DIELECTRIC CONSTANTS OF BIOLOGICAL OBJECTS

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HEIGHTENED attention has been paid to the application of physics to biological problems in recent years. Along this line, it is of interest to discuss the existing information on the electrical properties of living matter. The reactions of nerves and muscles to electrical influences have been studied intensively for a long time. There is also a vast amount of material on measurements of biocurrents and biopotentials. All of these are not problems of physics, but of physiology. Here, only the methods of applying influences and studying them are taken from physics, while the studied processes themselves involve the regulatory systems of the organism, which are a fundamental object for physiology. A new stage in the application of physics and chemistry to vital phenomena involves, in particular, the development of molecular biology, an approach having the goal of studying living matter, rather than the complex organism with its regulatory systems.

Studies in molecular biology widely use optical methods and electron and nuclear magnetic resonance, but make relatively little use of the electrical properties. This is due to the complexity of the electrical characteristics of biological objects, which often makes the interpretation of the results of electrical measurements ambiguous and dubious. Such an unsatisfactory state of the problem makes a further elucidation especially important and desirable.

This review is concerned basically with those electrical properties of living matter which do not involve the reaction of the regulatory systems of the organism. The most important of these is the dispersion of the dielectric constant.

1. THE DIELECTRIC CONSTANT AND CONDUCTIVITY

Living matter is a colloidal medium permeated with a physiological solution. Such a medium has a direct-current ionic conductivity. The value of the direct-current conductivity depends both on the concentration of the electrolytes and on the mobility of the ions, and is related to the biologically-important property known as the cell conductivity. On the other hand, when electric fields are applied to living matter, phenomena of internal polarization can take place, i.e., the displacement of charges, generating a spatially-distributed dipole moment. All these charge-displacement phenomena can be described in terms of the dielectric constant of the living matter. Here, a phase shift can take place between the applied field and the internal polarization, and thus the dielectric constant will generally turn out to be a complex quantity. The phase shift always involves dispersion, and in the simplest cases results from relaxation phenomena. The mechanism of internal polarization can vary: the charge displacement can occur either within a molecule, or by rotation of a polar molecule, or finally, within structural elements consisting of a large number of molecules (macrostructural polarization). In some cases the polarization involves the molecular structure, and in other cases, the macroscopic structure of the substance. However, in living matter the macroscopic structure is no less essential than the molecular structure. Hence, in speaking of the dielectric properties of living matter, we must include in this concept with equal claim both molecular and macrostructural polarization. This is all the more unavoidable, inasmuch as it is exceedingly difficult to distinguish experimentally the two kinds of phenomena, and this problem has not yet been solved for a number of the most important cases. Macrostructural polarization is commonly ascribed to the biological structure of the cells or the intercellular elements (e.g., the mitochondria) and their coverings (membranes). However, we cannot consider the possibility excluded of finding macrostructural effects in the study of biopolymers having colloidal, rather than biological structures. Lyophilic colloids tend to form gels, e.g., gelatin or agar. Such a gel is a porous medium containing an electrolyte solution. The displacement of the ions within the pores can lead to macrostructural polarization. Even molecular solutions of biopolymers can show, in addition to the molecular polarization, a polarization of the ionic atmospheres surrounding the polymer molecules, owing to their polyelectrolyte properties.[10] Such a polyelectrolyte polarization resembles macrostructural polarization in mechanism. We can completely eliminate all macrostructural effects only by complete removal of moisture by intensive drying in vacuo (so-called lyophilization).

Polarization involving a phase shift entails losses. These losses are superimposed on the ohmic losses due to the direct-current conductivity. In order to characterize completely the dependence of the properties of the medium on the frequency ω, it is convenient to introduce the complex conductivity

$$\Lambda = \sigma + i\frac{\omega}{4\pi}\varepsilon,$$

where σ is the direct-current ohmic conductivity, and ϵ is the complex dielectric constant. The real part of this quantity describes all the forms of losses.

Both the direct-current conductivity σ and the complex dielectric constant ϵ, in general, show dispersion.

Hence, the complex conductivity Λ can show a certain dispersion region. The reciprocal of the central dispersion frequency is generally called the relaxation time τ, even when it does not essentially involve relaxation phenomena.

2. THE LOW-FREQUENCY INCREMENT, THE HIGH-FREQUENCY DECREMENT, AND THE VIABILITY COEFFICIENT

As a rule, the dielectric constants of biopolymer solutions are higher than that of pure water at low frequencies, but lower at high frequencies. The increase in the low-frequency dielectric constant is called the increment, while the decrease at high frequencies is called the decrement (Fig. 1). Quantitatively, the increment or the decrement is given for unit concentration. The low-frequency increment can be explained by the orientation of the polar groups in the molecules of the biopolymer, which is manifested only at low frequencies, owing to the low mobility. The hypothesis has been advanced[48] that this increment may also involve a shift to lower frequencies in the dispersion region of the water itself, owing to the ordering action of the biopolymer molecules on the surrounding water, making its properties more like those of ice. We shall discuss this hypothesis of the ordering of the water structure further below. The high-frequency decrement is observed in the frequency range in which the internal rotations of the biopolymer molecules can no longer keep up with the external field, i.e., in practice, in the centimeter-wave region. In this region, the dielectric constant of the solution is determined by the rotation of water molecules. The biopolymer molecules bind a certain number of the surrounding water molecules, and prevent these molecules from rotating freely. Consequently, the polarizability and the dielectric constant of the solution are reduced. For these reasons, measurements of the high-frequency decrement can be used to determine the degree of hydration of biopolymer molecules, as has been done in [67,68]. In objects having biological structure, the low-frequency increment can be even many times larger than in solutions, owing to the macrosturctural polarization. When the biological structure is destroyed, the macrostructural polarization vanishes, and the difference between the low- and high-frequency dielectric constants is reduced. As a very crude measure of the presence of biological structure, several studies have used the dielectric-constant ratio between frequencies of the order of 10^4 to 10^6 cps. This ratio is sometimes called the "viability coefficient" or the "polarization coefficient." In the presence of biological structure, the viability coefficient can attain very large values, owing to the macrostructural polarization. When the biological structure has been completely destroyed, it falls to values of the order of unity.

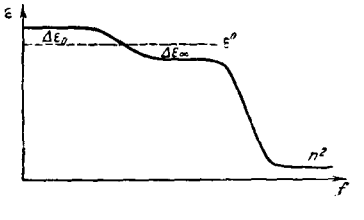

FIG. 1. The frequency-dependence of the dielectric constant of a solution of a biopolymer in water. ϵ^0 is the dielectric constant of water; n is the refractive index of the solution; $\Delta\epsilon_0$ is the low-frequency increment; and $\Delta\epsilon_\infty$ is the high-frequency decrement.

3. PHYSICAL MECHANISMS

All of the theories which have been developed in the literature on the nature of polarization phenomena in living matter can be reduced to the following five basic physical mechanisms:

1. Dipole orientation.
2. Macrostructural polarization.
3. Ordering of the water structure.
4. Polarization of the ionic atmosphere of polyelectrolytes.
5. Delocalization of electrons.

The orientation of molecular dipoles is the classical mechanism explaining the high dielectric constants of polar substances.[22] The relaxation time τ is determined here by rotational friction. Dispersion is observed near a frequency equal to the reciprocal of the relaxation time. Here, the real component of the complex dielectric constant falls from the high value ϵ_0 characteristic of dipole orientation to the low value ϵ_∞, which depends only on the internal polarizability of the molecules. For pure water, the dispersion region lies in the centimeter-wave region. The value of ϵ_0 is about 80, while $\epsilon_\infty \approx 3$. In polymers containing polar groups, we usually observe two dispersion regions involving two relaxation mechanisms.[72] The determining mechanism for the high-frequency dispersion is the relaxation of dipole groups, i.e., the rotation of individual dipole groups about the bonds linking them to the polymer chain. The relaxation mechanism for the low-frequency dispersion is the rotation of entire segments of the polymer molecule. This mechanism is termed dipole-elastic relaxation, since the same relaxation time determines also the mechanical properties of the polymers. The rotational relaxation times decrease with increasing temperature. This temperature-dependence is especially marked in dipole-elastic relaxation. Owing to the temperature-dependence of the relaxation time, when one makes measurements in a certain narrow frequency range, one observes the high-frequency dispersion (dipole-group relaxation) only at low temperatures, and the low-frequency (dipole-elastic) relaxation only at high temperatures. This has given rise to the widespread opinion that dipole-group relaxation occurs only in glassy states, and dipole-elastic relaxation only in rubbery states of a polymer. As the working range of frequen-

cies has been extended, it has become possible to observe both dispersion regions at a constant temperature.[72] The most important biopolymers, the proteins and nucleic acids, have large numbers of polar groups, and we naturally expect them to show dipole polarization. In the first studies of the electrical properties of biological objects, all of the observed dispersion phenomena were explained by the dipole mechanism. Later, it was noted that macrostructural polarization plays an important role, as we have mentioned above and shall discuss in more detail below. For objects having biological structure, the problem of distinguishing the dipole and the macrostructural mechanisms has not yet been solved at present, either from the experimental or the theoretical standpoint. Solutions of biopolymers should manifest dipole orientation in a purer form, but here specific mechanisms may be superimposed, which we shall discuss next.

The hypothesis has been advanced for a number of reasons that water occurs in an ordered state in the organism.[71,50,18] It is assumed that the biopolymer molecules induce long-range order in the surrounding water, bringing it into a certain quasicrystalline state approaching ice in properties. The application of this hypothesis of ordered water has been suggested to explain the electrical properties of biopolymer solutions. Here the low-frequency increment must be explained by assuming that the static dielectric constant of water, upon ordering, approaches the higher static dielectric constant of ice. The dispersion region for ice lies at much lower frequencies than for water. The cited studies considered the low-frequency dispersion observed in solutions of biopolymers to be the dispersion of the ordered water, which approached the dispersion region of ice. These arguments cannot be considered convincing, and have been subjected to a well-grounded criticism in [15]. A recent study has indicated another possible polarization mechanism is solutions of biopolymers, involving their polyelectrolyte properties.[10] A biopolymer molecule in solution gives off ions, and is converted into a polyelectrolyte ion bearing charges. Each ion attracts from the surrounding solution ions of the opposite charge, which form an ionic atmosphere about it. In the case of a polyelectrolyte, we can ascribe a surface conductivity to the ionic atmosphere. The action of the external field polarizes the ionic atmosphere. This polarization supplements that of the biopolymer itself, and contributes to the dielectric constant of the system. According to [15], the electrical properties of a polyelectrolyte can be described by the theory of macrostructural polarization; in a crude approximation, we can replace the biopolymer molecules by spheres of radius a, having a complex conductivity Λ_i, while the surface conductivity λ is taken into account by adding a correction term λ/a to Λ_i.

All of the discussed polarization mechanisms have involved dipole molecules or ions. Recently, the problem has been discussed in the literature[70,71] of the existence in living matter of semiconductor phenomena or the like. In such a case, the polarization could arise from displacement of electrons (or holes). It is not necessary for the material to be a true semiconductor for this to occur. It is sufficient that delocalization of electrons should take place. The simplest example of such a delocalization is given by conjugated bonds, i.e., the alternation of single and double bonds between carbon atoms in a chain or ring. The delocalization of electrons in systems containing conjugated bonds is manifested experimentally by the narrowing of the electron paramagnetic resonance (EPR) lines. This effect is also observed in biopolymers.[59,62] These data indicate that we can expect biopolymers to show semiconductor properties. Quantum-chemical calculations of the width of the forbidden band have been made[73] for proteins, and it was concluded thereby that semiconductor phenomena could appear. The direct-current experiments with proteins indicate a conductivity increasing exponentially with the temperature[74,75] and the moisture content.[76,85] This conductivity has been interpreted as a semiconductor phenomenon. We must note, however, that there are as yet no direct proofs of the electron or hole character of this conductivity for proteins (such as a Hall effect or photoconductivity). Thus, we cannot consider the existence of semiconductor phenomena in proteins to be rigorously proved, although a number of data favor their existence. Chlorophyll[82] and other photosynthetic pigments[69] show photoconductivity. The hypothesis has been advanced very recently[83] that a certain type of delocalization of electrons involving formation of charge-transfer complexes (CTC) can play a role in the properties of biopolymers. This type of complex denotes a molecular compound consisting of molecules having considerably differing electron affinities. Here, the formation of the molecular complex can involve an internal ionization, with transfer of an electron from one molecule to the other. Analogous phenomena can also be expected in a polymer chain in which two groups with differing electron affinities occur in sequence. All electron-delocalization phenomena can contribute to the polarizability and the dielectric constant, but it is difficult to detect them experimentally, since they are masked by the very strong ionic and dipole effects.

4. ELECTRICAL PROPERTIES AND CELL PERMEABILITY

One of the most important problems of the physiology of cells is that of the permeability. As is known, dissolved substances penetrate a living cell from the external medium slowly. After the cell dies, the permeability increases sharply. Such objects as muscle and nerve cells are known to show the phenomenon of excitation, which is accompanied by a certain increase

in the permeability. One might say that excitation is analogous to death; however, the increase in permeability in excitation is weaker and is reversible. The higher organisms, which possess regulatory systems, exhibit phenomena of active transport, in which diffusion occurs against a concentration gradient. Examples are the sodium pump which pumps sodium out of nerve cells, secretory phenomena in glands, and the high oxygen pressure in the swim bladder of deep-sea fishes.

There are two opposite viewpoints on the mechanism of cell permeability. The classical membrane theory holds that the cell is surrounded by a continuous membrane hindering diffusion. The increase in permeability upon death of the cell is explained, according to this theory, by the destruction (lysis) of the membrane. The membrane theory is the prevailing one in the Western literature. In this country, the studies of the school of D. N. Nasonov, V. Ya. Aleksandrov, and A. S. Troshin [8,12] have developed a contradictory viewpoint, which has been called the phase theory. According to this theory, the cell envelope is perforated by submicroscopic pores, and is not a poorly-permeable membrane. Morphological studies [77] have undoubtedly established the existence in the cell of numerous layered structures, including the external membrane as well as such important intracellular organelles as the mitochondria and the chloroplasts. The layered structures in most cases consist of alternating films of proteins and lipid (fat-like) substances. As expressed by one of the proponents of the phase theory, [7] "the lipid-protein structure, which is, as it were, the external skeleton of the cell separating the protoplasmic phase of protein dipoles from the intercellular liquid, plays an incomparably simpler role in cell physiology than the hypothetical cell membrane." According to the phase theory, the cell permeability is determined by the colloidal properties of the protoplasm. The protoplasm is a two-phase system consisting of colloidal micelles and intermicellar liquid. Dissolved substances are adsorbed from the liquid phase on the exposed surface of the colloidal particles; this explains the decreased permeability of the living cell. The increase in permeability upon death of the cell is explained by the phase theory by the irreversible coagulation of the colloidal systems of the cell. Analogous, but reversible, phenomena occur during excitation. According to this theory, the dielectric constant must involve molecular, rather than macrostructural polarization. The phase theory of cell permeability is commonly associated with the dipole theory of the dielectric constant, as we can see from the references cited above.

A final solution of the problem of the mechanism of cell permeability must be based on an analysis of a vast amount of experimental material, the interpretation of which is a highly difficult problem. As yet, the problem remains open to discussion. The study of biopotentials does not give an unambiguous answer. [78] The study of the electrical properties of cells is of great interest from this standpoint, since the membrane theory of the permeability corresponds to the theory of macrostructural polarization, while the phase theory of the permeability corresponds to a molecular mechanism of polarization, or, as is commonly considered, to the dipole theory. Unfortunately, the macrostructural and dipole theories give identical forms of dispersion formulas, so that the dispersion curve gives no clues whatsoever concerning the mechanism. The proponents of the membrane theory consider an important argument in its favor to be the agreement between the values of the membrane thickness, as calculated from the static dielectric constant and from the relaxation time. [32] The proponents of the phase theory use for their argument the temperature-dependence of the conductivity. The electrical conductivity of blood was studied [7] at 10^4 and 10^6 cps and at 25° and 56° C. It turned out that the low-frequency conductivity increases rapidly with temperature, while the high-frequency conductivity shows a temperature-dependence which becomes weaker as the concentration of erythrocytes increases. The ratio of the low-frequency to the high-frequency conductivity (the viability coefficient) falls to unity only at 75°C. Within the range from 25° to 56°C, the permeability of the membranes increases sharply, while the dielectric properties remain the same. This author considered that these results favor the phase theory of permeability.

The currently-existing information on the electrical properties of cells is clearly insufficient for a well-grounded solution of the problem of the mechanism of cell permeability. Nevertheless, the opinion is becoming more widespread in the literature that it is precisely electric measurements which can provide the most reliable data to solve this problem. However, for this we need a deeper and more detailed investigation of the complex conductivity of cells as a function of the various parameters, and also an elucidation of the internal mechanism of polarization.

5. THE ELECTRICAL PROPERTIES OF BIOPOLYMERS

Among the studies concerned with the electrical properties of isolated and purified biopolymers, we shall take up here only two groups having a certain definite theoretical approach. One group of studies has been performed on solutions, and the other on pressed tablets. The problem of study in the one case was the determination of the degree of hydration and the form of protein molecules in solution from the high-frequency decrement, while in the other case it was a search for anomalies in the electric properties of nucleic acids in connection with their special biological role.

a) The high-frequency decrement and the properties of protein molecules

Harris, Buchanan, et al[67,68] used measurements of the high-frequency decrement in protein solutions to determine the shapes and degrees of hydration of protein molecules in solution. The dielectric constant of the solutions was measured in the centimeter-wave region. The results were handled by use of the formula

$$\varepsilon - \varepsilon_w = \frac{\beta p}{1-p}(\varepsilon_p - \varepsilon_w),$$

where ϵ is the dielectric constant of the solution, ϵ_w is that of pure water, ϵ_p that of pure protein, p is the volume fraction of the protein in the solution, and β is a coefficient near $\frac{3}{2}$, the exact value depending on the shape of the protein molecule. Under the assumption that the molecule is an ellipsoid of revolution, the values of this coefficient were calculated as a function of the axial ratio. For dilute solutions, this formula can be written approximately as

$$\varepsilon \approx \varepsilon_w - \beta p(\varepsilon_w - \varepsilon_p) = \varepsilon_w - \delta C,$$

where C is the weight concentration of the protein in grams per 100 cm^3 of solution. The decrement δ is expressed as

$$\delta = \frac{\beta}{100}[(\varepsilon_p - \varepsilon_{\infty p})v + (\varepsilon_w - \varepsilon_{\infty w})w],$$

where v is the partial specific volume of the protein, and w is the weight of water bound per gram of protein. The latter formula is derived under the assumption that the bound water does not participate in dipole rotation. An analysis of the experimental data using these formulas led to the conclusion that the amount of bound water for all of the proteins studied amounts to about 0.3 g per gram of protein. This conclusion agrees with the results of other physicochemical measurements, and disagrees sharply with the hypothesis of ordering of water, according to which the amount of bound water must be many times greater. The determination of the shapes of the molecules turned out to be much more difficult, since the value of β is only slightly sensitive to the shape. Only for two of the studied proteins could a qualitative conclusion be drawn that the shape of the molecules is elongated, in agreement with other physicochemical data.

b) Electrical properties of nucleic acids

The electric properties of the nucleic acids have aroused a great deal of interest recently in line with the fundamental significance ascribed to these substances in modern molecular biology. Even in measurements[47] on the sodium salt of deoxyribonucleic acid (DNA)*, very large values were obtained for the dielectric constant, while the relaxation time was much shorter than would correspond to rotation of the long axis of the molecule. It was suggested that these results could be explained by assuming that there is no dipole moment along the long axis, but the molecule has a very high transverse electric moment of the order of 20,000 Debye units. Later investigators have obtained even much more complex and contradictory results. In [39], fibers of the sodium salt of DNA containing about 20% water were pressed in the form of a film of thickness about 1 mm between the plates of a flat condenser. At a frequency of 50 cps, at electric field amplitudes of 100—1000 V/cm and temperatures 30—50°C, hysteresis cycles were observed, similar to those characteristic of ferroelectric materials. These cycles disappeared either when the temperature was lowered to 0—10°C, or when it was raised to 60°C. The conclusion was drawn in this study that DNA is ferroelectric. It was suggested that the temperature 60°C could be considered as the Curie point. As this temperature was approached, the dielectric constant rose. At a frequency of 1000 cps, the values of the dielectric constant attained 500 at 30°C, and 140,000 as the "Curie point" was approached. However, a subsequent study in the same laboratory[40] showed that all these phenomena essentially involve the presence of water in the studied specimens. The fully dried sodium salt of DNA behaves, as these authors expressed it, "as an insulator." The nonlinear effects begin to appear at 20% water, and the hysteresis loop is most clearly marked at a water content about 40%. When the water content is increased above 50%, the direct-current conductivity becomes too large to permit interpretation of the results of the measurements. The "Curie point" near 50°C is explained simply by the thermal dehydration of the specimens. All these results were obtained at a frequency of 50 cps. When the frequency was increased to several kc, the dielectric constant dropped from several thousand to values below 10. We must note that the measurements were conducted with polished platinum electrodes, and no measures were taken to avoid electrode polarization. The authors themselves conclude that it will take further experimental studies to distinguish true dielectric effects from phenomena involving ionic conductivity.

Recently the problem of the electrical properties of the nucleic acids has gained further importance, in connection with some studies[59-60] in which these objects showed broad magnetic resonance bands. The measurements were made under the conditions commonly used in electron paramagnetic resonance (EPR) work. However, the broad absorption lines observed seem to be of ferromagnetic origin.[79,80] It was suggested[59] that these phenomena could be ascribed to a molecular structure of the biopolymers containing delocalized electrons, which would result in the appearance of semiconductor properties or the formation of charge-transfer complexes (CTC). In such cases, the anomalous magnetic properties should be accompanied

*The original reference used the old term "thymonucleic acid," corresponding to the modern term DNA.

by anomalous electrical properties. However, painstaking experiments [81] have shown the essential role played by ferromagnetic impurities (iron and its oxides) in the observed magnetic effects. The problem of the nature of the broad magnetic resonance lines in the nucleic acids is currently a topic of heated discussion. If these lines are completely due to ferromagnetic impurities, as has been shown in [81], it still has to be ascertained whether these impurities are introduced in the process of sample preparation or are a necessary constituent of living matter playing a definite biological role. [60] We must note that a change in the size of the magnetic effects at certain phases of the vital activity of cells does not prove that they have a biological significance. In fact, the fundamental role in vital activities of cells is played by the oxidative processes; as these processes take place, the iron present in ferromagnetic compounds can be oxidized and reduced, thereby changing its magnetic properties. Thus the processes of biological oxidation can affect the magnetic properties of the iron, even when this iron is only a chance impurity not playing any biological role. However, of course, the possibility is not excluded that ferromagnetic substances play an essential biological role. If the broad magnetic resonance lines arise from ferromagnetic impurities, then independently of their biological role, we could hardly expect any relation between them and the electrical properties.

Thus, the existing data on the electrical properties of the nucleic acids are extremely vague and contradictory. This problem requires a detailed experimental study.

6. THE ELECTRICAL PROPERTIES OF CELLS AND TISSUES

The objects of electrical measurements are both whole tissues and individual cells, as well as the constituent parts of cells. As is known, tissues and individual cells contain a large amount of water containing dissolved mineral salts occurring in the dissociated state. The continual exchange of ions between the cell and the external medium provides an ionic composition within the cell of an order of magnitude close to that in the external medium. However, in spite of this, the conductivity of cells and tissues at low frequencies is considerably lower than that of the medium. Thus, even in the last century, experiments were set up to measure the conductivity of suspensions of erythrocytes. The erythrocytes of the blood were replaced by an equal concentration of quartz sand. Here, the conductivities of the suspensions of erythrocytes and of sand turned out to be identical. This indicated that at low frequencies the conductivity of the erythrocytes is zero. [1] This result is confirmed by the measurement of the relation of the low-frequency conductivity σ_0 of a suspension of cells in a medium having a conductivity σ_a to the volume concentration p. Very good agreement was found with Maxwell's formula [2] derived for a suspension of non-conducting spheres in a medium of known concentration:

$$\sigma_0 = \sigma_a \frac{1-p}{1+\frac{p}{2}}. \tag{1}$$

The dielectric constant shows dispersion at frequencies of the order of 1 Mc, accompanied by a considerable increase in the active component of the conductivity. These facts have compelled investigators to advance several hypotheses on the internal structure of cells, [3] the most important of which are the membrane and dipole hypotheses.

According to the membrane hypothesis, [4,5] the cell is a drop of liquid containing ions in the free state, surrounded by an exceedingly thin membrane having a very low conductivity. Here, owing to the macrostructural polarization [2,6] (which is still called the Maxwell-Wagner mechanism), very large values of ϵ (of the order of 10^3 or greater) are obtained in measurements of the dielectric constant of tissues and cell suspensions.

According to the second, or dipole, hypothesis, [3,7-9] the cell consists of a set of dipolar protein molecules, while in place of the membrane, a lipid-protein lattice is assumed, devoid of any highly insulating properties.

Electron-microscope observations confirm the presence of a membrane. [11,77]

In addition to these theories, there is a theory postulating the existence of both mechanisms, [13,14] and also a theory which permits free movement of ions within limited regions in the cell, rather than throughout the cell. [15]

A study limited to the frequency-dependence of the dielectric constant cannot give a definite answer favoring any given hypothesis. Hence, experiments are important, which would give the dependence of the dielectric constant on the temperature, [7,16] on the functional state, [17] and on the content of ions in the external medium. [19-21]

a) Dispersion

In the general case, the dielectric properties of a material can be represented as a complex quantity:

$$\varepsilon = \varepsilon' - i\varepsilon'', \tag{2}$$

where ϵ' is the dielectric constant, and ϵ'' is the imaginary component associated with the losses; ϵ'' is related to the conductivity by the relation

$$\varepsilon'' = \frac{4\pi\sigma}{\omega}. \tag{3}$$

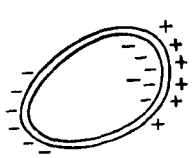

FIG. 2. Diagram of macrostructural polarization. The cell is situated in a solution through which a current is passing.

The ratio $\epsilon''/\epsilon' = \tan\delta$ is called the tangent of the loss angle. Debye has derived a very simple theory of dispersion for a polar liquid. As is now known, this theory is valid for any polarization mechanism having a single relaxation time τ. This theory gives the following form of the frequency-dependence of the complex dielectric constant ϵ, after dropping out the direct-current ohmic conductivity:

$$\varepsilon - \varepsilon_\infty = \frac{\varepsilon_0 - \varepsilon_\infty}{1 + i\omega\tau}, \tag{4}$$

where ϵ_0 and ϵ_∞ are the asymptotic values of the dielectric constant at frequencies respectively below and above the dispersion region.

We can write separate expressions for the real and imaginary components of ϵ:

$$\varepsilon' - \varepsilon_\infty = \frac{\varepsilon_0 - \varepsilon_\infty}{1 + (\omega\tau)^2}, \tag{5a}$$

$$\varepsilon'' = \frac{(\varepsilon_0 - \varepsilon_\infty)\,\omega\tau}{1 + (\omega\tau)^2}. \tag{5b}$$

Taking into account the direct-current ohmic conductivity σ_0, the latter equation can be rewritten in the form:

$$\sigma - \sigma_0 = \frac{(\sigma_\infty - \sigma_0)\,(\omega\tau)^2}{1 + (\omega\tau)^2}, \tag{6}$$

where σ_0 and σ_∞ are the asymptotic values of the conductivity at frequencies respectively below and above the dispersion region.

If we plot ϵ or σ on the complex plane, in both cases we should get a semicircle (Fig. 3). Thus, by plotting ϵ' as the abscissa and ϵ'' as the ordinate, we obtain a semicircle with its center on the axis of abscissas, and intersecting this axis at the points ϵ_0 and ϵ_∞. The point of maximum ϵ'' corresponds to the central dispersion frequency ω_c:

$$\omega_c = \frac{1}{\tau}. \tag{7}$$

In a number of cases, the agreement of experiment with the simple dispersion theory is very good. However, we often find a broader dispersion region than the theoretical, and here the maximum ϵ'' is smaller.

If in such a case we plot ϵ on the complex plane, we obtain an arc whose center lies below the axis of abscissas.[21,23-25] Such curves are described well by the semi-empirical formula

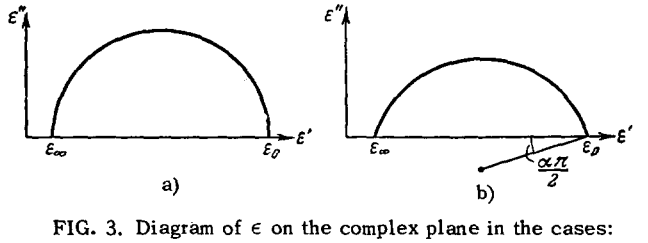

FIG. 3. Diagram of ϵ on the complex plane in the cases: (a) simple dispersion mechanism; (b) the mechanism of Cole and Cole.

$$\varepsilon - \varepsilon_\infty = \frac{\varepsilon_0 - \varepsilon_\infty}{1 + (i\omega\tau)^{1-\alpha}}. \tag{8}$$

The meaning of the parameter α is obvious from the diagram. When $\alpha = 0$, we get Debye's formula. A large number of experiments have shown good agreement with Eq. (8).

If we represent the measuring capacitor filled with the substance in terms of an equivalent circuit, we arrive at the circuit diagram[23] of Fig. 4, in which $\tilde{Z} = [\tau(i\omega\tau)^{-\alpha}/(\epsilon_0 - \epsilon_\infty)]$; when $\alpha = 0$, $\tilde{Z} = R = \tau/(\epsilon_0 - \epsilon_\infty)$. $\tilde{Z}$ has the property that only its modulus varies with the frequency, while the phase-shift angle remains constant. The parameter α is often ascribed to the distribution of relaxation times τ. However, an attempt to derive from Eq. (8) the spectrum of relaxation times did not give the natural Gaussian curve

$$F(s)\,ds = \frac{b}{\sqrt{\pi}}\, e^{-b^2 s^2}\, ds \tag{9}$$

but rather, a formula to which no physical meaning could be given:[23,26]

$$F'(s)\,ds = \frac{1}{2\pi}\,\frac{\sin\alpha\pi}{\cosh(1-\alpha)s - \cos\alpha\pi}\,ds; \tag{10}$$

where

$$s = \ln\left(\frac{\tau}{\tau_0}\right).$$

Equation (9) is the Gaussian distribution law for the relaxation times, and b is the width of the distribution.

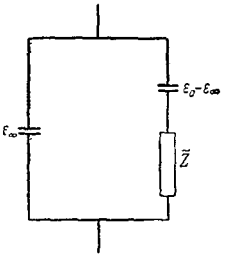

FIG. 4. Equivalent circuit diagram for the condenser filled with the substance being studied.

The literature contains some attempts[23,24,33] to explain the impedance $\tilde{Z} = Z(i\omega)^{-\alpha}$, which is called the polarization impedance, since we encounter a similar phenomenon in studying the conductivity of a metal-electrolyte boundary layer. An analogous phenomenon also occurs in the thin film of a photocell and in certain poor dielectrics.

It is assumed that the impedance of the membrane itself is not a capacitive impedance, but a polarization impedance. Such a hypothesis explains the experimental results. When $\alpha = 1$, the membrane exhibits a purely capacitive character. Then the membrane is impermeable to ions.

According to the Nernst-Warburg theory of the diffusional nature of the polarization impedance, this phenomenon can be explained[24] by the differing permeabilities of the membrane for oppositely-charged ions.

For a surface which is completely impermeable for one type of ion, and completely permeable for the other type, this theory gives $\alpha = 0.5$. For complete permeability for all ions, we find $\alpha = 0$. This theory has not been worked out for the case of incomplete selectivity, but presumably we can get any value of α between zero and unity under suitable assumptions as to the nature of the permeability of the membrane. In certain cases, the value of the central frequency obtained from the dispersion of the real component of the dielectric constant does not agree with the value of the central frequency obtained from the dispersion of the imaginary component. Here, the central frequency $\omega_0 = 1/\tau_0$ has been taken[21] to be the geometric mean of $\omega_{c\epsilon}$ and $\omega_{c\sigma}$:

$$\omega_0 = \frac{1}{\tau_0} = \sqrt{\omega_{c_\varepsilon}\omega_{c_\sigma}}. \tag{11}$$

In dielectric measurements of biological objects, three dispersion regions have been found, termed α, β, and γ.[27]

The nature of the α dispersion is least known. It occupies the frequency range from 0 to 10^4 cps. It is very difficult in this range to obtain precise values of ϵ', owing to the effect of electrode polarization, which complicates the experiment greatly.

Most of the experiments[5,21,24,27] have been performed on the β dispersion, which occurs in the range from 10^4 to 10^8 cps. As we see from Eq. (5), the dispersion region occupies two decades. However, we have cited a broader frequency range here because the location of the dispersion region depends on the type of object. It is precisely in the study of the β dispersion that the dispute arises between the proponents of the membrane and the dipole hypotheses.

The γ dispersion occurs in the centimeter range. It is the dispersion of the dielectric constant of the water.

b) Estimate of the membrane thickness

The membrane hypothesis was advanced first by Fricke[4] in 1925. He was the first to calculate the capacitance C_M of the membrane per square centimeter. The values of C_M from various measurements[21,28-31] are about the same: 0.5—1.5 $\mu F/cm^2$.

The experiments to determine C_M are carried out in suspensions of cells in a weak solution of an electrolyte. This sort of measurements has been performed for the red blood cells of various animals,[30] for certain types of bacteria,[25] for yeast cells,[28] and for mitochondria,[21,32] which are a constituent part of the cell. In many cases the particles are nearly spherical in form. Hence the theory of the conductivity of suspensions of shielded spheres is applicable to them.[36] This theory is based on a formula first derived by Maxwell[2] for the conductivity of a suspension of spheres of conductivity σ_i in a medium of conductivity σ_a:

$$\frac{\sigma-\sigma_a}{\sigma+2\sigma_a} = p\frac{\sigma_i-\sigma_a}{\sigma_i+2\sigma_a}. \tag{12}$$

Here σ is the conductivity of the suspension. The formula remains correct if we replace σ by the complex conductivity Λ or the dielectric constant ϵ. Here p is the volume concentration of the spheres. The formula is valid for $p \ll 1$.

Certain investigators have derived formulas which are also correct for $0 \le p \le 1$,[34] and also valid for suspensions of objects of ellipsoidal shape.[34,35] The formula relating the complex conductivity Λ_H of a cell to the parameters of the cell is analogous to Eq. (12). The cell is represented in the form of two concentric spheres. The interior of the smaller sphere is filled with a medium having the complex conductivity Λ_i, while the space between the spheres (i.e., the membrane) is represented as a medium having Λ_M. The external radius of the cell is R, and the membrane thickness is d:

$$\frac{\Lambda_H-\Lambda_M}{\Lambda_H+2\Lambda_M} = \left(\frac{R-d}{R}\right)^3\frac{\Lambda_i-\Lambda_M}{\Lambda_i+2\Lambda_M}. \tag{13}$$

If one substitutes for Λ_a and Λ_i the active conductivities σ_a and σ_i, or else the dielectric constants ϵ_a and ϵ_i, and assumes that the membrane has a very small active conductivity which can be neglected, together with a dielectric constant ϵ_M, one obtains the following formulas[36] from (12) and (13):

$$\varepsilon_0 - \varepsilon_a \approx 9\pi pRC_M, \tag{14}$$

$$\varepsilon_\infty = \varepsilon_a\frac{(1+2p)\,\varepsilon_i+2(1-p)\,\varepsilon_a}{(1-p)\,\varepsilon_i+(2+p)\,\varepsilon_a}, \tag{15}$$

$$\sigma_\infty = \sigma_a\frac{1+2p\,\frac{\sigma_i-\sigma_a}{\sigma_i+2\sigma_a}}{1-p\,\frac{\sigma_i-\sigma_a}{\sigma_i+2\sigma_a}}, \tag{16}$$

$$\tau = RC_M\frac{\sigma_i+\sigma_a}{2\sigma_i\sigma_a}, \tag{17}$$

$$C_M = \frac{\varepsilon_M}{4\pi d}. \tag{18}$$

Here the quantities ϵ_0, ϵ_∞, σ_0, and σ_∞ characterizing the suspension are obtained directly from experiment. We can immediately find p from Eq. (1). Then, knowing p, we can find σ_i from Eq. (16). Further, knowing $\tau = 1/\omega_c$ from experiment, we can find C_M. Of course, for this purpose we also need a microscope, since we have to determine R. A second way[37] to find C_M is to use Eq. (14). In this case also, we need to know the radius of the suspended cells. However, most often the cells being studied do not have a single value of the radius, but a distribution about some mean value.[21] Then we can study this distribution under the microscope by counting the number of cells having a given radius R_i. Knowing the volume fraction p of all the cells, we can find the volume fraction p_i of the cells of radius R_i. Thereupon, Eq. (14) is transformed into

$$\varepsilon_0 - \varepsilon_a = 9\pi C_M\sum_i p_iR_i. \tag{19}$$

As we see from Eq. (18), we can say nothing about the membrane thickness d from a knowledge of C_M unless

we know ϵ_M. The thickness of the membrane, if referred to a substance having $\epsilon_M = 1$, is of the order of 10 Å. Certain authors assume that ϵ_M is of the order of 3. They rely on the optical refractive-index data [25,30] where $n = \sqrt{\epsilon}$. These data give a value of the order of 3 for ϵ_M. Here they assume that ϵ_M does not vary with the frequency at which C_M is measured, up to the optical frequencies. We can obtain the value of three by assuming that the membrane consists of fats. Other authors [3] who assume that an appreciable part of the membrane consists of proteins give values greater than 30 or even 100 for ϵ_M.

7. THE STUDY OF THE DIELECTRIC PROPERTIES OF MACROMOLECULES

The study of the constituent parts of the cell is of great interest from the standpoint of their dielectric behavior. The importance of a knowledge of the configuration of the electric charges lies in the possibility of explaining various physicochemical properties of macromolecules. [38] Here the investigation may take several pathways: 1) the study of the polar molecules at concentrations such that the interaction of the macromolecules becomes small; and 2) the study of the properties of the macromolecules in bulk, i.e., the study of tablets prepared from the given substance. Typical of the second method are the studies of Sadron and his associates on the dielectric properties of tablets consisting of DNA with a 20% water content. [39] As we can see from [40], these studies are difficult to interpret. Most probably, the water plays a very essential role here.

Now, the first method can be divided into two aspects:

a) the study of substances having a low dielectric increment, basically proteins; and

b) the study of substances having a high dielectric increment, the so-called high-polymeric electrolytes or simply polyelectrolytes.

We shall now take up the study of the dielectric properties of proteins in solution. The best-known representative of this approach is Oncley. [41,42]

If we have a mixture of two types of dipoles, then in measuring the frequency-dependence of the real component of the dielectric constant $\epsilon'(\omega)$ (which we shall simply denote as ϵ below) we shall get a curve showing two dispersion regions. [41] This happens in the study of solutions of polar protein molecules in water (Fig. 1). Here ϵ^0 is the dielectric constant of the pure solvent (water). The value of ϵ^0 of water is very large (80.3 at 25°C), and hence at frequencies above the dispersion region of the protein, but below the dispersion region of the water, the dielectric constant of the solution will be less than ϵ^0 by an amount $\Delta\epsilon_\infty$. At low frequencies, owing to the large dipole moment μ of the macromolecules, ϵ_0 of the solution will exceed ϵ^0 by an amount $\Delta\epsilon_0$.

The quantities $\Delta\epsilon_0/g$ and $\Delta\epsilon_\infty/g$ are called, respectively, the low-frequency dielectric increment and the high-frequency decrement. Here g is the concentration of the proteins in solution in grams per liter.

For a solution of polar molecules in a non-polar liquid, the Clausius-Mosotti formula holds:

$$\frac{\varepsilon-1}{\varepsilon+2}=\frac{4\pi}{3}n\beta=p, \tag{20}$$

where n is the number of polar molecules per cm^3, and β is the polarizability of a single molecule as a function of temperature.

Debye has derived an expression for the polarizability of a molecule due to its rotation, $\beta_0-\beta_\infty$, where β_0 and β_∞ are respectively the polarizabilities at frequencies below and above the dispersion region:

$$\beta_0-\beta_\infty=\frac{\mu^2}{3kT}; \tag{21}$$

and since $n = gN/1000M$, where N is Avogadro's number and M is the molecular weight of the polar molecule, we can derive from Eqs. (20) and (21)

$$\mu^2=\frac{27\cdot10^3kTM(\varepsilon_0-\varepsilon_\infty)}{4\pi Ng(\varepsilon_0+2)(\varepsilon_\infty+2)}. \tag{22}$$

When $\Delta\epsilon_0/\epsilon^0 \ll 1$, and $\Delta\epsilon_\infty/\epsilon^0 \ll 1$, we obtain

$$\mu=\alpha\sqrt{M\frac{\varepsilon_0-\varepsilon_\infty}{g}}, \tag{23}$$

where α is a parameter depending on the temperature and the type of solvent.

In systems where the solvent has a high dielectric constant ϵ^0, the Clausius-Mosotti equation is not obeyed, but one can use instead the equation [43]

$$p=\frac{\varepsilon-\varepsilon^0-a}{b},$$

where a and b are parameters. On the basis of these arguments, one obtains the same result

$$\mu=\alpha\sqrt{M\frac{\varepsilon_0-\varepsilon_\infty}{g}}.$$

Thus, if we know the molecular weight of the protein being studied, we can find the dipole moment μ of the molecule from measurements of the low-frequency increment and the high-frequency decrement. The measurements of ϵ are usually performed at several concentrations of the solution, and the increment is determined from the slope of the curve $\Delta\epsilon(g)$ as $g \to 0$.

Taking the disorienting influence of the temperature into account is equivalent to taking into account the resistance of the macromolecule to rotation [86] in a medium of viscosity η. To do this, one introduces the rotational diffusion constant [41,44]

$$\theta=\frac{kT}{\xi}, \tag{24}$$

where ξ is the Stokes inner-frictional constant for rotation of a sphere of radius r in a medium of viscosity η,

$$\xi=8\pi r^3\eta. \tag{25}$$

For molecules differing in shape from spherical, a form factor ψ is introduced. Here,

$$\xi = 8\pi r^3 \eta \psi.$$

Debye has shown that the relaxation time $\tau = 1/\omega_c$ is related to the rotational constant by the expression

$$\tau = \frac{1}{2\theta}. \quad (26)$$

Thus, if we know ω_c, the central dispersion frequency, and the viscosity, we can find the volume of the molecule. The difference between the given dimensions from the values obtained in the ultracentrifuge or in other measurements can be explained by the effect of hydration.[45,46]

Attempts to apply the theory of rotation of dipoles in a viscous medium to explain the results of studies of aqueous solutions of DNA and certain other biopolymers have led to certain contradictions.[53] Thus, the molecular weight of DNA as determined[47] by the method of dielectric measurements disagreed with the molecular weight data using sedimentation and diffusion methods. Some very essential points here are that, first, these results gave a low value for the molecular weight, and second, that this value depended on the concentration of NaCl in the water: as the NaCl concentration was raised to 0.002 M, the "molecular weight" varied from 135,000 to 35,000. The authors explained this by polar association, i.e., the combination of smaller molecules into larger ones. This does not sound convincing.

Jacobson has given an original explanation for this phenomenon. He used the results of measurements of ϵ for mixtures of cellulose and water,[49] the data on the proton magnetic resonance of DNA solutions,[50] and the results of a study of the dielectric properties of flow-oriented DNA molecules,[51] and concluded that the dispersion in the region of 1 Mc/sec of aqueous DNA solutions can be explained by the formation of a new water structure in the vicinity of the macromolecules.[49] The helical model of the DNA molecule of Watson and Crick fits very well into a tetrahedral water structure. According to Jacobson, the hydrogen-bonding atoms of the DNA molecule upon solution in water link with the nearby water molecules, thus orienting them. This results in a further orientation of more distant water molecules, and consequently, an ordered structure of tetrahedra is formed within a certain neighborhood of the macromolecule. The structure of the water in this region even resembles the structure of ice, and since ice has an elevated value of ϵ at low frequencies (i.e., ϵ_0)[52] as compared with ϵ_0 for water, we can also rightly expect here an increase in ϵ for the solution. The distance at which the orientation is still appreciable is of the order of 500—1000 Å for solutions of DNA when the molecules of the latter are 6000 Å long and 20 Å wide. Of course, this distance depends considerably on the degree of fit of the macromolecule and the water structure.

8. THE EXPERIMENTAL BASIS FOR THE ORDERED-WATER HYPOTHESIS

a) Studies have been made[51,49] of the dielectric constant of an aqueous solution of DNA flowing perpendicular to the electric-field direction, and of its dependence on the velocity gradient. The value of ϵ decreased with increase in the velocity gradient, but still remained greater than ϵ for water. For other substances (hemocyanin), ϵ did not depend on the velocity gradient.

b) A study[49] has been made of the value of ϵ for cellulose-water mixtures as a function of the frequency. Soft porous filter paper was taken and washed for four days in distilled water, and then placed in a measuring condenser, and measurements were taken at 20°C. The paper content was about 20%, the rest being distilled water. As we see, an appreciable increase in ϵ is observed, although ϵ for paper is of the order of 5 (Fig. 5).

c) A study[16] of the dielectric constant of thin films of water ($\sim 2-5\mu$) between mica plates has given values of ϵ of the order of 10—20, depending on the film thickness.[54] This also indicates that the properties of water are affected considerably by the presence of a second phase.

d) The changes in ϵ of aqueous solutions of DNA when salts are added to the solution should be ascribed to the fact that the length of the DNA molecule can change appreciably upon change in the electrolyte concentration (the change can be of the order of 30%). Here the fit with the water structure is destroyed, and the dimensions of the region occupied by ordered water diminish.

e) Experiments on the proton magnetic resonance of water also confirm this hypothesis.[50] The intensity of the line decreased appreciably when DNA was dissolved in the water in 1.6% concentration, but increased again upon addition of 0.04 M NaCl to this solution.

Measurements of the viscosity and the osmotic pressure also favor this hypothesis, since the viscosity of the water increases upon ordering.

Dispersion. The low-frequency increment for proteins[46] is of the order of 0.1—2, while for DNA molecules it is 10—2000.[55,57] The greater the increment is, the more the given polymer increases the viscosity

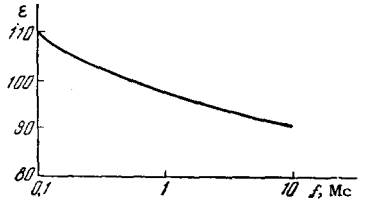

FIG. 5. Frequency-dependence of the dielectric constant of a mixture of paper and water.

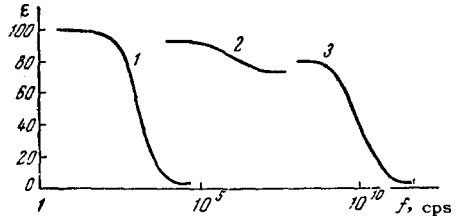

FIG. 6. Frequency-dependence of the dielectric constant of: 1 – ice; 2 – a solution of a polyelectrolyte in water; 3 – water.

of water upon solution. As the polyelectrolyte concentration is increased, the increment declines, indicating the overlap of the ordered regions belonging to different macromolecules. The greater the value of the increment is, the more nonlinear is its concentration dependence. Such a nonlinearity is also observed in proton magnetic resonance. Thus we can draw conclusions on the dimensions of the ordered shell.

The dispersion in pure water is found at a frequency of 10^{10}cps, and for ice at 10^2—10^4 cps, while that of the solution lies midway between these values at 10^6 cps. The central dispersion frequency ω_c increases with the concentration: for DNA, ω_c shifts from 0.3 to 0.9 Mc as the concentration is varied from 0.03 to 0.25 grams per liter; this is explained by a decrease in the dimensions of the ordered regions due to overlap. It is assumed that the value of ω_c is less for a larger water shell.

At frequencies above the dispersion region in ordinary proteins, the decrement is of the order of 0.06—0.1. This is twice as large as the decrement calculated theoretically from the volume occupied by the molecule. When the water shell is small, its value of ϵ at high frequencies is less than that of ordinary water at the same frequency. However, when the shells have large dimensions, the situation is more complicated, since the dielectric properties of the ordered water can vary appreciably as a function of the distance from the macromolecule. Here we sometimes find an increment instead of a decrement. In the water-cellulose mixtures, the value of ϵ exceeds that of water even at 50 Mc.

9. EXPERIMENTAL METHODS

In order to obtain a picture of the dielectric properties of a substance, we can use two approaches:

1) A study using transient effects.[24,58] This is done by studying the reaction of the given element to a jump in either the voltage or the current, or to a linear increase in the voltage or current.

2) Determining the frequency-dependence of the impedance, e.g., by bridge methods.

Both methods are equivalent with regard to the information obtained, since a Fourier transformation permits us to transform a transient characteristic of the element into a spectral characteristic, and vice versa.

In most cases in studying biological objects, one uses the second method, since it permits greater accuracy. However, when speed of measurement rather than accuracy is important, the transient-effect method turns out to be better. If we aim to determine the frequency-dependence of the impedance at very low frequencies (below 10 cps), in a number of cases the transient-effect method is more practicable.

However, the fundamental method is the bridge method. Most measurements are performed in the range 10^4—10^8 cps, i.e., encompassing the β-dispersion region. The study of biological objects has the peculiarity that tan δ is very large, being of the order of 10. Hence, the bridges used for measurement must permit measurements on samples of high conductivity. However, there have been many designs of such bridges, and since many authors in their studies have used a bridge having inductive coupling between the arms, we shall describe here such a bridge[61] (Fig. 7).

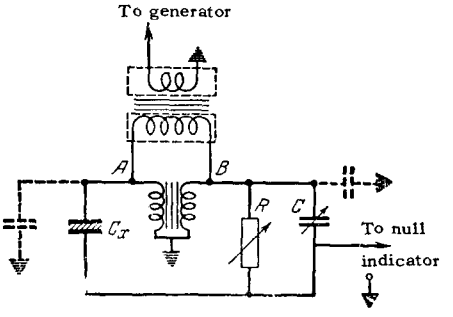

FIG. 7. Circuit diagram of a bridge having inductive coupling between the arms, permitting the reduction of stray capacitances.

The first transformer is used to apply a potential which is symmetrical with respect to ground to both sides of a second transformer. The coils of this transformer must be carefully shielded. The second transformer has identical coils with a coupling coefficient between them of unity. This makes it possible to reduce appreciably the effect of stray capacitances and leaks from points A and B to ground. Such a bridge can operate over the range from 20 cps to 10 Mc, if one has two or three pairs of replaceable transformers.

In working at the highest frequencies (above 5—10 Mc), we cannot apply a variable resistance to balance the bridge with respect to the active component, owing to the large effect of stray capacitances. Hence, in the frequency range from 1 to 300 Mc, one uses a Schering bridge, in which both the capacitive and the active components are balanced by using variable capacitances.

The designs of the measuring cells are extremely varied. However, a general feature of all the cells is the fact that they have all been constructed either of platinum, or of silver, and have been coated with platinum black by electrolysis in an H_2PtCl_6 solution. This

is done to reduce the harmful effect of electrode polarization.[63,84]

Electrode polarization affects the results of measurement of the real component of the dielectric constant at frequencies below 10^5 cps.

In bridge measurements of the capacitance and the conductance of the cell containing the substance, we obtain two quantities at a given frequency: the capacitance C and the conductance G. That is, our condenser containing the substance can be represented in terms of an equivalent circuit, which is a parallel combination of a capacitance and a conductance (Fig. 8a).

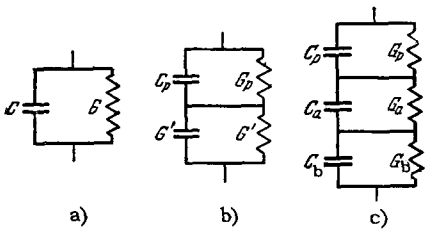

FIG. 8. Equivalent circuit diagram for taking the electrode polarization into account. C and G are the instrument readings, C_p and G_p are the capacitance and leak resistance of the electrode surface layer, and C′ and G′ are the parameters of the substance being studied.

However, the true characteristics of the substance being studied are C′ and G′, while the appreciable capacitance C_p of the surface film of the electrode and the finite conductance G_p of this film introduce a large error into the measurements. The equation relating the parameters of these circuits has the form

$$\frac{1}{G+i\omega C}=\frac{1}{G_p+i\omega C_p}+\frac{1}{G'+i\omega C'}. \tag{27}$$

This equation is equivalent to the following:

$$\frac{G'}{G'^2+\omega^2C'^2}=\frac{G}{G^2+\omega^2C^2}-\frac{G_p}{G_p^2+\omega^2C_p^2},$$
$$\frac{C'}{G'^2+\omega^2C'^2}=\frac{C}{G^2+\omega^2C^2}-\frac{C_p}{G_p^2+\omega^2C_p^2}. \tag{28}$$

There are several methods of taking the electrode polarization into account.

The method of using two inter-electrode spacings.[63] The original spacing is a, and the second spacing is a+b. In the first case, the bridge gives readings C_1 and G_1, and in the second case, C_2 and G_2. The equivalent circuit is shown in Fig. 8c. Here we can obviously derive from Eq. (27):

$$\frac{G_2-i\omega C_2}{G_2^2+\omega^2C_2^2}=\frac{G_1-i\omega C_1}{G_1^2+\omega^2C_1^2}+\frac{G_b-i\omega C_b}{G_b^2+\omega^2C_b^2}; \tag{29}$$

and since we always have

$$\left(\frac{\omega C_2}{G_2}\right)^2\ll 1,\quad \left(\frac{\omega C_1}{G_1}\right)^2\ll 1,\quad \left(\frac{\omega C_b}{G_b}\right)^2\ll 1 \tag{30}$$

(being interested in the low frequencies), we obtain

$$\frac{1}{G_2}=R_2=\frac{1}{G_1}+\frac{1}{G_b}=R_1+R_b; \tag{31}$$

Hence,

$$R_b=R_2-R_1,$$
$$C_b=\frac{R_2^2C_2-R_1^2C_1}{(R_2-R_1)^2}=\frac{(a+b)^2C_2-a^2C_1}{b^2}. \tag{32}$$

This method has been discussed in detail in a paper by Shaw,[63] where it was also compared with the method of applying corrections used by Oncley.[42] In this method, one uses an empirical law according to which

$$C'=C-C_0-AG^2f^{-(2-m)}, \tag{33}$$

where A and m are empirical constants.

Oncley considers that m = 0.5, while Shaw obtains m = 0.3. This method has a theoretical basis. According to Newman[64] and Warburg,[65] the polarization capacitance C_p depends on the frequency as $f^{-1/2}$, with $G_p = \omega C_p$. Since we most often have to deal with electrolytes having a large conductivity G, the quantity ΔG involving the polarization phenomena is small in comparison with G. Hence, one most often takes into account only the change in the capacitance for which one can use Eq. (33).

According to Shaw, both methods of taking into account the polarization phenomena give equally good results. The second method is more practicable when it is difficult to change the spacing between the electrodes in the measuring cell.

In order to obtain reproducible results, it is also very important to note the value of the current in the measuring cell. Hence, we can compare the results obtained by different authors provided that the values of the current are comparable.

In working with such objects as muscle and nerve cells, one must not operate at high potentials, since excitation takes place at currents exceeding a certain value, and the parameters of the cells are altered thereby.[3,24]

10. RESULTS OF INVESTIGATIONS

We shall present in greater detail one of the most recent and most painstaking studies by Schwan and his associates[21] on the electrical properties of mitochondria. Since mitochondria change in dimensions, depending on the external medium, the studies were conducted both with swollen and shrunken mitochondria.

Swollen rat-liver mitochondria gave values $C_M = 0.5\ \mu F/cm^2$, while guinea-pig-heart mitochondria gave the same value, $1.3\ \mu F/cm^2$, in both the swollen and shrunken states (within experimental limits of error not exceeding 30%). This is an amazing fact, since if we assume that swelling of the mitochondria (with a fourfold increase in the membrane surface) reduces the thickness of the membrane by a factor of four, then C_M should increase by a factor of four. The authors concluded that the thickness of the membrane does not change during swelling, while the increase in the surface involves a supply of membrane material

contained within the mitochondria, which is used up in proportion to the swelling. This is confirmed by electron-microscopic observations.[11]

In order to explain the above-mentioned lowering of the center of the semicircle in the diagram of ϵ on the complex plane, they had to assume that the internal conductivity is anisotropic. Citing the electron-microscopic data indicating the presence of transverse partitions in mitochondria, these authors suggested that the conductivity is four times smaller in the direction of the axis than in perpendicular directions.

The distribution of relaxation times was even broader for shrunken mitochondria. C_M can be calculated by two methods: from the static dielectric constant and from the relaxation time. In this study, the value of C_M calculated for swollen mitochondria from the value of ϵ_0, $C_{M_\epsilon} = 1.3\ \mu F/cm^2$, did not depend on the conductivity of the external medium as the latter increased tenfold, while $C_{M_{\omega_0}}$ increased from 0.5 to 1.2 $\mu F/cm^2$. However, for shrunken mitochondria, as the external conductivity increased by a factor of fifty, the value $C_{M_{\epsilon_0}} = 1.1\ \mu F/cm^2$ did not vary, but $C_{M_{\omega_0}}$ decreased from 1.8 to 0.4 $\mu F/cm^2$.

The authors considered the $C_{M_{\epsilon_0}}$ data to be more valid, ascribing the variations in $C_{M_{\omega_0}}$ to the distribution of relaxation times.

The data of the same authors on the relation of the internal to the external conductivity are also of interest. For swollen mitochondria, the ratio σ_i/σ_a varied from 0.64 to 0.32 as σ_a was varied by a factor of 30. However, for shrunken mitochondria, as σ_a was increased by a factor of 50, σ_i/σ_a decreased from 1.4 to 0.08, with σ_i hardly varying.

This was explained by the binding of a certain portion of the ions by proteins, whose concentration is large in the shrunken mitochondria. This effect disappears in the swollen mitochondria.

We see from the example of this study that the membrane and phase theories can be combined.

Fricke[25] has made analogous measurements on several types of cells, including suspensions of intestinal bacilli. He obtained a typical curve. The rise in ϵ' at low frequencies, or so-called α dispersion, is to be noted. As the conductivity of the external medium was varied, the β dispersion region shifted in accordance with theory:[20]

$$C_M = 0.7\ \mu F/cm^2.$$

In Fricke's study on yeast,[20] an attempt was made to study the α dispersion region at various values of the conductivity of the external medium:

$$C_M = 0.6\ \mu F/cm^2.$$

These authors came to the conclusion that the values of C_M in different types of cells are very similar, thus indicating a common structure of the membranes of different cells.

The problem is more complex in the study of the impedance of tissues and nerve fibers,[24] since it is harder here to interpret the data obtained. Indeed, in the case of nerve fibers oriented perpendicular to the field, we can treat the given fibers as cylinders of known radius and infinite length. If we adopt the membrane hypothesis here, we can even calculate the capacity of the membrane.[24]

In studies with tissues and nerve fibers, we must always note the value of the current, since large currents can bring on excitation, resulting in a change in the measured parameters.[3,24]

If one measures the impedance of a muscle having its fibers oriented parallel to the electric field, one obtains on the complex plane very good arcs having considerably lowered centers, the angle γ being $\sim 45-60°$. In analogous measurements of the impedance of a nerve fiber, one does not get a good arc. This has been explained by the existence of three dispersion regions in the regions of 100 cps, 5 kc, and 1 Mc. These results are characteristically poorly reproducible.

In transverse measurements (with the field perpendicular to the fiber axis), good arcs with considerably lowered centers have been obtained. When the sheath of the nerve fiber is removed, the reactive component of the impedance of the fiber is considerably reduced.

The relation of the impedance of tissues to their functional state is very complex. However, we may note the following features: during death of the tissue the difference between the low- and high-frequency conductivities begins to decline, and vanishes when the death of the tissue becomes final. An analogous, but reversible, increase in the low-frequency conductivity is observed in excitation. The proponents of the phase theory explain these phenomena by the decomposition of complexes resulting in liberation of ions.

The proponents of the membrane theory say that permeability of the membrane increases.

Not many experiments have been performed on the temperature-dependence of the impedance of tissues.[7,66] In a study by Aladzhalova and Maslov,[66] the position of the maximum of tan δ shifted to higher frequencies as the temperature was raised from 2° to 32°C.

[1] M. Oker-Blom, Arch. Ges. Physiol. 79, 28 (1900).

[2] J. C. Maxwell, A Treatise on Electricity and Magnetism, 3rd Ed., Vol. 1, p. 437, Oxford, Clarendon Press (1892).

[3] B. I. Tarusov, Osnovy biofiziki i biofizicheskoĭ khimii (Fundamentals of Biophysics and Biophysical Chemistry), Part I, M., "Vysshaya shkola," (1960).

[4] H. Fricke, J. Gen. Physiol. 9, 137 (1925).

[5] H. Fricke, Cold Spring Harbor Symposia on Quantitative Biology, Vol. 1, p. 117, The Biological Laboratory, Cold Spring Harbor (1933).

[6] K. Wagner, Arch. Electrotechn. 2, 371 (1914).
[7] K. S. Trincher, Uspekhi sovremennoĭ biologii 45, 261 (1958).
[8] D. N. Nasonov and V. Ya. Aleksandrov, Reaktsiya zhivogo veshchestva na vneshnie vozdeĭstviya (The Reaction of Living Matter to External Influences), M., (1940).
[9] K. S. Trincher, Biofizika 1, 113 (1956).
[10] O. B. Ptitsyn, Usp. Fiz. Nauk 69, 371 (1959), Soviet Phys. Uspekhi 2, 797 (1960).
[11] Schulz, Löw, Ernster, and Sjöstrand, Proceedings of the European Regional Conference on Electron Microscopy, Stockholm, Sept. 17-20, 1956, p. 134, Academic Press, New York (1956).
[12] A. S. Troshin, Problema kletochnoĭ pronitsaemosti (The Problem of Cell Permeability), M.-L., Izd. AN SSSR (1958).
[13] D. L. Rubinshteĭn and R. A. Rutberg, DAN SSSR 71, 109 (1950).
[14] A. I. Polivoda and A. A. Mikhaĭlova, Biofizika 5, 612 (1960); Engl. Transl., Biophysics (Pergamon Press) 5, 693 (1960).
[15] C. T. O'Konski, J. Phys. Chem. 64, 605 (1960).
[16] K. S. Trincher, Fiziol. Zhur. SSSR 3, 293 (1956).
[17] N. A. Aladzhalova and S. N. Mertsalova, DAN SSSR 94, 213 (1954).
[18] P. L. Privalov, Biofizika 3, 738 (1958); Engl. Transl., Biophysics (Pergamon Press) 3, 691 (1958).
[19] N. M. Maslov and S. M. Sverdlov, DAN SSSR 71, 49 (1950).
[20] H. Fricke, J. Phys. Chem. 59, 168 (1955).
[21] Pauly, Packer, and Schwan, J. Biophys. Biochem. Cytol. 7, 589 (1960).
[22] P. Debye, Polar Molecules, Chemical Catalog Co., New York (1929) and Dover (1945).
[23] K. S. Cole and R. H. Cole, J. Chem. Phys. 9, 341 (1941).
[24] K. S. Cole and H. J. Curtis, Cold Spring Harbor Symposia on Quantitative Biology, Vol. 4, p. 73, The Biological Laboratory, Cold Spring Harbor (1936).
[25] Fricke, Schwan, Li, and Bryson, Nature 177, 134 (1956).
[26] R. M. Fuoss and J. G. Kirkwood, J. Am. Chem. Soc. 63, 385 (1941).
[27] H. P. Schwan, Advances in Biological and Medical Physics, Vol. 5, p. 148, Academic Press, New York (1957).
[28] H. Fricke and H. J. Curtis, Nature (Lond.) 134, 102 (1934).
[29] H. Fricke and H. J. Curtis, J. Gen Physiol. 18, 821 (1935).
[30] H. Fricke, Nature (Lond.) 172, 731 (1953).
[31] K. S. Cole, J. Gen. Physiol. 18, 877 (1935).
[32] H. Pauly and L. Packer, I. Biophys. Biochem. Cytol. 7, 603 (1960).
[33] K. S. Cole, Science 79, 164 (1934).
[34] D. Polder and J. H. Van Santen, Physica 12, 257 (1946).
[35] J. G. Kirkwood, J. Chem. Phys. 4, 592 (1936).
[36] H. Pauly and H. P. Schwan, Z. Naturforsch. 14b, 125 (1959).
[37] H. Fricke, J. Appl. Phys. 24, 644 (1953).
[38] E. J. Cohn, Ann. Rev. Biochem. 4, 93 (1935).
[39] Polonsky, Douzou, and Sadron, Compt. rend 250, 3414 (1960).
[40] Douzou, Francq, Polonsky, and Sadron, Compt. rend. 251, 976 (1960).
[41] J. L. Oncley, J. Am. Chem. Soc. 60, 1115 (1938).
[42] J. D. Ferry and J. L. Oncley, J. Am. Chem. Soc. 63, 272 (1941).
[43] J. Wyman, Jr., Chem. Revs. 19, 213 (1936).
[44] Oncley, Ferry, and Shack, Ann. N. Y. Acad. Sci. 40, 371 (1940).
[45] J. L. Oncley, Crystalline Protein Molecules, Ann. N. Y. Acad. Sci. 41, 77 (1941).
[46] E. J. Cohn and J. T. Edsall, Proteins, Amino Acids, and Peptides, p. 460, Reinhold, New York (1943).
[47] Jungner, Jungner, and Allgén, Nature 163, 849 (1949); G. Jungner and I. Jungner, Acta Chem. Scand. 6, 1391 (1952).
[48] B. Jacobson, Nature (Lond.) 172, 666 (1953).
[49] B. Jacobson, J. Am. Chem. Soc. 77, 2919 (1955).
[50] Jacobson, Anderson, and Arnold, Nature 173, 772 (1954).
[51] J. Jacobson, Rev. Sci. Instr. 24, 949 (1953).
[52] R. P. Auty and R. H. Cole, J. Chem. Phys. 20, 1309 (1952).
[53] Oncley, Ellenbogen, Gitlin, and Gurd, J. Phys. Chem. 56, 85 (1952).
[54] Palmer, Cunliffe, and Hough, Nature 170, 796 (1952).
[55] Dintzis, Oncley, and Fuoss, Proc. Natl. Acad. Sci. USA 40, 62 (1954).
[56] I. Jungner, Acta Physiol. Scand. 20, Suppl. 69 (1950).
[57] L. G. Allgén, Acta Physiol. Scand. 22, Suppl. 76 (1950).
[58] S. Bishop, Am. J. Physiol. 89, 618 (1929).
[59] L. A. Blyumenfel'd, Biofizika 4, 515 (1959); Engl. Transl., Biophysics (Pergamon Press) 4, No. 5, 2 (1959).
[60] Blyumenfel'd, Benderskiĭ, and Kalmanson, Biofizika 6, 631 (1961).
[61] Mel'yanovskiĭ, Mikhaĭlenko, and Kotenko, PTÉ No. 4, 92 (1961).
[62] L. A. Blyumenfel'd, Izv. AN SSSR, ser. Biol. 3, 255, 285 (1957).
[63] T. M. Shaw, J. Chem. Phys. 10, 609 (1942).
[64] F. H. Newman, Electrolytic Conduction, Wiley, New York (1931).
[65] E. Warburg, Ann. Physik, Ser. 4, 6, 125 (1901).
[66] N. A. Aladzhalova and N. M. Maslov, DAN SSSR 115, 407 (1957).
[67] Haggis, Buchanan, and Hasted, Nature 167, 607 (1951).

[68] Buchanan, Haggis, Hasted, and Robinson, Proc. Roy. Soc. **A213**, 379 (1952).
[69] W. Arnold and R. K. Clayton, Proc. Natl. Acad. Sci. U.S. **46**, 769 (1960).
[70] A. Szent-Györgyi, Nature **148**, 157 (1941).
[71] A. Szent-Györgyi, Bioenergetics, Academic Press, New York (1957); Russ. Transl., M., Fizmatgiz (1960).
[72] G. P. Mikhailov and T. I. Borisova, Uspekhi Khimii **30**, 895 (1961).
[73] M. G. Evans and J. Gergely, Biochim. Biophys. Acta **3**, 188 (1949).
[74] D. D. Eley and G. D. Parfitt, Trans. Faraday Soc. **51**, 1529 (1955).
[75] M. H. Cardew and D. D. Eley, Faraday Soc. Disc. **27**, 115 (1959).
[76] B. Rosenberg, Nature (Lond.) **193**, 364 (1962).
[77] Sovremennye problemy biofiziki (Modern Problems of Biophysics), Vol. 2, p. 58 et seq., M., IL (1961).
[78] E. A. Liberman, Biofizika **6**, 177 (1961).
[79] Shulman, Walsh, Williams, and Wright, Biochem. Biophys. Res. Commun. **5**, 52 (1961).
[80] Walsh, Shulman, and Heidenreich, Nature **192**, 1041 (1961).
[81] Aleksandrov, Gavrilov, Kiselev, Lazurkin, and Mokul'skiĭ, DAN SSSR **141**, 1483 (1961), Soviet Phys. Doklady **6**, 1121 (1962).
[82] W. Arnold and H. K. Sherwood, Proc. Natl. Acad. Sci. USA **43**, 105 (1957).
[83] A. Szent-Györgyi, Introduction to a Submolecular Biology, Acad. Press, New York, 1960.
[84] G. Jones and S. M. Christian, J. Am. Chem. Soc. **57**, 272 (1935).
[85] B. Rosenberg, J. Chem. Phys. **36**, 816 (1962).
[86] S. Takashima, J. Polymer. Sci. **56**, 257 (1962).

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