

POTENTIAL, IMPEDANCE, AND RECTIFICATION IN MEMBRANES

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INTRODUCTION

Since the early recognition of the value of electrical measurements on biological cells and tissues, the resting potentials of many cells have been determined and have been shown to vary with the degree of activity and with the composition of the external medium. When a steady current is passed, the potential takes on a new value. The ratio of the current to the change in potential, namely the conductance, has also been measured for many cells and has also been found to depend on physiological state and environment. Since ion motion is equivalent to an electric current, the conductance may be taken as an index of the "ion permeability."

The introduction, by Kohlrausch, of A.C. measurements in place of D.C. has allowed more reliable results to be obtained. Further, one is then able to determine not only the conductance, but the capacitance as well. The use of a range of frequencies of the measuring current (Höber, 1912; Gildemeister, 1919), has resulted in a picture of the cell membrane as electrically equivalent to a parallel resistance-capacitance combination, the capacitance having a constant phase angle less than 90° (Philippson, 1921; Cole, 1932).

If the magnitude and direction of the current is varied, it is found that the conductance may change. In particular, it may be large with the current in one direction and small with the current in the other and the system then shows electrical rectification. This phenomenon has been studied in *Valonia* (Blinks, 1930*a, c*), in the squid giant axon (Cole and Baker, 1941; Cole and Curtis, 1941), and in frog muscle (Katz, 1942).

According to the membrane hypothesis of Bernstein, the permeability of the cell membrane increases during activity. Corresponding changes in conductance have been found in many systems and it has recently become possible to study them quantitatively in *Nitella* and in the squid giant axon (Cole and Curtis, 1938, 1939). By contrast, the capacitances appear to be remarkably constant and independent of physiological state and environment. They have thus been interpreted as representing the dielectric properties of fixed elements of the membranes to which they are referred while the conductances and potentials depend primarily on ion mobilities and concentrations and their distributions.

In so far as any of these properties may involve physical rather than metabolic

factors, attempts have been made to duplicate them in artificial systems. Of these, collodion membranes have received the lion's share of attention. In addition to diffusion properties, potentials and conductances have been studied chiefly by Michaelis under a number of conditions (Michaelis, 1929). Blinks (1930 *b*) observed rectification in very thin membranes in certain combinations of environmental solutions, and Sollner and his coworkers (1940, 1941, 1943) have studied some relationships between potentials and structural characteristics. Teorell (1935) and Meyer and Sievers (1936) have pointed out the importance of fixed ions in the determination of potential properties. Structural investigations on biological membranes have proved difficult. However, it seems likely that they consist largely of protein and phospholipid and that there may be an organized arrangement of these elements.

In spite of the amount and variety of information at hand, there is, so far, only one system, the squid axon, on which potentials, conductances, and rectification properties have all been studied. Correspondingly, the analytical methods customarily adopted in interpreting the results are limited in their scope. Thermodynamics, for example, is quite inadequate for handling conductance and rectification phenomena. The kinetic method outlined by Planck (1890) appears capable of fulfilling the desired function. As ordinarily applied, however, it has proved only moderately successful in explaining the relations found in biological systems between the membrane potential and the composition of the medium. Several examples are cited by Steinbach (1940) of cells for which the logarithmic relation between potential and concentration does not hold.

The work to be reported here represents an attempt to obtain simultaneous measurements, in some simple artificial systems, of the electrical properties mentioned and to modify and extend the kinetic theory of ion motion to cover the observed phenomena to the extent that they may be physical rather than metabolic. This will involve a consideration of the fixed structural characteristics of the membrane and of the ionic configuration of the medium. Obviously radical simplifications will have to be adopted thereby vitiating the results somewhat. Nevertheless rough agreement may be hoped for in some cases and such an analysis may also help in clarifying the influence of some of the controlling factors.

Preliminary experiments were made with protein membranes prepared by the method of Dean (Dean, Curtis, and Cole, 1940) measuring the current and potential directly.

An I and J tube, each containing dilute KCl with a trace of tannic acid, were suspended from rack and pinion mounts in a beaker of butyl acetate so as to produce a pair of oil-water interfaces one over the other. Flecks of solid protein were placed on the interfaces and the system was allowed to stand for about a half hour for spreading and stabilization to occur. The J tube was then raised so as to press the interfaces

gently together and the system allowed to stand, this time for several hours. Thereafter a slow change in properties continued but the arrangement could be kept as long as 3 days provided that precautions were taken to avoid mechanical shocks and evaporation of liquid. Scrupulous care was required in handling glassware and solutions.

The best results were obtained with dried, powdered blood serum or egg albumin. It was not found possible to make stable membranes from any of several carefully purified proteins. The membranes could be made in various thicknesses depending on the amount of protein placed on the interfaces. Whether the butyl acetate played an important part in determining the properties of these membranes could not be ascertained.

The rectification observed in these membranes under moderate concentration gradients of salt was rather small indicating that a method of greater sensitivity was needed. The A.C. bridge described below was then introduced and was found quite satisfactory. However, the instabilities were thus magnified considerably. In addition only one pair of solutions could be used with any membrane so that no intercomparison of results was possible. Recourse was then had to thin, dried membranes of collodion to which cephalin or lecithin had been added, the phospholipid introducing a large number of fixed dipolar ions into the membrane. A few measurements were also made on membranes of collodion and of polystyrene, and on onion and tomato cuticles.

Impedances were then determined over a wide frequency range as a function of membrane current in various arrangements of environmental solutions (NaCl, KCl, HCl). Membrane potentials were also measured.

Material and Methods

The plastic membranes were prepared by the method used by Blinks (1930b). The materials were Mallinckrodt Parlodion, a commercial sample of polystyrene, which may have contained a plasticizer, Merck egg lecithin, and cephalin prepared from calf brain. The collodion was dissolved in alcohol-ether. The phospholipid desired was added to the solution. Most of the membranes studied were made from a solution containing 5 per cent collodion and $\frac{1}{2}$ per cent cephalin. Butyl acetate was the solvent for the polystyrene. A small amount of a solution of the material was pressed between two clean glass coverslips and allowed to dry thoroughly. On placing the coverslips in water, the membranes came loose and were then dried and stored between sheets of filter paper. The thicknesses as measured with a micrometer were from 2 to 8 micra.

The measuring cell is shown in Fig. 1. The impedance electrodes were platinized platinum bands 8 mm. wide by 10 mm. in diameter and sealed to the glass 2 mm. from the membrane. Constant current was supplied through a pair of chlorided silver wire coils placed 4 or 5 cm. from the membrane and supported by the stoppers which also held inlet and outlet tubes for running solution through the cell. The membranes were mounted directly between the ground glass lips of the tubes or first placed between a pair of celluloid washers of a convenient size. Of the perfusion tubes, one pair con-

nected each to a reservoir and the other pair to waste beakers. It was thus possible to control independently the solutions bathing the two sides of the membrane. Much of the data was, however, obtained with the electrode tubes used for the protein membranes which had a similar arrangement of electrodes but no facilities for perfusion.

The plant cuticles were stripped, boiled to destroy living tissue, and gently scraped to remove debris.

When impedance measurements without current flow were desired, another cell could be used which had large platinum disc electrodes. It is described in detail by Cole and Guttman (1942).

Measurements were made at room temperatures (20–25°C). The variation in any experiment was less than 1°C.

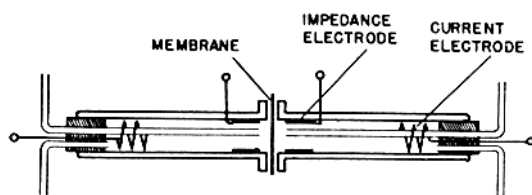


FIG. 1. Measuring cell for solid membranes. The small tubes are for perfusion, the upper pair connecting with reservoirs and the lower pair acting as waste outlets.

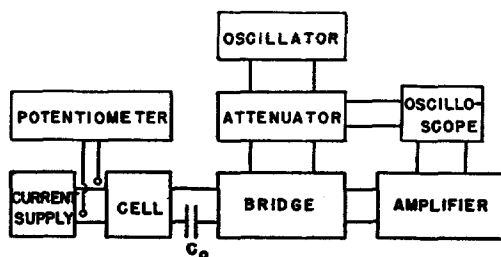


FIG. 2. Block diagram of measuring equipment.

Apparatus

The measuring equipment (Fig. 2) consisted of:

1. A grounded point, a. c. Wheatstone bridge including a Leeds and Northrup "Campbell-Shackleton" ratio arm assembly.
2. A Western Electric telephone type oscillator adjusted for a frequency range of 35 cycles to 100 kc. and a harmonic content of 1 per cent.
3. An attenuator for controlling the input to the bridge.
4. A 4-stage output amplifier, resistance-capacity coupled except for the second stage which could be tuned to the measuring frequency. This was necessary in order to maintain adequate sensitivity by cutting down noise and harmonics.
5. A 3 inch oscilloscope as an indicator. The amplified bridge output was applied to the vertical plates and a portion of the oscillator output applied directly to the

horizontal plates thus providing a 1-1 Lissajous figure which at bridge balance was a horizontal line.

The "unknown" arm of the bridge contained, in addition to the membrane cell, a pentode circuit for supplying constant current independent of membrane and electrode impedances and through a very high internal impedance (10 megohms in parallel with $35 \mu\text{mf}$). The blocking condenser, C_0 , served to keep the membrane system open-circuited and to prevent d. c. from entering the bridge circuit. It was large enough ($21.9 \mu\text{f}$) to have a very small impedance except at the lowest frequencies.

A Leeds and Northrup K-2 potentiometer could be switched in to determine the potential difference between the silver chloride electrodes to within 0.2 mv.

The sensitivity of the bridge was about 0.05 per cent over most of the useful range with a falling off at the extremes of frequency and impedance. Since the "unknown" arm of the bridge may be represented as in Fig. 3, it will be seen that to obtain the equivalent impedance of the membrane a rather complicated set of calculations is, in general, necessary. However, the high value of the current supply circuit and the

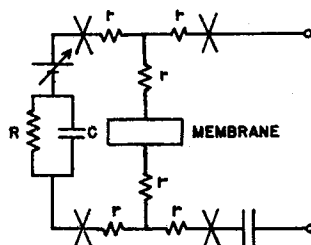


FIG. 3. Equivalent circuit of membrane, cell, and accessories. R and C represent the current supply circuit including stray capacities. Resistances r are for the solution. The X 's are electrode impedances.

low values of the electrodes and blocking condenser allowed, for most of the membranes, the neglecting of these corrections and impedance values were then readily obtained which were accurate to about 1 per cent.

EXPERIMENTAL

For purposes of analysis, a large number of measurements were required on a single membrane. It is therefore pertinent to discuss the experimental procedure in some detail. The concentrations of the environmental solutions were chosen largely with an eye to bringing the resistances within the optimum range of the bridge (10^3 to 10^6 ohms). This usually resulted in the use of 0.01 to one molar solutions approaching the condition found in biological systems rather than the high dilution which has been found most useful in electrochemical studies. Also, the areas and thicknesses represented a compromise between the factors of uniformity, mechanical strength, and impedance values.

When the membranes were clamped in position and the solutions added to the cell, some time was required for the impedance to reach a steady value.

With fresh, dry membranes this was 2 or 3 hours, varying with the type and thickness of the membrane used. Replacement by another solution was followed by a more rapid attainment of a steady value. If the same solution bathed both sides it was occasionally replaced before beginning the measurements. If different solutions were used, a continuous flow of solution was kept up ($\frac{1}{2}$ to 1 cc./minute) to maintain as nearly as possible constant boundary values of the concentrations. However, the effectiveness of such low rates of flow is questionable and it seems worth while to attempt an estimate of the diffusion rate of salt when there is no perfusion.

The diffusion rate, S , in mols/cm.²-second is related to the concentration difference across the membrane by the equation

$$S = \alpha \frac{n' - n^0}{a}$$

where α is the diffusion constant, n' and n^0 are the membrane concentrations at the two sides, and a is the thickness. Also the mobility $u = \frac{F}{RT} \alpha$ where F , R , and T have their usual significance of Faraday, gas constant, and absolute temperature. Then the relation may be written.

$$S = \frac{RT}{F^2} \left(\frac{uFn'}{a} - \frac{uFn^0}{a} \right)$$

But $\frac{unF}{a}$ is the conductance, Λ , of the membrane (per cm.²) at a salt concentration n . Hence

$$S = 2.5 \times 10^{-7}(\Lambda' - \Lambda^0)$$

Λ , however, is known. At a concentration of 1 molar in the adjacent solution, it is usually less than 10^{-3} mhos/cm.² and decreases with dilution. Thus $S < 10^{-10}$. If the solution on the other side is at 0.1 molar, this means an increase of 0.1 per cent in its concentration during the 1st second. Reference to Fig. 4 shows that the estimate is roughly consistent with the observed changes. It will also be seen that changes in conductance with current are much less affected. Hence, except with membranes of high conductance under a large concentration gradient, perfusion does not appear to be of fundamental importance within the limits of reproducibility attainable.

The most important limitation encountered was that due to prolonged soaking of the membranes most of which appeared to undergo a slow, steady dissolution resulting, perhaps, from swelling or from loss of phospholipid or other material. There was a gradual increase in conductance which could amount to as much as 20 per cent in 24 hours. While this was relatively unimportant for a single set of impedance-frequency or impedance-current data, it destroyed

any hope of obtaining more than an approximate intercomparison of results such as is required for any extended analysis of the membrane characteristics.

The impedance measurements were made with a very small bridge input corresponding to 1 or 2 microamperes through the membrane. Compared with the polarizing currents of up to 30 microamperes and in view of the magnitudes of the conductance changes, this is small enough for the impedances so determined to represent quite well the slope of the current-voltage curve of the membrane at the "operating point" specified by the steady current. It is also small enough to avoid any interference of the non-linearities with the bridge balance. A set of data could usually be obtained in about 15 minutes, the 1 kc. values being checked after the run to make sure that no large drifts had occurred.

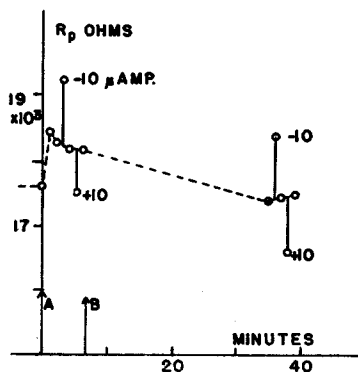


FIG. 4. Changes of membrane resistance with flow of solution. Collodion-cephalin membrane. Left hand side of membrane in 0.1 N KCl, right side in 1 N KCl. At A the flow was started, at B it was cut off.

The impedance-current runs were taken with the D.C. in alternate directions and flowing only long enough to allow rebalancing the bridge. This served both to minimize residual accumulation effects and to maintain the current supply electrodes in a symmetrical condition.

While the current supply circuit had a capacity of more than 1 milliampere, it was found that if the applied field in the membrane exceeded a few thousand volts per centimeter there was a tendency for the membrane conductances to undergo irreversible changes. Accordingly the results obtained here correspond to a small part only of the entire current-voltage curves of the membranes and preclude the attainment of any limiting conductance values. However, the intrusion of possible Wien effect was thereby ruled out as were also any effects due to heating.

The blocking condenser, C_0 , provided a convenient alternative to a counter E.M.F. in series with the membrane. It also acted as a delay device to the

passage of d.c. through the membrane. When the membrane resistance changed with current flow, the rise in current through it on switching in the supply circuit was distorted from the simple, exponentially delayed form although the time to reach a practical steady state was not usually altered very much. This effect of C_0 could be checked by changing its value or by shorting it out altogether. In the latter case it was necessary to correct for the current lost into the bridge network. It was then found that except for membranes of the very highest resistance the steady state was reached too rapidly to be seen on the oscilloscope face. This indicated that the "time constant" was less than 0.1 second. The procedure was also useful in showing the absence of slow accumulation effects of current flow as it served to spread out on a linear time scale the impedance-current curve on the oscilloscope.

In general the non-linearities of the membranes studied were not large and it was necessary to make sure that the apparatus itself did not contribute to any of the effects seen. When the membrane cell was replaced by a conventional R-C network, and the behavior of the electrodes and solutions observed without the membrane, it was found that within the range of measurement used such effects were negligible. Two further points should be made. First, with the a.c. equipment used, steady potentials have no effect on the bridge balance. Second, the solution resistances are so much smaller than the membrane resistance that changes in the former due to current flow are very small. It follows that any impedance variations observed must be due to changes within the membrane itself.

Membrane potentials were determined from time to time. As the membranes were allowed to stand in solution, the potentials underwent slow, irregular changes of a millivolt or two so that the same limitation applies here as to the impedance measurements. At the end of each experiment control runs were made.

The impedances were recorded as parallel resistance, R_p , and capacitance, C_p . For ease in handling corrections and for convenience of plotting, the data were frequently converted to the equivalent series resistance and reactance using the formulae

$$r_s = \frac{R_p}{1 + (R_p C_p \omega)^2}$$

$$x_s = -\frac{R_p^2 C_p \omega}{1 + (R_p C_p \omega)^2}$$

Elimination of ω between these two equations leads to the impedance locus while elimination of R_p leads to a locus showing the path followed by the impedance when the resistance varies. Since C_p and ω occur together, the impedance locus is also the locus for varying capacitance (Cole, 1932; Cole and Curtis, 1938).

RESULTS

When the same solution bathed both sides of the membrane and in the absence of current flow, the observed impedance properties were entirely consistent with the results already obtained by other investigators.

1. The membranes behave like parallel resistance-capacity combinations.
2. The capacitances have a phase angle less than 90° and independent of frequency. For the protein membranes the values found were $65-75^\circ$, for collodion-lecithin $79-82^\circ$, for collodion-cephalin $84-86^\circ$, for collodion alone $88-89^\circ$, and for onion cuticle $83-85^\circ$.
3. The conductances are roughly proportional to those of the environmental solutions (*cf.* Green, Weech, and Michaelis, 1929) but are much smaller, although somewhat larger than the estimated values for the membrane material in bulk. For example, conductances in mhos/cm.² in 0.01 normal KCl were: for protein membranes $10^{-2}-10^{-4}$, for the plastics $10^{-5}-10^{-6}$, depending on the thickness and for onion skin about 5×10^{-5} . Bulk nitrocellulose has a specific conductance* of $10^{-10}-10^{-11}$ mhos/cm.
4. The capacitances vary slightly from one solution to another. The dielectric constants are somewhat larger than those of the bulk membrane material. As to values in $\mu\text{f}/\text{cm}^2$ at 1 kc. we find for the protein membranes 0.005-0.5, for the plastics $5 \times 10^{-4}-8 \times 10^{-3}$, for onion cuticle 0.1-0.2. Bulk nitrocellulose has a dielectric constant* of about 8.
5. The phase angles show little if any variation with kind or concentration of salt.

The above statements should be qualified somewhat. The membranes containing phospholipid and those made of dried blood serum showed a slight but definite tendency to depart at the lowest frequencies from the simple impedance loci (circular arc) in such a way as to indicate the presence of a low frequency component beyond the range of the bridge (*cf.* Fig. 5). Since phospholipids and proteins are known to have large electric moments (Hausser, 1935; Oncley, 1940) and since these materials are rather heterogeneous, this is not surprising. However, it emphasizes the danger of interpreting extrapolated zero frequency values obtained from loci as the actual D.C. resistances of the membranes. In any case the extrapolated value may be regarded as representing a resistance component whose variations are to be considered.

Nothing is known of the values or variations of partition coefficients and, in view of the deterioration of the membranes, the relation between membrane and solution resistances cannot be further analyzed at present.

When the two sides of the membrane were in solutions of different salts or different concentrations of the same salt, the observed impedances were in

* Handbook of chemistry and physics, Cleveland, Chemical Rubber Publishing Company, 24th edition, 1940.

between the values seen in either solution alone. The mode of variation was not a direct function of concentration or conductance and will be considered later.

The membrane impedance varies with the current; and the variation is such that it can be interpreted as a change in conductance only. The parallel capacitances as read on the bridge showed very little change and, since the corrections are small, these values are close to the actual equivalent capacitances. The loci show that the impedance changes with current flow follow closely the theoretical arc for a resistance change only (Fig. 5). Furthermore since the equivalent circuit can be represented as a membrane conductance in parallel

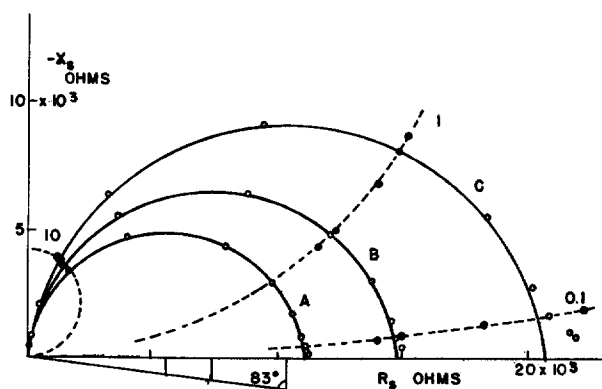


FIG. 5. Impedance loci for a collodion-cephalin membrane in three arrangements of KCl. Curve A, 1 N on both sides; B, 0.3 N on left, 1 N on right; C, 0.1 N on left, 1 N on right. Open circles are locus points with no current flow; solid circles, ± 15 microamperes. Numbers outside large arc refer to measuring frequency in kilocycles. The dashed arcs are theoretical loci for changes in resistance only. All data taken within 48 hours.

with a membrane capacitance having a constant phase angle, and since the series conductance of the adjacent solution is very much larger than the membrane conductance, it can be shown that, except at very high frequencies, the conductance changes with current are independent of frequency. Within the limits of reproducibility of the data, this was found to be true. One is thus allowed the use of a single frequency in making impedance-current measurements. Most of the data were obtained at 1 kc.

A set of conductance-current curves is given in Fig. 6 for a collodion-cephalin membrane in several arrangements of KCl and more complete data on this membrane are given in Table I. This behavior is typical of these membranes. At higher currents, however, there was frequently a tendency for the high conductance side of the curves to show a reversal. Unfortunately the onset of irreversible changes makes these measurements unreliable.

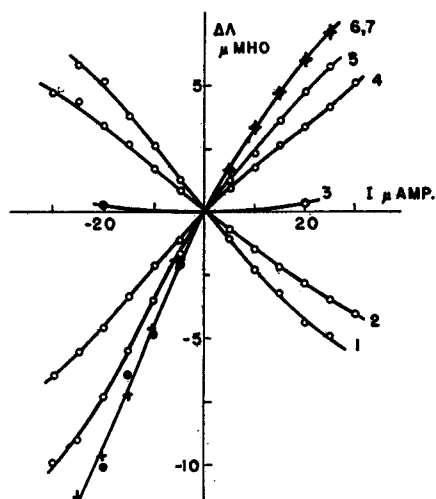


FIG. 6. Conductance as a function of current in a collodion-cephalin membrane for different concentration ratios of KCl. The concentration on the right hand side of the membrane was kept fixed at 0.2 N and that on the left side was varied from curve to curve. Ratio r is concentration on right hand side of membrane to that on left hand side. For curve 1, $r = 0.2$; curve 2, 0.4; curve 3, 1; curve 4, 2; curve 5, 3; curve 6, 5; curve 7, 7. No perfusion. All data taken within 30 hours. Conductance values for no current and no concentration gradient; 82.5 micromhos initially, 98 micromhos at end of experiment. The positive direction of the current is from left to right through the membrane. For further data on this membrane see Table I.

TABLE I

Collodion membrane containing 10 per cent cephalin. KCl solutions. Right hand side 0.2 N, left side variable. Area 0.8 cm.² Thickness 3×10^{-4} cm. Sign of potential is that of left side with respect to right side as measured in external circuit.

$$V_0 = V - 58 \log r$$

| Conc. ratio right/left | Specific conductance | ϵ at 1 kc. | $\frac{d\Delta}{dI} _0$ per volt | V total | V_0 membrane |
|---------------------------|-------------------------|---------------------|-------------------------------------|--------------|-------------------|
| | <i>mhos/cm.</i> | | | <i>mv.</i> | <i>mv.</i> |
| 0.2 | 8.4×10^{-8} | 21 | -0.24 | -37.5 | 3.1 |
| 0.4 | 5.4 | 20 | -0.17 | -19.8 | 3.3 |
| 1.0 | 3.3 | 18 | 0.0 | 0.0 | 0.0 |
| 2 | 2.6 | 18 | 0.20 | 15.5 | -1.9 |
| 3 | 2.2 | 18 | 0.29 | 25.5 | -2.2 |
| 5 | 1.9 | 18 | 0.38 | 37.5 | -3.1 |
| 7 | 1.8 | 19 | 0.37 | 44.5 | -4.6 |

In some systems the reversal occurred at smaller current values. An onion cuticle in HCl gave the curve shown in Fig. 7. This also happened with some of the collodion membranes especially after long soaking. The data on the latter membranes and on those from the polystyrene sample are incomplete, but suggest that a somewhat more complicated situation exists, especially with respect to the variation of rectification properties with concentration gradient. Even greater complexities were sometimes seen in the plant cuticles.

When different chlorides bathed the two sides of the membranes similar variations were found. The non-linearities were, however, usually greater even though the concentrations or conductances of the two solutions might be the same. In general, the amount of rectification appeared to vary with the membrane potential rather than with concentration or conductance gradient.

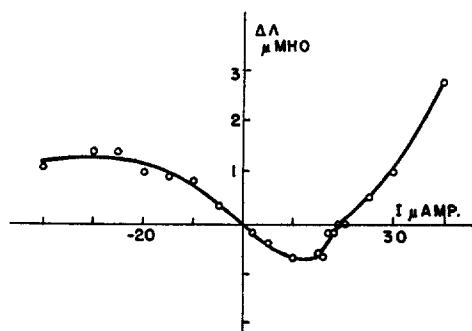


FIG. 7. Change in conductance with current in an onion cuticle in HCl, 0.01 N on left side of membrane, 0.1 N on right side. Conductance at no current, 132 micromhos. Positive current flows from left to right through membrane.

The potentials as measured were the sums of the membrane and electrode potentials. In most cases these total potentials were not far from the calculated concentration potentials of the electrodes. This, in addition to the drifts, made the membrane potential values quite unreliable. Nevertheless, there appeared to be a definite departure, especially in the membranes containing phospholipid, from the proportionality seen in solution between potential and the logarithm of the concentration ratio. It is, of course, impossible at present to make activity coefficient corrections for the membranes. Data for three membranes are given in Fig. 8.

It is evident from the foregoing that the results are not yet sufficiently complete or reproducible to justify more than a first approximation in an analytical treatment of the problem of the current flow in such systems. The experimental results may be summarized as follows. The smallness of the membrane potentials does not allow a definite statement as to whether or not the logarithmic relation between potential and concentration gradient is followed.

These membranes are thus quite non-selective in their action contrasting with many of those studied by Michaelis. The difference may be due either to the presence of the phospholipid plasticizers or to the thinness of the membranes. Sollner and Carr (1943) have shown that concentration potentials in collodion membranes decrease as the membrane is made thinner.

The conductances are proportional to those of the solutions and are small enough to suggest that the membranes have a relatively tight packing of structural elements. The dielectric constants then suggest a definite effect of the phospholipid. As to the rectification, the conductances appear to vary di-

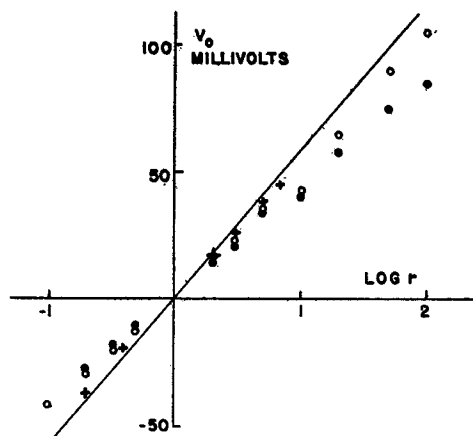


FIG. 8. Potential of membrane plus electrodes *vs.* log of concentration ratio r . Crosses, collodion-cephalin in KCl; open circles, collodion-cephalin in NaCl; solid circles, collodion in NaCl. Sign of potential is that measured in external circuit. The actual membrane potential is the difference between the total potential and the electrode potential calculated from the simple Nernst formula as given by straight line. The slope of the line is 58 mv. per 10:1 ratio but would be somewhat less if activity coefficients in solution were included.

rectly with the current within the range studied and for the collodion-cephalin membranes at least. Taking the initial slope of the conductance-current curves as an index of the amount of rectification, this is found to increase with the membrane potential. For larger currents, other factors may enter and in some types of membrane may obscure the simpler behavior by appearing at small current values.

Theory

Consider a solid membrane, of thickness a , immersed in solution and containing ions moving under the combined influence of diffusion and electrical forces. The membrane is assumed to be a uniform system having a dielectric

constant ϵ , and in which the ion mobilities and activity coefficients are constant. Let V be the potential at a point x ; n_i , the concentration of the i th ion; z_i , its algebraic valence; and $z_i u_i$, its mobility. Then the contribution, j_i , to the total current density, J , is given by the relation

$$j_i = -z_i u_i RT \left(\frac{dn_i}{dx} + z_i n_i \beta \frac{dV}{dx} \right) \quad (1)$$

where

$$\beta = \frac{F}{RT}$$

The potential distribution is related to the net charge density ρ through Poisson's equation

$$\frac{d^2V}{dx^2} = -\frac{4\pi}{\epsilon} \rho \quad (2)$$

ρ being expressed in terms of the ion concentrations, fixed as well as mobile.

$$\rho = F(\sum z_i n_i + \bar{N}) \quad (3)$$

where the summation extends over the mobile ions and \bar{N} is the contribution of the fixed ions.

The assumptions call for some comment. The membranes dealt with consist of large molecular elements some of which may be ions. There appears to be a rather tight packing. If, then, the membranes are to be regarded as having a sieve-like structure, the "holes" must be almost of molecular dimensions (*cf.* Michaelis, 1929). It therefore seems reasonable to suppose that the current carriers pass through more or less randomly distributed interstices in the structure which is thus assumed uniform normal to the direction of flow. For many biological membranes this assumption is made in the absence of evidence to the contrary. There is less ground for postulating uniformity in the direction of flow. The method of preparation of the artificial membranes suggests that there may be oriented layers at the surfaces. Moreover, biological membranes may be highly organized or have a periodic structure.

The influence of the dielectric constant may be great. If ϵ is small, ion association is promoted (Bjerrum, 1926) and the Wien effect augmented (Onsager, 1934) although the presence of a water shell around the ions would tend to counteract this. However, for the present purpose, we have only to assume ϵ constant. Mobilities and activity coefficients actually depend on concentration. For ranges which are not too large, their constancy may reasonably be postulated.

We must also adopt simple boundary conditions. Since the system as a whole is electrically neutral,

$$\int \rho \, dx = 0$$

and if one assumes that the influence of external surface charges is small, the limits of integration are the boundaries of the membrane. Further, there must be a point at which $\rho = 0$, such that the total charge on one side of it, equal and opposite to that on the other, represents the equivalent condenser charge on the membrane, whether it be confined to the surfaces or distributed throughout. Evidently boundary layers may play a considerable part especially in the determination of the resting potential of the system. We shall, however, neglect them entirely in order to keep the problem in relatively simple form. The values of the concentrations and potentials at the boundaries will be considered as fixed, and the surface charge as zero.

We now have a set of equations and conditions from which, ideally, the current-voltage relation of the membrane may be derived. This set is not integrable in closed form as it stands however, and we are forced to introduce still further simplification if usable results are to be reached. The nature of the equations does suggest that, in general, a linear relation will not occur and that rectification will be more evident the greater the asymmetry of mobilities and concentrations. These same factors also determine the membrane potential and so the finding that the initial slope of the conductance-current curve increases with the membrane potential may be expected in any case.

Since equations (1) are linear in n_i , an explicit expression for the field distribution will lead directly to an equation for J . Practically, this means that we must find a simple treatment for equation (2). This appears to be possible in two ways. First, if there are no fixed ions present and if we assume microscopic electroneutrality, *i.e.* $\rho = 0$ everywhere, we have the situation dealt with by Planck in studying liquid junction potentials. However, the equations may also be solved for a finite fixed ion concentration, \bar{N} , and although we shall not make use of the results in the general form, it is of some interest to see how the presence of the fixed charges affects the various distributions. We consider univalent ions only.

Write equations (1) for each ion, divide each through by the corresponding mobility, and add separately the positive and negative contributions. Then

$$-\sum + \frac{j_i}{u_i RT} = \frac{d}{dx} \sum + n_i + \beta \sum + n_i \frac{dV}{dx}$$

$$\sum - \frac{j_i}{u_i RT} = \frac{d}{dx} \sum - n_i - \beta \sum - n_i \frac{dV}{dx}$$

Add and subtract these, introduce equation (3) remembering that $\rho = 0$, and let

$$\sum + n_i + \sum - n_i = N$$

(the total concentration of mobile ions). Then

$$\frac{dN}{dx} - \beta\bar{N} \frac{dV}{dx} = B \quad (4)$$

$$\beta N \frac{dV}{dx} = -gB \quad (5)$$

where B and g are given from the previous equations but are to be determined from the boundary conditions.

(4) integrates at once to

$$N - \beta\bar{N}V = Bx + \text{constant} \quad (6)$$

We now introduce the limits

$$\text{Let } N = N^0 \text{ and } V = 0 \text{ at } x = 0.$$

$$N = N' \text{ and } V = -\Delta V \text{ at } x = a, \quad N' - N^0 = \Delta N$$

Then

$$B = \frac{\Delta N + \beta\bar{N}\Delta V}{a} \quad (7)$$

Eliminate $\frac{dV}{dx}$ between (4) and (5) and integrate.

$$\beta V = -g \ln \frac{N - g\bar{N}}{N^0 - g\bar{N}} \quad (8)$$

and

$$\beta\Delta V = g \ln \frac{N' - g\bar{N}}{N^0 - g\bar{N}} \quad (9)$$

which determines g .

We may now return to equations (1), substitute from (5) and (8) to eliminate x and N , integrate, and rearrange.

Then, finally,

$$j_i = \frac{u_i RT}{a} (\Delta N + \beta\bar{N}\Delta V)(g - z_i) \frac{n_i' e^{-z_i\beta\Delta V} - n_i^0}{N' e^{-z_i\beta\Delta V} - N^0 - z_i\bar{N}(e^{-z_i\beta\Delta V} - 1)} \quad (10)$$

where $z_i = \pm 1$ and, of course, $J = \sum j_i$. Certain features of this result are of interest. When $\bar{N} = 0$ (the Planck case), the potential distribution is

logarithmic and the total concentration is linear although the individual ion concentrations are not. If \bar{N} is not zero, the potential distribution is more complicated and the total concentration distribution is no longer linear. Evidently the current-voltage relation is, in general, non-linear as well. One might also be tempted to speculate on the biological implications of such a curve were it justified either by experimental work or by the nature of the analytical simplifications. We shall consider only the case where $\bar{N} = 0$, but before doing this we take up an entirely different method of handling the differential equations.

We assume that the membrane contains a large number of dipolar ions near the isoelectric point and that these can act to minimize distortion of the field especially at low currents. We then approach a situation in which the field is constant and are led to a solution analogous to that given by Mott (1939) for electronic conduction in the copper-copper oxide rectifier. If the field is constant, equations (1) may be integrated directly and the limits introduced. Then

$$j_i = \frac{u_i F}{a} \Delta V \frac{n'_i e^{-z_i \beta \Delta V} - n_i^0}{e^{-z_i \beta \Delta V} - 1} \quad (11)$$

We may now proceed to indicate some of the consequences of the two types of treatment as being very rough approximations but as being simple enough to yield readily workable relations with experiment.

Consider a single electrolyte with a concentration gradient across the membrane. Then equation (10) reduces to an expression of Ohm's law, and we obtain

$$J = \frac{F}{a} (n' - n^0) \left[(u_+ + u_-) \frac{\Delta V}{\ln r} - \frac{u_+ - u_-}{\beta} \right] \quad (12)$$

where

$$r = \frac{n'}{n^0}$$

Further, if $J = 0$, we get the usual Planck formula for the liquid junction potential

$$V_0 = \frac{1}{\beta} \frac{u_+ - u_-}{u_+ + u_-} \ln r \quad (13)$$

Correspondingly, we obtain from equation (11)

$$J = \frac{F}{a} \Delta V \frac{(u_+ n^0 + u_- n') - (u_+ n' + u_- n^0) e^{-\beta \Delta V}}{1 - e^{-\beta \Delta V}} \quad (14)$$

and

$$V_0 = \frac{1}{\beta} \ln \frac{u_+ n' + u_- n^0}{u_+ n^0 + u_- n'} \quad (15)$$

The Planck equation (12) then predicts no rectification at all and a linear relation between V_0 and $\ln r$ while the constant field assumption yields relations which are non-linear in both cases.

Another situation arises when the membrane separates two different electrolytes having a common ion. Then both hypotheses result in non-linear current-voltage curves. If the total concentration is the same on both sides, the two are identical. This is to be expected since it is then possible for ions of one sign to replace each other freely without distorting the field.

The constant field case has the advantage that a general expression may be given, for any salt combination, by writing

$$\Lambda_+ = \frac{F}{a} \left[\sum_+ u_i n_i^0 + \sum_- u_i n_i' \right]$$

and

$$\Lambda_- = \frac{F}{a} \left[\sum_+ u_i n_i' + \sum_- u_i n_i^0 \right] \quad (16)$$

Then

$$J = \Delta V \frac{\Lambda_+ - \Lambda_- e^{-\beta \Delta V}}{1 - e^{-\beta \Delta V}} \quad (17)$$

Evidently, Λ_+ and Λ_- represent limiting conductance values for large potentials in one direction or the other. Also,

$$V_0 = \frac{1}{\beta} \ln \frac{\Lambda_-}{\Lambda_+} \quad (18)$$

For comparison with experimental data, we want Λ and $\frac{d\Lambda}{dJ}$ at $J = 0$. By differentiation,

$$\Lambda_0 = \beta V_0 \frac{\Lambda_- \Lambda_+}{\Lambda_- - \Lambda_+} \quad (19)$$

$$\left. \frac{d\Lambda}{dJ} \right|_0 = -\beta L \left(\frac{\beta V_0}{2} \right);$$

$$L(x) = \operatorname{ctnh} x - \frac{1}{x}$$

or, for V not too large,

$$\left. \frac{d\Lambda}{dJ} \right|_0 = -\frac{\beta^2 V_0}{6} \quad (20)$$

The corresponding Planck expressions for the single electrolyte are

$$\Delta_0 = \frac{F}{a} (u_+ + u_-) \frac{n' - n^0}{\ln r}$$

$$\frac{d\Delta}{dJ} = 0 \tag{21}$$

DISCUSSION

Having obtained explicit expressions for the membrane potential and for the conductance and its variation with current, we are in a position to see how far the data justify the above simplifications. First as to the membrane potentials. Equation (15) predicts a limiting value of the potential when the concentration ratio becomes very large or very small. It can be seen from Fig. 8 that the membrane potentials of the artificial systems are quite small. Then the ion mobilities are nearly equal and there is little difference between the two theoretical treatments. The results are not accurate enough to allow conclusions to be drawn.

On the other hand, the giant axon of the squid, *Loligo pealii*, in common with many biological membranes, appears to allow K^+ to pass much more easily than other ions. On the basis of our previous assumptions, we use data given by Curtis and Cole (1942 and unpublished) where the membrane potential was measured directly in solutions equivalent to sea water in which the sodium was replaced by varying amounts of potassium. The 10 mv. liquid junction correction was made as given in the paper cited and a small correction for a sea water-experimental solution junction was ignored. We may then calculate a mobility ratio $m = u_-/u_+$ on either theoretical picture, since both yield

$$\frac{dV_0}{d \ln r} = \frac{1 - m}{1 + m}$$

near $V_0 = 0$, and so obtain a complete curve. Fig. 9 shows such a curve together with the experimental data. Further, the value of r then found at the resting potential is 34. From chemical analysis, Bear and Schmitt (1939) give $r = 26$, and Webb and Young (1940) give $r = 29$ for the closely related species, *Loligo forbesi*. The data cited by Steinbach (1940) on potential variations with environmental K^+ concentration, appear to be of the same type.

The low value of 0.06 for the mobility ratio is consistent with the assumptions. Osterhout (1930) has found an even lower value (0.012) in *Nitella*. For such a ratio, enormous values of r would be required to produce any great deviation of the theoretical curve (15) from the linear relation.

The variation of conductance, at no current, with concentration gradient

might also be used for comparison. If, in equations (12) or (14), we take out a factor

$$\Lambda^* = \frac{F}{a} n^0(u_+ + u_-)$$

this should be independent of r . However, this procedure is subject to serious interference from drift effects both for the artificial membranes and for the squid axon.

When we turn to the interpretation of the rectification properties, we find the Planck treatment useless since for the single salt it predicts no rectification

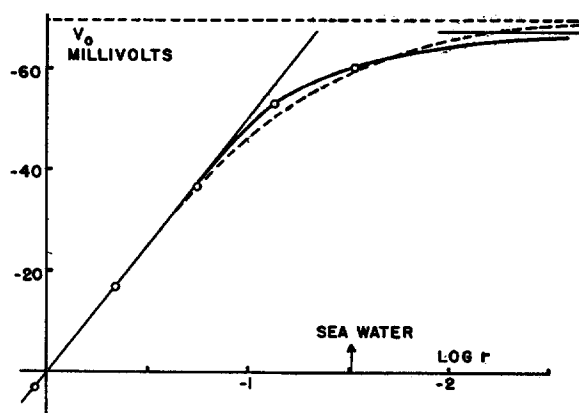


FIG. 9. Membrane potential of squid axon as a function of the ratio r of external to internal K^+ concentration. Circles and solid lines are experimental data. Dashed line is theory for $u_-/u_+ = 0.064$. Straight line is Planck equation.

at all and for the equal concentration case of two different salts it reduces to the same form as the constant field equation.

Equation (20) is a linear relation between the membrane potential and the initial slope of the conductance-current curves. Fig. 10 shows that for the artificial membranes this relation is followed only to an order of magnitude.

Blinks' (1930 *b*) results on collodion membranes for two different salts seem to fit the general picture rather well although his data are given in terms of d.c. measurements.

The only biological system on which adequate measurements have been made is, again, the squid axon (Cole and Curtis, 1941, and unpublished) and here also there is the difficulty of obtaining enough simultaneous data. There are several ways in which a comparison may be made. The ratio of limiting conductances may be put into equation (18) to get V_0 . This yields values of 70 to 100 mv. The resting potentials actually found ranged from 55 to 70 mv. Inspection of equation (17) reveals that it has two asymptotes intersecting at

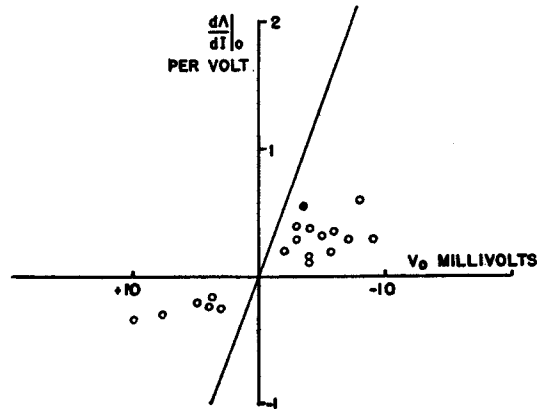


FIG. 10. Rate of change of conductance with current as a function of membrane potential for several artificial membranes and solutions. Open circles, concentration gradient of a single salt. Solid circle, two different chlorides at the same concentration. Two other such points could not be plotted as they fell outside the area of the diagram somewhat below the theoretical curve. Sign of potential as measured in external circuit.

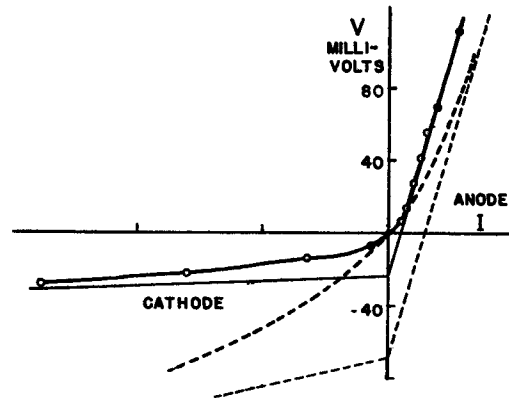


FIG. 11. Current-density *vs.* potential for squid axon. Circles and solid lines are experimental data. Dashed lines, theory for $V_0 = 70$ mv. The straight lines are the corresponding asymptotes. Since the actual membrane resistances are imperfectly known, the abscissa units are arbitrary.

$J = 0$ and $\Delta V = V_0$. In this way we obtain from the experimental curves 15 to 30 mv. Further, the ratio of limiting slopes may be used in equation (19) to get Λ_0 and here values are found 50 to 200 per cent of the actual ones. A curve is given in Fig. 11. It is thus evident that while the simple theory can account fairly well for the variation of resting potential with the external K^+ concentration, the axon is a much better rectifier than is predicted.

The rectification properties of the axon fade away as it dies or is narcotized (Guttman and Cole, 1941). This indicates either that the integrity of the membrane is affected or that the rectification depends on metabolic phenomena which supply a source or sink of K^+ . The present study appears, however, to strengthen the idea that the so called subthreshold electrical properties are interpretable in terms of a physical picture which does not involve metabolic activity. Cole and Marmont (1942) have shown that the impedance properties can vary considerably with the constitution of the environmental medium. As another possibility, Danielli (1941*a*) has suggested an interpretation of the "permeability" changes of nerve in terms of a bimolecular layer which could be profoundly altered by the enormous electric fields which might then be present.

The foregoing analysis has been applied to systems for which a relatively simple structure might conceivably be postulated. In the presence of an asymmetrical membrane or of large charge asymmetries the complexities are much greater. Indeed, the more simple systems are themselves a severe strain on the analytical treatment and the rough correspondence observed may be fortuitous. The neglect of surface and body charges has been justified on the grounds of convenience, and while it is easy to show that external surface effects cannot contribute appreciably to the rectification, the internal charges cannot be so lightly dismissed. For example, insulating oils have been found to have a markedly non-linear field distribution (Whitehead and Minor, 1935) where the indifferent electrodes refused to allow the passage of ions and so caused a back diffusion into the body of the fluid. Labes (1932) has given a discussion similar to the Planck treatment for a membrane penetrated only by cations and has included surface charges and phase boundary potentials. The results do not appear to differ markedly from the simpler case.

Another approach is possible. If the material is dense enough, the ions may have to jump from point to point over potential barriers. This idea has been successfully applied to conduction in glass by Maurer (1941) and has been suggested by Danielli (1941*b*) for biological membranes. Whether such dense, rigid structures occur in any of the cases considered here is doubtful.

From the experimental point of view, the wide range of possibilities and the lack of uniform, stable material has both necessitated and interfered with the obtaining of a maximum of relevant data for each membrane. With biological systems this difficulty must be met in terms of the system studied. On the other hand, the present state of the technology of synthetic plastics encourages the belief that it should be possible to find materials for artificial membranes relatively free from this disadvantage. It is evident that materials are needed whose composition can be accurately stated and it would be particularly helpful to have data on membranes of a completely inert nature.

Diffusion studies have yielded much information about membrane properties. The analysis given here indicates that non-linearities are to be expected in

this case as well and that Fick's law may be no more valid than Ohm's law when applied to the entire membrane. This may have a bearing on some diffusion processes in biological systems.

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SUMMARY

Impedance and potential measurements have been made on a number of artificial membranes. Impedance changes were determined as functions of current and of the composition of the environmental solutions. It was shown that rectification is present in asymmetrical systems and that it increases with the membrane potential. The behavior in pairs of solutions of the same salt at different concentrations has formed the basis for the studies although a few experiments with different salts at the same concentrations gave results consistent with the conclusions drawn.

A theoretical picture has been presented based on the use of the general kinetic equations for ion motion under the influence of diffusion and electrical forces and on a consideration of possible membrane structures. The equations have been solved for two very simple cases; one based on the assumption of microscopic electroneutrality, and the other on the assumption of a constant electric field. The latter was found to give better results than the former in interpreting the data on potentials and rectification, showing agreement, however, of the right order of magnitude only. Although the indications are that a careful treatment of boundary conditions may result in better agreement with experiment, no attempt has been made to carry this through since the data now available are not sufficiently complete or reproducible. Applications of the second theoretical case to the squid giant axon have been made showing qualitative agreement with the rectification properties and very good agreement with the membrane potential data.

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