this undissociated part would not affect the diffusion potential), cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 26, 293 (1942-1943).

If sufficient $K^+$ is present in the external solution there is no recovery since the accumulative forces do not produce a sufficiently great outwardly directed activity gradient of $K^+$.

I.e., cations tend to flow across the protoplasm from the sap to the external solution.

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SOME PHYSICAL ASPECTS OF BIOELECTRIC PHENOMENA

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The structure and activity of living systems present many problems of physics, chemistry and mathematics and the electrical aspects of these problems have been investigated almost continuously for the past century and a half. As increasingly powerful concepts, techniques and instruments have been developed they have been applied to the study of these phenomena and, as a result, we now have available a vast store of information on the bioelectric phenomena of organisms, tissues and cells—both plant and animal. Much of this information has been correlated into the general principles of electrophysiology and, in some cases, in surprisingly successful quantitative form. But the further description and interpretation of the various bioelectric phenomena in terms of physical and chemical concepts has been, in general, less successful. Although it has been almost impossible to design and execute experiments on living materials which disclose their physical characteristics in simple and direct form, some of these characteristics have been measured and found to be strikingly similar to phenomena of non-living systems. This is highly gratifying to the physical scientist and very useful to the biologist except for the several important instances in which the behavior of these non-living systems has itself not been satisfactorily explained. Thus, as is found for other biological phenomena, the physical aspects of the electrical phenomena may present problems of experiment and theory which are not confined to the living systems alone but extend into the physics and chemistry of non-living liquid and solid materials.

This contribution to the Conference on Bioelectric Potentials presents
some of the simpler electrical characteristics of living cell membranes which have come from the application of physical principles and techniques to the measurement and analysis of the electrical properties of cells and tissues. These membrane characteristics may serve the Conference both as background information and as examples of the many unsolved physical problems of biological systems.

Although the experimental methods used in these investigations of the electrical characteristics of the cell membranes cannot be discussed in detail they should not be ignored completely. The design of the experiments and the equipment and the analysis of the results have usually been rather straightforward applications of conventional techniques such as those of potential theory, circuit analysis, electrochemistry, and communication and electronic engineering. But as often as not the conditions imposed by living cells have required that the necessary techniques be pushed beyond those achieved in current practice and in some cases the equipment has had to reach to the natural limits of sensitivity and resolution.

Membrane of the Cell.—It is well established not only that the interior of the living cell is very different from the external inanimate environment in composition, structure and electric potential, but also that these differences are maintained by a barrier at the surface which is necessary for the life of the cell. Although this barrier may not be positively identified under the microscope—as for example a part of the plasma membrane—it is definitely recognized by other characteristics—such as a rather small permeability to water and to many solutes. The structure which constitutes the barrier to the free flows of ions in and out of the cell is most probably the seat of the immediate source of electrical energy and the origin of the principal bioelectric effects. This structure is our primary concern and for convenience we shall refer to it as the membrane of the cell.

Capacity of the Membrane.—The cell membrane was thought of electrically as a "leaky condenser" before 1900 and by 1925 the capacity of this condenser had been measured in the red blood cell\(^1\) and found to be about one microfarad per square centimeter. Since that time the membrane capacity of an adequate number of cell types has been measured\(^2\) with sufficient accuracy to justify the hypothesis that all living cells have a membrane capacity and that it is of the order of one microfarad per square centimeter. On the assumptions of a macroscopic behavior and a dielectric constant of three, the membrane has a thickness of 30 A. This thickness is clearly of molecular dimensions and it suggests the need for more detailed information and analysis on thin films. A further complication is that only a few of these membranes exhibit a pure static capacity, it being more usual to find a behavior which resembles the dielectric loss of engineering insulators.

The alternating-current characteristics of the membrane capacity are
conveniently considered in two parts—\( C' \), the conservative or "real" component and \( C'' \) the dissipative or loss, component—which are combined as the complex capacity,

\[
C^* = C' - iC''
\]

where \( i = \sqrt{-1} \). The membrane capacities are adequately expressed empirically by

\[
C^* = \overline{C} (i\omega)^{-\alpha}
\]

where \( \overline{C} \) is constant, \( \omega/2\pi \) is the frequency and \( \alpha \) is a loss index, \( 0 \leq \alpha \leq 1 \), which is related to the phase angle \( \phi = \alpha\pi/2 \). These results can be expressed in terms of a resistance and a capacity, both of which vary with frequency, or, as shown in figure 1, in terms of \( C' \) and \( C'' \).

This behavior of cell membranes is similar to the polarization of electrode systems such as platinized platinum, silver–silver chloride and calomel, and this similarity has led to the attractive suggestion that this membrane capacity also is a phenomenon of ion transport. Although it may not be possible either to accept or reject the hypothesis without a better understanding of the electrode systems, several factors make it seem rather improbable:

(a) This analytical description of the membrane and electrodes is not unique to them but can be applied with equal success to the electrical properties of dielectrics and a barrier layer photovoltaic cell and to the mechanical characteristics of some viscoelastic materials.

(b) The capacities of the electrode systems are usually one or more orders of magnitude larger than those of the cell membranes.

(c) Although the electrode characteristics depend to a considerable de-

![Figure 1](image-url)
gree upon the method of manufacture and the composition and concentration of the electrolyte, the membrane characteristics seem quite stable and remain relatively unchanged as the cell becomes active, is injured or is placed in a chemically modified medium.

(d) The anomalous reactance, to be mentioned later, has some characteristics which are more closely analogous to those found in ion transport processes than have these membrane capacities of one microfarad per square centimeter.

The membrane capacity may—as has been indicated—be dielectric in origin. For a dilute solution of dipoles the complex dielectric factor $\epsilon^*$ is given by

$$\epsilon^* = \epsilon - i\epsilon = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/(1 + i\omega\tau),$$

(2)

where $\epsilon_0$ and $\epsilon_\infty$ are the dielectric constants at zero and infinite frequencies, respectively, and $\tau$ is the relaxation time. An equivalent circuit is readily interpreted with one capacity for $\epsilon_\infty$, the deformation polarization, another capacity for $\epsilon_0 - \epsilon_\infty$, the rotation polarization, and a resistance for $\tau/(\epsilon_0 - \epsilon_\infty)$, the viscous dissipation in rotation. For concentrated solutions of dipoles and polar liquids and solids one often finds

$$\epsilon^* = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/[1 + (i\omega\tau)^{1-\alpha}].$$

(3)

In the equivalent circuit the resistance is now replaced by an impedance $z^* = z(i\omega)^{-\alpha}$. If now $\epsilon_\infty << \epsilon' < \epsilon_0$ and $\alpha$ is near unity the element $z^*$ is the only one of importance and equation (3) approaches the membrane characteristic of equation (1) and figure 1. Then, since $\epsilon_\infty$ has a minimum value of unity, the real part of the dielectric factor $\epsilon'$, for the membrane must be considerably larger than unity. Furthermore, since the static dielectric constant, $\epsilon_0$, in turn is much larger than $\epsilon'$ it must be at least ten and may be hundreds. In case the static dielectric constant is as low as ten, the membrane thickness then becomes about 100 A.

In the case of dilute solutions and those liquids and solids which follow equation (2) the dipole rotation is opposed by only a pure viscous resistance and $\alpha = 0$. But as the concentration of a solution such as beta lactoglobulin is increased, or the temperature of a liquid such as glycerin or a solid such as ice is decreased, the value of $\alpha$ increases. This suggests a gradual replacement of the viscous opposition to rotation by intermolecular forces of an elastic nature which becomes almost complete for some solid polymers—and for the Arbacia egg membrane. The index $\alpha$ and the intermolecular forces may be expressed as a rather special distribution of relaxation times which varies from a single component for $\alpha = 0$ to an increasingly broad and flat distribution as $\alpha$ approaches unity. If then the cell membrane capacity is of a dielectric nature and this interpretation of liquid and solid dielectrics is correct, the cell membranes are to be thought of as rather
solid structures in which the energy of thermal agitation is small or negligible in comparison with the intermolecular potential energy.

**Conductance of the Membrane.—**If equation (1) is a complete and correct description of its electrical characteristics, the cell membrane has no direct-current conductance and consequently is completely impermeable to ions. And indeed it has not been possible to demonstrate a conductance of the membrane with any certainty in the large number of experiments which have been done with small cells of irregular or poorly defined shapes and in media of rather high conductivity. As a result, serious consideration has been given to the possibility that materials can only be transported across the membrane as neutral molecules. However, by the use of long cells such as *Nitella* and various nerve fibers,13–16 a large cell such as frog egg in pond water16 or an internal electrode as in *Valonia* it has been possible to measure resistances ranging from 100 ohm-cm.2 to 100,000 ohm-cm.2 for the membranes of a number of cells in a resting state. Since membrane resistances as high as these could not have been detected in the other experiments it is possible to assume that all cell membranes have a similar conductance and ion permeability.18

When a cell becomes active,19 is narcotized,20 is subjected to changes in the composition of the external medium,21 is injured or dying or is polarized by current flow22 this membrane conductance changes. The conductance change correlates closely with the physiological state of the cell and may amount to several orders of magnitude while the change of the capacity, if any, is usually not more than 10 or 20%19. On this basis one may picture the capacity as characteristic of an ion-impermeable, inert structure composing usually more than 90% of the effective membrane area with the functional ion permeable aspect relegated to the small remaining area of the membrane.

On the assumption that the membrane has a thickness of 100 A its specific resistance is about $10^{10}$ ohms-cm or about $10^8$ times that of protoplasm and the normal environments of many cells. Although this high resistance may be a result of either a very low concentration or a very low mobility of the ions in the membrane, it is more probably that both of these factors are involved to a considerable extent.

It is to be expected that the resting potential and the conductance of the membrane are both functions of the ion permeability and so should be closely related. At the present time there are only a few cells in which the resting potential has been measured in the absence of current flow and practically none in which the resting potential and the membrane conductance have been measured simultaneously.

The indications in the squid axon21 are that the membrane resistance decreases for all increases of external potassium concentration, although the membrane potential is but slightly altered until the external potassium is
about three times its normal value. Changes of the external calcium seem to be almost without effect on the potential but the resistance becomes very high for a tenfold increase and practically vanishes as the external calcium is reduced toward zero. A more extensive use of internal electrode techniques should produce more satisfactory and complete evidence as to the nature of the relation between these two characteristics of the cell membrane.

Rectification of the Membrane.—The current flow through a membrane is proportional to the change of the potential difference for small changes—a few millivolts for the squid axon—and the ratio of current to potential change is quite properly expressed as the conductance discussed above. But several lines of evidence have made it highly probable that the relationship is not so simple for larger changes of potentials as has been verified by direct measurements on nerve fibers\textsuperscript{14, 22} and plant cells.\textsuperscript{23} As the potential difference was decreased more than a few millivolts the outward current increased more rapidly than it did for small changes, while for the large increases of the potential difference the inward current increased more slowly than for the small changes of potential. This highly non-linear relationship between the current and the potential difference is of the type which characterizes a rectifier. In the squid axon the ratio of the maximum conductance for outward flow to the minimum conductance for inward flow is about a hundred to one.\textsuperscript{22} If this effect is ascribed to an ion, such as potassium, which has a higher concentration inside than out, the concentration of this ion should be higher for an outward than for an inward current flow at every point in the membrane. As a result the outward conductance should be higher than the inward which is in qualitative agreement with the experiments.

The problem may be approached quantitatively by means of the equation for the ion concentration, $n$, under the influence of diffusion and electric forces,

$$\frac{\partial n}{\partial t} = - \frac{\partial}{\partial x} \left( n e X - kT \frac{\partial n}{\partial x} \right), \quad (4)$$

where $\rho$ is the friction coefficient and $X$ is the electric field. This equation has been applied to the barrier layer rectifier and solved on the assumption of a negligible space charge. Although this assumption does not appear to be justified in the present problem and a more rigorous solution is not available, it has been possible to obtain reasonably good agreement with some of the experimental results on the rectification characteristics and the membrane potential of the squid axon.\textsuperscript{24}

Anomalous Reactance of the Membrane.—When definitive data were taken on the membrane of squid axon at frequencies below 1 kc. another physical characteristic made its appearance.\textsuperscript{25} This is a reactance in addition to the
membrane capacity of one microfarad per square centimeter which, unlike the latter, is closely correlated with physiological and chemical factors and may be either capacitative or inductive. The present indications are that it is normally present in the squid axon membrane and somewhat capacitative although it is rather variable. A cursory inspection of the data on other biological systems suggests rather strongly that this anomalous reactance is not a unique feature of the squid axon membrane although indications of an inductive reactance have not been found except in nerve fibers.

The reactance of the squid axon membrane becomes highly capacitative with excess calcium in the external medium and highly inductive as the normal calcium is removed from the medium. An excess of external potassium up to five times the normal value gives rise to an inductive reactance which then disappears for still higher concentrations. The investigations of the effect of membrane current flow on this anomalous reactance have not been satisfactory but the preliminary results indicate that the reactance tends to become inductive for outward current flow and capacitative for an inward flow.

The most attractive explanation of this reactance is that it is a perfectly general consequence of the non-linear characteristic of the membrane. Let us assume that the mechanism which is responsible for the non-linearity of a system cannot respond to instantaneous or sufficiently fast variations. In electrical terms we have \( e = r_\infty i \), where \( e \) and \( i \) are small rapid variations of the potential difference and the current, respectively, and \( r_\infty \) is the instantaneous variational resistance. We will now assume that this instantaneous response is modified by the non-linear mechanism to approach the steady-state response, \( e = r_0 i \) at a rate \(-\beta (e - r_0 i)\), where \( r_0 \) is the steady state of variational resistance and \( \beta \) is the rate constant of the mechanism. The phenomenon is then described by the equation

\[
\frac{de}{dt} = r_\infty \frac{di}{dt} - \beta (e - r_0 i).
\]

The transient solution for a constant current \( i_0 \) applied at \( t = 0 \) is

\[
e(t) = [r_0 - (r_0 - r_\infty) e^{-\beta t}] i_0
\]

and the steady-state impedance is

\[
z(i\omega) = r_\infty + \frac{r_0 - r_0}{1 + i\omega \tau},
\]

where \( \tau = 1/\beta \). According as \( r_0 - r_\infty \) is greater or less than zero both the transient and the steady-state solutions are represented by equivalent circuits containing resistances and a single capacity or inductance, respectively. The values of the reactive elements for one circuit are

\[
C = \frac{\tau}{(r_0 - r_\infty)} \quad \text{and} \quad L = (r_\infty - r_0)\tau.
\]
This formal consideration indicates that an anomalous reactance is to be expected in a non-linear system, but for an interpretation of the velocity constant it is necessary to make a detailed analysis of the mechanism involved. Although either a chemical reaction or a transport process may be primarily responsible for this velocity constant we shall use only the simple potassium diffusion model already discussed as an illustration.

In this model, the distribution of ions at equilibrium is such that the outward diffusion is prevented by the inwardly directed electrical field. At the instant when the field is changed by a sudden increase or decrease of the potential difference across the membrane the initial current flow is determined by the ion distribution which was present before the potential was changed. But as time goes on the ion distribution and the current change to approach the steady-state values required by the alteration of the potential. For an increase of potential, the current decreases from its initial value to a lower steady-state value which corresponds to the presence of an equivalent capacity in the membrane. For a decrease of potential, the current rises to a higher value indicating an equivalent inductance. The details of these processes have been calculated from equation (4) and it is found, as can be seen by normalizing the equation, that the order of magnitude of velocity constant is given by

$$\beta = \frac{X^2e^2}{\rho kT}.$$  

The available data indicate that $\beta$ is of the order $100 \text{ sec.}^{-1}$ for the squid axon. A crude combination of this value and the conductance data leads to an ionic concentration in the membrane $n = 5.10^{17}$ ions/cc. or about 0.001 $N$ and a friction coefficient $\rho = 3.10^{-4}$ dyne sec./cm. which is larger than the value for the potassium ion in water by a factor of about $10^6$.

**Conclusion.**—Although this discussion has been limited to a few of the simpler physical aspects of bioelectric phenomena, it is apparent that no one of the membrane properties mentioned has as yet been adequately explained in terms of physical or chemical principles and that the description of the structure and function of the living cell membrane in elementary terms is still far from complete.

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THE SOURCE OF THE BIOELECTRIC POTENTIALS IN LARGE PLANT CELLS

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The use of large plant cells for the study of bioelectric potentials was originated by Professor Osterhout, and received much of its advance in his laboratory. The advantages of such multinucleate cells are several: they occur either singly, or easily separable from their neighbors with a minimum of dissection or injury, and they survive well in the laboratory, often for days or even weeks with a fine glass tube making connection with the cell sap. The large and measurable surface allows expression of resistance and capacity in definite terms; the capacity being often as high as several microfarads in a single cell, and resistances as high as one megohm (100,000 ohms per square centimeter of surface). New solutions may be quickly applied over the whole surface, or at definitely separated areas for the study of concentration effects, etc. The cell sap may be analyzed for its constituents, or obtained in sufficient quantity to apply to the exterior (see below); in two genera it may be replaced within the vacuole by perfusion with sea water or other new solutions. Through these means the