THE POTENTIOMETRIC ANALYSIS OF MEMBRANE STRUCTURE AND ITS APPLICATION TO LIVING ANIMAL MEMBRANES

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In 1911 Loeb and Beutner concluded from experiments on the concentration potentials of the apple-skin that it is specifically permeable for cations. Since then electromotive forces produced by living membranes and organs in contact with salt solutions have often been used in analysing living membranes. In particular Osterhout has succeeded in elucidating the fine structure of the membrane of large plant cells by potentiometric analysis, while Höber (1926) and others investigated the ion permeability of animal membranes. In spite of such important results, it is not yet quite clear, whether the cation or anion selectivity which can be deduced directly from the measured potential is due to the sieve structure of the membrane or its charge or its specific dissolving power, or to all these factors taken together. A more detailed analysis of these properties will therefore be possible only if it is based on quantitative relations between the measured potential and those properties of the membrane or of the living tissue which influence the passage of ions. In order to make a survey of all these factors it seems reasonable to visualize first of all the molecular structure of living membranes.

Living membranes, or any other living matter, can be considered as a network of flexible primary valence chains, in particular protein chains (K. H. Meyer, 1928, 1929; Seifriz, 1928). These chains are interlinked at definite places by secondary valences or cross-linkings, called by us "regions of contact" (Haftstellen) and later by Frey-Wyssling (1938) "points of contact" (Haftpunkte). Intracellular fluid, ions, lipoids, and soluble globular proteins are located inside this network. The lipoids are mostly linked to apolar groups of the protein chains, for which the term lipophilic groups was proposed, while the hydrophilic groups of the chains are surrounded by water or aqueous cellular liquid and the ionised groups, e.g. --COO− groups, are neutralised by mobile "counter-ions."

A natural membrane can moreover consist of layers of different composition under which lipoid layers can exist, and these layers can in turn be formed of areas of different properties like a mosaic.

Calculation of the Membrane Potential

If a membrane, i.e. a layer of any kind separates two solutions of a binary electrolyte, an electromotive force E results from diffusion of the two solutions...
through the layer. According to the Nernst equation its value is determined by the ratio of the transport numbers \( \frac{n_K}{n_K + n_A} : \frac{n_A}{n_K + n_A} \) which is equal to the ratio \( n_K / n_A \): \( n_K \) being the number of cations and \( n_A \) the number of anions passing through the membrane per time unit.

\[
\frac{dE}{dc} = \frac{RT n_K - n_A}{F (n_K + n_A c)}
\]

The ratio \( n_K / n_A \), and therefore the value of \( E \) depends upon several factors.

1. The ratio \( U_K / U_A \) of "migration velocities" or "mobilities" of cations (\( U_K \)) and anions (\( U_A \)) through the membrane. A network with narrow pores allows the passage of small ions and holds up the larger ions ("sieve effect"). \( U_K / U_A \) assumes thus a different value than it has in water. In a membrane, the pores of which are filled with a non-aqueous liquid, e.g. a lipoid, the ratio, \( U_K / U_A \) is also different from its value in water.

2. Membrane Selectivity. A network of chain molecules with laterally affixed ionised \(-\text{COO}^-\) groups neutralised by mobile cations ("counter-ions") is permeable for cations. For the cations of the solution outside can change place with the mobile cations (counter-ions) inside the membrane and thus permeate. A membrane with fixed ionised basic groups, (e.g. \(-\text{NH}_3^+\)), neutralised by mobile ions is permeable for anions. A membrane consisting of amphoteric molecules, e.g. protein chains, is not selective at the isoelectric point; it is permeable for cations on the alkaline side, for anions on the acid side of this point.

The concentration of fixed ions expressed in equivalents per liter with respect to the liquid in the pores is a characteristic constant for a membrane, called below "selectivity constant" and indicated by \( A' \) (K. H. Meyer and coworkers, 1936, 1945).

3. The Concentration of Electrolytes. While in a membrane permeable for cations at a low exterior concentration only the cations permeate and carry electricity, at greater concentrations an increasing amount of neutral electrolyte penetrates into the pores, so that anions also participate in carrying electricity. If the migration velocity of these anions is greater than that of the cations, more anions can pass through the membrane in the time unit than cations, so that beyond a certain concentration the electromotive force changes sign. (Teorell, 1935; Meyer, 1935; Meyer and Sievers, 1936). This phenomenon has long been known as "concentration effect" and nearly always erroneously attributed to a "reversal" of the membrane selectivity.

4. The Solubility of Ions in the Membrane. If the pores are wide and filled with water, it can be assumed that ions possess the same solubility in the pore liquid as in water. In membranes with narrow pores however, the attracting or repelling forces of the groups on the pore surface can no longer be neglected.
Increase or decrease of salt concentration in the pore liquid may follow. The salt will thus seem either more or less soluble than in the exterior aqueous phase. Moreover membranes can also be filled with a liquid other than water in which ions have a different solubility. We can take this into consideration by introducing the partition coefficients $l_x$ (cation) and $l_a$ (anion), which are equal to concentration in the pore liquid divided by concentration in water, or to activity coefficient in water divided by the activity coefficient in the pore liquid.

To begin with it is best to make the following simplifying assumptions.

1. The membrane is homogeneous; i.e., it does not consist of layers of different properties and is not a mosaic.

2. The concentration of the electrolytes in the liquid layers which are in direct contact with the membrane surfaces is constant, so that an unvarying flow of ions is established within the membrane (and a stationary state on both surfaces).

3. The partition coefficients are independent of the concentration of the salt.

We then arrive at the following equation:

$$E = \frac{RT}{F} \left[ u \cdot \ln \frac{z_x/A + u + \frac{1}{2} \ln \frac{(z_x/A + 1)(z_x/A - 1)}{(z_x/A - 1)(z_x/A + 1)}}{x/A + u} \right]$$

where

$$u = \frac{U_k - U_a}{U_k + U_a}$$

$$z = \sqrt{4e^2 + A^2}; \quad A = \frac{A'}{\sqrt{l_{k}^{*}l_{a}}},$$

**The Membrane Potential in the Study of Membranes**

By measuring the membrane potential at various concentrations of the electrolyte one obtains several equations from which the unknown quantities

$$U_k/U_a$$

and

$$A = \frac{A'}{\sqrt{l_{k}^{*}l_{a}}}$$

can be deduced.

By comparing the values of $A$ for the same membrane and salts with the same anion one obtains:

$$A_1 : A_2 = \frac{A'}{\sqrt{l_{k1}^{*}l_{a}}} : \frac{A'}{\sqrt{l_{k2}^{*}l_{a}}} = \sqrt{\frac{l_{k2}}{l_{k1}}}$$

The absolute values of $A'$ and of $l_k$ and $l_a$ cannot be determined. One relates these values to $l_{k+}$ and $l_{cl-}$ taken as units. $A$, determined with KCl, is thus by definition equal to $A'$, the "selectivity constant."

Similarly the migration velocities $U_k$ and $U_a$ are related to $U_{cl}$ taken as unity. The ratio $U_k/U_a$ to $U_{k1}/U_a$ for two salts with the same anion gives the value for $U_{k1}/U_{x1}$. It is thus possible to relate migration velocities of all cations to that of $K^+$ and this to that of $Cl^-$. 
Serious objections to this theory have been recently formulated by Sollner (1944). He compared the values of $A_p$ (concentration of fixed ions in the pore liquid determined by the potentiometric method) with $A_b$ (the concentration of fixed ions determined by base exchange capacity and the water content of the membrane). He finds large discrepancies between $A_p$ and $A_b$ which he attributes to some "inherent weakness" of the theory.

We wish to emphasize in the first place that according to our theory one cannot determine by potentiometric analysis the absolute value of $A_b$, but only the value of $\frac{A'}{\sqrt{k_e \cdot l_d}}$. Secondly, Sollner neglects, in deriving the value of $A_b$, the well known Donnan hydrolysis of insoluble acids. His simplified Equation 3 is thus erroneous. In the third place, the experimental method used to analyse the base exchange capacity is open to severe criticism. The fact that the presence or absence of salt does not alter in any appreciable manner the pH reading, proves that the observed variations of pH are within the limits of experimental error. Indeed if cation absorption is very small, considerable errors may arise from the solubility of glass or impurities in the solutions or the water. We think therefore that Sollner's results do not in any way invalidate our theory.

**Determination of $A$ and $U_K/U_A$ by Means of Graphs**

In order to simplify the evaluation of the potentials one can use a system of graphs based upon the following principles. As shown in Equation 2 the value of $E$ depends on $u$ (i.e. on $U_K/U_A$), on $x_1/A$, and $x_2/A$ (i.e. on $c_1/A$ and on $c_2/A$). If the ratio of concentrations is kept constant, e.g. $c_1/c_2 = 1:2$, then $E$ depends on two parameters only, namely $U_K/U_A$ and $c_1/A$. For a given value of $U_K/U_A$ (e.g. $U_K/U_A = 1$) a curve is drawn of $E$ as a function of $\log A/c_1$. For every other value of $U_K/U_A$, one obtains another curve (Fig. 1). This graph serves as a basis for the evaluation.

A series of measurements of $E$ are now made keeping the ratio of concentration of electrolytes equal to 1:2, i.e. 0.01/0.02 N; 0.02/0.04 N; 0.04/0.08 N; 0.08/0.16 N; 0.16/0.32 N. These readings of $E$ are entered in the graph as functions $\log 1/c_1$. We then obtain a curve, the "selectivity curve," which is characteristic for the membrane and the electrolytes chosen.

The experimental curve is now compared with the curves of the graph. We ascertain which curve it can be made to fit by displacement parallel to the abscissae. This gives the value of $U_K/U_A$. We then measure the displacement: it is equal to the difference $\log A/c_1 - \log 1/c_1$ and therefore equal to $\log A$. As already mentioned the real "selectivity constant" $A'$ cannot be determined by this method, but only the value $A = \frac{A'}{\sqrt{k_e \cdot l_d}}$. Here $A$ measured with KCl is the "selectivity constant" of the membrane.

**Selective Permeability for H Ions**

Where several electrolytes are involved, all cations participate in the transport of positive electricity and contribute thus, according to their concentra-
tion, partition coefficient, and migration velocity to $n_K$. The same occurs with the anions.

If a Na phosphate buffer solution of pH 6 diffuses directly in a similar solution of pH 7, there results practically no electromotive force as the action of H ions, in spite of their greater velocity, is extremely small with respect to the influence of Na ions, the concentration of which is $10^8$ times greater. If, however, a membrane permeable only to H ions and impermeable to all other ions is set between the two solutions, there results a membrane potential, such as would be the case, if only the H ions were present, the others being excluded from carrying electricity and therefore from influencing the membrane potential. The existence of such specific H ion permeability is thus best revealed by means of buffer solutions.

One cannot, however, distinguish whether a membrane is permeable for H ions

![Fig. 1. Potential difference $E$ as a function of log $A/c_1$ for different values of $U_K/U_A$.](image-url)
only or OH ions, or both. One can only say that a membrane is impermeable
to all ions except H or OH. For, as the product $[H^+][OH^-]$ remains constant,
the effect of the H ions in the two solutions equals that of the OH ions:

$$E = \frac{RT}{F} \ln \frac{[H^+]_h}{[H^+]_l} = \frac{RT}{F} \ln \frac{[OH^-]_h}{[OH^-]_l} = \frac{RT}{F} 2.30(pH_2 - pH_1)$$

(3)

at $15^\circ$ one obtains: $E = 57 (pH_2 - pH_1)$ mv. For greater simplicity, we shall
speak of this as H ion permeability.

A membrane of this type is the well known glass membrane of the “glass
electrode.” Many synthetic organic membranes, e.g. the collodion membrane
of Michaelis (1926) exhibit the phenomenon of specific H ion selectivity in a
very imperfect way: the migration velocity of all other ions is only greatly
diminished with respect to the velocity of H ions, but is not reduced to zero.

**Fig. 2. Analysis of outer layers of membrane.**

**Analysis of a Membrane Consisting of Layers**

In the case of membranes consisting of layers of different permeability, the
total potential observed can be considered as the sum of the individual poten-
tials of each single layer. But only the outer layers can be analysed in the
following way. The concentration of the solution on one side of the membrane
is kept constant, while on the other side solutions with increasing concen-
trations are successively brought to bear, each concentration doubling the previous one;
\( i.e., c_1, c_2 = 2 c_1, c_3 = 2 c_2, \) etc. \( E \) is measured immediately after the change of
the solution. Only in the outer layer \( q - G_2 \) (Fig. 2) is equilibrium immediately
obtained, while between \( G_1 \) and \( q \) the preceding ion distribution remains at
first unchanged, so that the sum of individual potentials is kept constant.
The potential \( c_1/outer layer/c_2 \) is given by the difference \( E (c_n/c_2) - E (c_n/c_1) \).
Thus by plotting this number and the following: \( E (c_n/c_3) - E (c_n/c_2) \), etc., the
selectivity curve can be drawn. The value \( A \) and the ratio \( U_K/U_A \) for the
boundary layer can then be obtained as described above.

H ion selectivity can be tested by putting buffer solutions of different pH
successively in contact with one surface of the membrane and measuring the difference between the potentials obtained.

If the pores of the outer layer are wide, the adjacent solution soon penetrates towards the inner layers. Hence the electromotive force is obviously influenced and may slowly change.

If, after a while, the electromotive force reaches a constant level, it can be assumed that the solution has reached a deeper narrow pored layer through which diffusion is very slow.

If a membrane in layers is bathed by the same solution on either side, no potential will be observed if equilibrium is attained. If this is not the case the membrane presents the phenomenon of "asymmetric potential." This can be explained in the following way. If the ions of the inner layers of the membrane, on their way into the adjacent solutions or vice versa from the solutions into the interior have to pass through layers of different selectivity on both sides of the membrane, a concentration potential will arise. This asymmetric potential disappears more or less rapidly with death, while living processes maintain the disequilibrium.

If a membrane is of the "mosaic type," its permeability and selectivity are determined by the areas of greatest permeability.

Application of the Method to the Analysis of the Frog Skin

A great number of synthetic membranes have been analysed according to the principles explained above. We shall now undertake to apply this method to a living membrane, namely the skin of a frog's belly. As the membrane is in layers, only the relevant methods can be employed. A general remark must, however, be made. The electromotive force obtained, after having changed the solution on one side of a living organ, often does not remain constant, but changes slowly. This variation might be caused by permeation through an inner layer as mentioned above. But in dealing with living membranes, an entirely different phenomenon may occur; i.e., excitation can provoke biological reactions which involve a changing of ionic concentration or permeability inside the living membrane. Thus the true cause of variation cannot always be determined.

Experimental Method, Material, and Treatment

Experiments were carried out on healthy, well fed specimens of frogs (Rana temporaria) caught in May or September. The frogs are beheaded, the skin of the belly is cut out, and stretched with needles on a flat cork ring K of 32 mm. inner diameter. The skin H is then firmly placed in the apparatus shown in Fig. 3 between two rubber stoppers C with cylindrical holes of 10 mm. diameter. The two rubber stoppers are pressed against each other by means of three screws S, through two parallel brass plates P with holes, so that the frog skin separates the two inner chambers in the rubber stoppers. Through these holes two glass vessels A and B are brought to within 7
mm. of the membrane. In cell A a solution of constant concentration flows slowly along the membrane without interruption during the whole series of readings. In cell B too the solution flows slowly over the membrane; this solution can be rapidly changed. The apparatus is slightly inclined so that tube R, slightly curved downwards at its end and brought as near as possible to the membrane has its orifice at the lowest point of cell B. Through this tube the solution $L_1$ of cell B can be drawn away in a few seconds by means of a water pump. The new solution $L_2$ can then be turned on from one of the reservoirs $Z$, with a wide tap. By again drawing off the solution through tube $R$ and refilling with solution $L_2$, cell B is rapidly and thoroughly washed and finally filled with solution $L_2$ which flows in slowly from a reservoir $V$ through tube $R$ after turning the three-way tap $D$. Excess of solution is drawn away by the water pump through a capillary tube $M$. Oxygen bubbles through the solution through another capillary tube $O$ reaching close to the membrane. Cell $A$ is fitted
with a T-tube $T$ one end of which goes close to the membrane. Through the other end protruding above, a solution is let in from reservoir $G$, and the excess flows away through tube $U$. Through the third branch of tube $T$ a capillary tube $N$ is set close to the membrane to allow bubbling of oxygen. The gas escapes through the water level tube $W$. Cells $A$ and $B$ are connected by means of strings soaked with dilute KCl solution with vessels containing saturated KCl solution; the latter are connected to calomel electrodes. Readings are carried out by means of a compensation bridge. As zero instrument an electrometer triode is used, the grid-circuit of which is linked to the compensation bridge. A galvanometer is placed in the anode circuit. By means of a double-pole switch in the grid-circuit it can be observed—Independently of the slow variations of anodic current—whether the potential is exactly compensated or not. The sensibility of the bridge is about $\pm 0.5$ mv. (cf. Fig. 4).

In order to study the outer epithelial layer of the skin, the inner side is brought in contact with Ringer solution (6.5 gm NaCl, 0.2 gm KCl, 0.2 gm CaCl$_2$, 0.32 gm NaHCO$_3$, 1 gm glucose made up to 1 liter with water). The pH of 7.7 to 7.8 is checked with a quinhydrone electrode. The outer side of the skin is brought into contact with solutions of NaCl or KCl, beginning with the concentration of 0.01 $\times$, then this is doubled and so on.

In order to study the inner layer of connective tissue (corium), the epithelial side is brought into contact with tap water. Water in Geneva contains about 50 mg Ca and 12 mg Mg per liter as hydrogen carbonates, chlorides, and sulfates, pH = 8.
To determine the specific H ion permeability, the following buffer solutions—practically isotonic with Ringer solution—were used.

<table>
<thead>
<tr>
<th>Solution of pH</th>
<th>2N NaCl cc.</th>
<th>0.1N NaHPO₄ cc.</th>
<th>0.1N HCl cc.</th>
<th>Filled to cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.7-7.8</td>
<td>50</td>
<td>50</td>
<td>2</td>
<td>1000</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>50</td>
<td>50</td>
<td>5.5</td>
<td>1000</td>
</tr>
<tr>
<td>pH 6.8-7.0</td>
<td>50</td>
<td>50</td>
<td>19</td>
<td>1000</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>50</td>
<td>50</td>
<td>28.5</td>
<td>1000</td>
</tr>
</tbody>
</table>

In these solutions the concentration of Na⁺ and phosphate ions remains constant, that of Cl⁻ ions varies not more than from 0.1000 to 0.1030, and hydrogen ion concentration alone increases to twenty times its value. The pH values of the solutions are checked by means of the quinhydrone electrode. They vary slightly with the water used and the age of the solution, due to variations of CO₂ content.

In several experiments Ringer solution with which CO₂ had been mixed, was used as acid solution. Its pH was each time potentiometrically determined (pH 6.8 to 6.0).

The Influence of Respiration

When the skin of a freshly killed frog is bathed on both sides by Ringer solution through which oxygen bubbles to maintain respiration, the liquid touching the inner surface (corium, connective tissue) is charged positively. The potential rises gradually and after an hour at most it reaches a practically constant level of 50 to 100 mv. If, however, the skin is bathed by Ringer solution on the inside and tap water on the outside, the potential immediately obtained is of a lower value and sometimes even of opposite sign. It changes progressively in the same manner as stated above; i.e., the inner surface (corium) becomes more positive till a practically constant level is reached after about an hour. This potential is, however, from 30 to 60 mv. less positive than in the first case. Reactions which slow down combustion processes in the cell, e.g., deprivation of oxygen or cooling, cause the resting potential to become progressively more negative with respect to the inner surface. After letting in oxygen or after heating to room temperature it regains its former value. Hydrocyanic acid which interrupts all combustion processes in the cell, makes the inner surface rapidly more negative. If then Ringer solution is applied on both sides, the potential may differ considerably from zero; thus the HCN does not lead to a destruction of cell membranes which would cause the potential to sink to zero.

All these observations are in good agreement with the important statement made by several previous authors that the resting potential depends upon respiration. As Mansfeld (1910) and later Leuthardt and Zeller (1934)
pointed out, lack of oxygen renders the potential on the inner side (corium) more negative. It is shifted in the same direction by all influences such as removal of glucose which slacken the combustion process. Huf (1935) showed that the potential falls in spite of the presence of glucose, when the skin has been poisoned with bromoacetic acid and can therefore no longer oxidize glucose. On adding lactate which can be utilized even by poisoned cells, the potential rises and returns to normal.

The influence of temperature studied in detail by Barnes (1939) is most typical. Cooling down to 4-5°C causes a drop of potential in the course of 15 to 30 minutes. Further cooling to 0°C produces another slow decrease which finally ceases. Heating to 20°C causes the potential to rise very rapidly to normal. The increase can amount to considerably more than 50 mv. The relative rise of the potential is thus many times greater than the relative rise of T. There is in fact no proportionality between T and the potential observed, as should be the case if the effect of temperature on the potential was merely a physical one. According to Barnes, inhibition and acceleration of combustion processes caused by variation of temperature, make the potential fall and rise.

Narcotics such as chloroform or fatty alcohols (Leuthardt and Zeller) and poisoning by deuterium oxide (Barnes) also lead to a fall in potential; if the narcotics or the deuterium oxide are washed away, the potential returns to its normal value.

Before beginning experiments with salt solutions, one must wait till a practically constant resting potential is attained. Temperature and oxygen supply must be kept constant throughout the experiment in order to exclude all possible variations of potential due to influence of respiration.

Sometimes the resting potential fails to become entirely constant, but decreases slowly and regularly. This is probably due to a certain slowing down of combustion processes in the living cells. However, the change of potential generated by varying the solutions is so rapid that this slow decrease does not interfere with the readings.

If the potential takes time to stabilize, for instance if buffer solutions are applied to the inner surface, the double influences of metabolism and buffer solution are superposed. Each value can, however, be evaluated from the curves obtained (v/ Fig. 8, curve 6, page 370).

The skin remains alive in the apparatus about 12 hours. The living condition can be tested by cutting off the oxygen; this ought to cause a drop of potential if the skin is alive.

Study of the Outer Surface of Frog Skin

When the outer epithelial surface is bathed in tap water and the inner one (corium) in Ringer solution, a low negative potential with respect to the outer
surface is generally produced. By substituting distilled water for tap water, the electromotive force changes sign. This can be explained if tap water ions penetrate into the outer layer and pass out again through a membrane which is chiefly permeable for cations, thus imparting to the adjacent solution a positive charge. The outer epithelial layer is thus normally permeable for cations.

For a further study of the outer layer, Ringer solution was applied inside and salt solutions outside, beginning with the concentration of 0.01 N, then 0.02, 0.04, 0.08, etc. The salt solutions were rendered isotonic with Ringer

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**Fig. 5.** Selectivity curves of frog skin. I, NaCl without buffer; II, treatment with Ringer, then KCl buffered to pH 7.8; III, KCl after treatment with CHCl₃; IV, KCl buffered to pH 7.8 without preliminary treatment; V, KCl without buffer, without preliminary treatment; A, reference curve for cation selectivity; B, reference curve for anion selectivity.
by addition of sucrose. By means of the differences of the potentials measured, the selectivity curve was drawn (Fig. 5 and the following table). When, at the end of each series of readings, the first solution was applied again, the first potential was restored immediately or very rapidly.

**Curve I, Fig. 5**

<table>
<thead>
<tr>
<th>Interior</th>
<th>Exterior</th>
<th>E</th>
<th>Differences relative to exterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Ringer</td>
<td>0.01 N NaCl</td>
<td>-62</td>
<td>+ 0.01/0.02 - 12</td>
</tr>
<tr>
<td>+ Ringer</td>
<td>0.02 N NaCl</td>
<td>-74</td>
<td>+ 0.02/0.04 - 6</td>
</tr>
<tr>
<td>+ Ringer</td>
<td>0.04 N NaCl</td>
<td>-80</td>
<td>+ 0.04/0.08 - 3</td>
</tr>
<tr>
<td>+ Ringer</td>
<td>0.08 N NaCl</td>
<td>-83</td>
<td></td>
</tr>
</tbody>
</table>

For curve 1, Fig. 5, A was found equal to 0.02, the membrane being cation-permeable. All other frogs gave similar values.

The selectivity curves for KCl are quite different. If the skin is bathed by Ringer inside and KCl outside, no constant potential is attained and the potential differs with each specimen tried. This can only be caused by an active reaction of the skin.

If on the other hand KCl solution is applied on both sides of the skin, constant and reproducible potentials are observed. But as is shown by the following table and the selectivity curve V (Fig. 5), the exterior epithelial membrane has become permeable for anions.

**Curve V, Fig. 5**

<table>
<thead>
<tr>
<th>Interior</th>
<th>Exterior</th>
<th>E</th>
<th>Differences relative to exterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Beginning)</td>
<td>+ 0.16 N KCl</td>
<td>0.16 N KCl</td>
<td>-1</td>
</tr>
<tr>
<td>+ 0.16 N KCl</td>
<td>0.01 N KCl</td>
<td>-52.5</td>
<td>+ 0.02/0.04 + 14</td>
</tr>
<tr>
<td>+ 0.16 N KCl</td>
<td>0.02 N KCl</td>
<td>-37.5</td>
<td>+ 0.04/0.08 + 12.5</td>
</tr>
<tr>
<td>+ 0.16 N KCl</td>
<td>0.04 N KCl</td>
<td>-23.5</td>
<td>+ 0.08/0.16 + 10</td>
</tr>
<tr>
<td>+ 0.16 N KCl</td>
<td>0.08 N KCl</td>
<td>-11</td>
<td>+ 0.16/0.32 + 6</td>
</tr>
<tr>
<td>(End)</td>
<td>+ 0.16 N KCl</td>
<td>0.16 N KCl</td>
<td>-1</td>
</tr>
</tbody>
</table>

The membrane has thus changed from being cation-permeable and acid in character to anion-permeable and basic. The epithelial surface of the skin must therefore be amphoteric and consist of amphoteric proteins. Amberson (1936) found this in dead frog skin. Its isoelectric point is given by him as pH 5.1. On its alkaline side it is permeable to cations, on the acid side to anions. This anion permeability which occurs under the influence of K ions, can only be explained by production of acid under the action of potassium which brings the intercellular fluid to the acid side of the isoelectric point. This explanation
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accounts also for the curious and varying results obtained by placing Ringer against KCl: the alkalinity of Ringer more or less neutralises the newly formed acid.

Distinct cation selectivity, however, can be obtained also with KCl, if the skin is placed for a while before the experiment in Ringer or 0.1 M NaCl buffered with phosphate to pH 7.8 and if the KCl at pH 7.8 is applied outside (curve II, Fig. 5). That the acidifying process must be considered as an active reaction of the skin, can be shown by treating the skin beforehand with chloroform vapor: it then retains its normal cation permeability in the presence of KCl without the alkaline treatment (cf. curve III, Fig. 5).

The selectivity curves show that the selectivity constants for cation permeability and therefore the concentration of fixed acid groups is considerably lower (A ≈ 0.02) than when we have anion permeability (A = 0.05 to 0.1). It can be inferred that either the proteins of the epithelium possess more bound basic than acid groups, or that the water content of the membrane is reduced in an acid milieu.

The ratio $U_A/U_A$ is found to be near unity for NaCl as well as for KCl, while in water $U_A/U_A$ equals 0.6. The migration velocity Na$^+$ in the membrane is thus relatively higher than in water, and practically as high as that of K$^+$.

Solubilities of K$^+$ and Na$^+$ in the epithelial layer cannot differ very much since the selectivity constants $A'_{K^+}/A'_{Cl^-}$ and $A'_{Na^+}/A'_{Cl^-}$ are practically the same.

The influence of different H ion concentrations on the potentials was studied by means of buffers from pH 6 to 8. There was no potential difference between the different buffers. The epidermis is thus not specifically permeable for H ions; it behaves quite differently from the inner surface of the skin as we shall see below.

Study of the Inner Surface of Frog Belly Skin

The curves of selectivity were determined with tap water on the epithelial side of the skin and with NaCl or KCl solutions on the inner surface. Within the limits of error, no potential difference was generated upon changing the concentration of the salts. The selectivity curves are straight lines parallel to the abscissae. Hence $A = 0$: there is no cation or anion selectivity.

In order to investigate the membrane with respect to H ion selectivity, tap water was applied to the outer surface and buffer as described above of pH 7.8 to the inner surface (corium). When the potential had remained constant for half an hour which was generally the case after 1 hour, a more acid solution, e.g. of pH 6.8, was applied. After a period of latency lasting several minutes, often even a quarter of an hour, the potential dropped at first slowly, then rapidly, the inner surface becoming more negative, till a potential difference
was obtained which corresponds almost exactly to exclusive H ion permeability, according to the equation (mv. at 15°).

\[ E = \frac{RT}{F} \ln \frac{[H^+]_1}{[H^+]_2} \quad E = \frac{RT}{F} 2.30(pH_1 - pH_2) \quad E = 57(pH_1 - pH_2) \]

When the first solution of pH 7.8 is again applied, \( E \) rises after a short period of latency to its former value. The whole cycle can be repeated several times (Figs. 6 and 7). The following measurements were read inter alia:

<table>
<thead>
<tr>
<th>pH changes</th>
<th>( E ) observed</th>
<th>( E ) calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.8/6.8</td>
<td>-63</td>
<td>-57</td>
</tr>
<tr>
<td>6.8/7.8</td>
<td>+60</td>
<td>+57</td>
</tr>
<tr>
<td>7.8/6.8</td>
<td>-53</td>
<td>-57</td>
</tr>
<tr>
<td>6.8/7.8</td>
<td>+55</td>
<td>+57</td>
</tr>
<tr>
<td>7.8/6.8</td>
<td>-54</td>
<td>-57</td>
</tr>
<tr>
<td>6.8/7.8</td>
<td>+48</td>
<td>+57</td>
</tr>
</tbody>
</table>
In the case of large, thick-skinned, September frogs, the potential falls and rises more slowly. We assume that this latent period is due to a porous, non-selective layer through which the buffer solution must pass before reaching the membrane selective for H ions further inside.

Fig. 7. Effect of phosphate buffer of different pH on the potential of frog skin. Buffer applied to corium, tap water to epidermis. Sign related to corium.

The membrane of these frogs soon loses its selectivity as shown; e.g. in curve 2, Fig. 6, and curve 3, Fig. 7.

<table>
<thead>
<tr>
<th>pH changes</th>
<th>$E_{observed}$</th>
<th>$E_{calculated}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.7/6.5</td>
<td>-61</td>
<td>-68</td>
</tr>
<tr>
<td>6.5/7.4</td>
<td>+48</td>
<td>+51</td>
</tr>
<tr>
<td>7.4/6.5</td>
<td>-16</td>
<td>-34</td>
</tr>
<tr>
<td>6.8/7.7</td>
<td>+18</td>
<td>+51</td>
</tr>
<tr>
<td>7.8/7.0</td>
<td>-41</td>
<td>-46</td>
</tr>
<tr>
<td>7.0/7.8</td>
<td>+17</td>
<td>+46</td>
</tr>
<tr>
<td>7.8/7.0</td>
<td>-8</td>
<td>-46</td>
</tr>
<tr>
<td>7.0/7.8</td>
<td>+5</td>
<td>+46</td>
</tr>
</tbody>
</table>

In some cases the resting potential did not remain quite constant; the gradual linear decrease seems due to a progressive slackening of combustion processes. If this descending straight line serves as starting point for calculations such as
in curve 4, Fig. 7, the following values are obtained for that part of the potential (indicated by a dotted line) for which the solutions are solely responsible.

<table>
<thead>
<tr>
<th>pH changes</th>
<th>( E ) observed</th>
<th>( E ) calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.8/6.9</td>
<td>-46</td>
<td>-51</td>
</tr>
<tr>
<td>6.9/7.8</td>
<td>+46</td>
<td>+51</td>
</tr>
</tbody>
</table>

In order to shorten as much as possible the latent period caused by the slow penetration of the electrolyte through the porous layer, Ringer brought to an acid pH by CO₂ was used instead of the phosphate buffer. Its pH was determined potentiometrically each time. As this solution shows a considerable CO₂ tension, it might be supposed that CO₂ would rapidly penetrate through the porous layer of connective tissue and thus make the intercellular fluid which is in contact with the selective membrane inside, rapidly more acid. In fact, the fall and rise of potential followed almost immediately after the change of solution (Fig. 8 and following table).

<table>
<thead>
<tr>
<th>pH changes</th>
<th>( E ) observed</th>
<th>( E ) calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.7/6.9</td>
<td>-47</td>
<td>-46</td>
</tr>
<tr>
<td>6.9/7.7</td>
<td>+48</td>
<td>+46</td>
</tr>
<tr>
<td>7.7/6.9</td>
<td>-37</td>
<td>-37</td>
</tr>
<tr>
<td>6.9/7.7</td>
<td>+23</td>
<td>+23</td>
</tr>
<tr>
<td>7.9/6.0</td>
<td>-53</td>
<td>-108</td>
</tr>
<tr>
<td>6.0/7.9</td>
<td>+51</td>
<td>+108</td>
</tr>
<tr>
<td>7.9/6.0</td>
<td>-46</td>
<td>-108</td>
</tr>
<tr>
<td>6.0/7.9</td>
<td>+44</td>
<td>+108</td>
</tr>
<tr>
<td>7.9/6.0</td>
<td>-51</td>
<td>-108</td>
</tr>
</tbody>
</table>

In one experiment hydrocyanic acid in a 0.5 per cent solution was applied to the outer side and replaced after 10 minutes by tap water. This caused a considerable fall of potential due to the inhibition of respiration. But in spite of hydrocyanic acid poisoning, substituting at the inner surface pH 7.9 for pH 6, caused the potential to rise, while return to pH 6 made it fall, in exactly the same manner as with a normally breathing skin (curve 6, Fig. 8).

<table>
<thead>
<tr>
<th>pH changes</th>
<th>( E ) observed</th>
<th>( E ) calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0/6.0 (HCN)</td>
<td>-36</td>
<td>-</td>
</tr>
<tr>
<td>6.0/7.9</td>
<td>+74</td>
<td>+108</td>
</tr>
<tr>
<td>7.9/6.0</td>
<td>-47</td>
<td>-108</td>
</tr>
</tbody>
</table>
This confirms the hypothesis that the observed variations of potential are not due to a variation in respiration which is suppressed by hydrocyanic acid, but are caused by a direct physical-chemical action of the solution containing CO₂ on the H⁺ selective membrane.

**Fig. 8. Effect of Ringer + CO₂ of different pH on the potential of frog skin.**
Ringer applied to corium, tap water to epidermis. Sign related to corium.

**Conclusions Concerning Skin Structure**

These experiments combined with known data lead to the following conclusions concerning the multilayered structure of the skin. The interior surface consists of a non-selective layer which is porous and easily permeable for large ions. Then comes a membrane which is practically exclusively permeable for H ions. This layer is very thin as the electrical resistance of the entire skin is rather low. Immediately adjoining this there is a layer in which carbonic acid is generated by respiration and passes in part into the serum and is then carried away. The acid diffuses out as CO₂. This involves a greater concentration of CO₂ and therefore of H⁺ in the CO₂-producing layer than in the intercellular liquid on the other side of the thin selective membrane. The potential
difference caused by the different concentrations of $H^+$ on both sides of the thin membrane depends obviously on the rate of production of $CO_2$ and therefore on the intensity of the respiration. Next to the $CO_2$-producing layer, there are cells which under the influence of KCl can produce acid. Finally comes a layer of amphoteric proteins which form the outer surface.

We shall compare this picture built upon potentiometric measurements with histological data. Frog skin, 0.1 to 0.2 mm. thick, consists of an inner layer of connective tissue about half as thick as the whole skin and containing several "chromatophores" (layer 5, Fig. 9). This layer corresponds to the inner porous layer mentioned above. Next follows a very thin homogeneous membrane (4, Fig. 9), called basal membrane, which is obviously identical with the $H^+$ selective layer. It stains with methyl red and must therefore consist of proteins of the collagen type. Close against the basal membrane is located the stratum germinativum, a layer of prismatic epithelial cells about 7µ thick and 10 to 20µ long (3, Fig. 9). These prisms are perpendicular to the skin surface. The cells are connected by protoplasmic fibres and constantly dividing to form other epithelial cells. These cells must have an intense metabolism and are probably the main source of carbonic acid. Then follow 5 to 7 layers of epithelial cells of equal size which are alive but do not divide. Finally at the outer surface there is a layer of flat dead epithelial cells (stratum squamosum). There is thus a large measure of agreement between our own conclusions and the histological picture of skin structure.

As has been pointed out, variation of the pH at the outer surface had no effect on the skin potential; only the variation at the corium side led to the
detection of the membrane permeable to H ions. When the corium was thick, the potential followed only slowly the pH variation. There can be little doubt that permeability to H ions of the basal membrane will be perfectly hidden, if the corium is very thick and tight. The absence of any effect on the potential, when the pH of the adjacent solution is changed, therefore does not prove the absence of an interior layer permeable to H⁺ in a multilayered membrane or organ.

Conclusions Concerning the Resting Potential of Frog Skin

As pointed out above the corium is separated from the living cells of the stratum germinativum by the thin basal membrane which is specifically permeable to H⁺ ions. Carbonic acid is continuously produced in respiring cells; the CO₂ whichdiffuses out through the basal membrane is carried away by the serum and blood. At constant temperature and oxygen supply a stationary state will thus arise which involves a gradient of CO₂ and therefore of H ions on both sides of the basal membrane. Owing to this gradient a potential must exist across the membrane, the corium side being positive. This potential probably accounts for a great part of the asymmetrical potential which is observed when the skin is bathed in Ringer on both sides. It seems possible, however, that other ions produced in respiration processes contribute to the observed potential.

Any variation of the respiration and the CO₂ production of the living cells must influence the gradient of CO₂ and therefore the potential across the membrane. The very marked influence of respiration processes on the potential is thus explained.

If, on the other hand, the skin is bathed in Ringer inside and tap water outside, the observed potential is near zero. The potential across the membrane must, therefore, be compensated by a potential of opposite polarity which is located between the respiring cells and the tap water. If the fluid and the water are separated from each other by a layer which is permeable to cations, as is indeed the case (stratum squamosum), a potential must occur which is of opposite polarity to the potential across the basal membrane and can therefore compensate it.

Previous Work on the Ion Permeability of Skin

(a) Frog's Skin.—Leuthardt and Zeller (1934) studied the ion permeability of frog skin by means of concentration potentials. They found the outer layer to be selectively permeable for cations even in the presence of KCl. Polyvalent electrolytes in medium concentration and univalent electrolytes in high concentration lead to a “reversal of the membrane selectivity.” The observed reversal of polarity which occurs when passing from a lower to a higher concentration cannot be attributed to a reversal of the selectivity, but should be explained as follows: If the migration velocity of the anions is much greater than that
of the cations as in the case of Cl\textsuperscript{-} and slow polyvalent cations, then beyond a certain concentration more anions pass through than cations, and the electromotive force changes sign.

(b) Human Skin.—The ion permeability of human skin was studied in particular by Rein (1926). Two fingers of the same hand are placed in two vessels containing KCl solution. The skin of one finger is injured, while the other is left intact. The solutions are connected in the usual manner to unpolarisable electrodes by means of concentrated KCl solution.

The concentration of the solution bathing the injured finger is kept constant (1 N KCl), while the other is increased from 0.001 N to 0.01, 0.1, and 1 N. The differences between successive readings can be used in plotting the selectivity curve; the results of five series of experiments are given in the following table.

<table>
<thead>
<tr>
<th>KCl</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 0.001/0.01</td>
<td>+10</td>
</tr>
<tr>
<td>+ 0.01 /0.1</td>
<td>+8</td>
</tr>
<tr>
<td>− 0.1 /1.0</td>
<td>−2</td>
</tr>
</tbody>
</table>

These potential differences may be recalculated for a concentration ratio 1:2. The following values are thus obtained:

<table>
<thead>
<tr>
<th>KCl</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 0.003/0.006</td>
<td>+3</td>
</tr>
<tr>
<td>+ 0.03 /0.06</td>
<td>+2</td>
</tr>
<tr>
<td>− 0.3 /0.6</td>
<td>−1</td>
</tr>
</tbody>
</table>

The outer surface of the human skin is thus found to be always cation-permeable, though the selectivity is very small (A = 0.001) as is evident from the curve of selectivity which was drawn with the values of the last table. In addition a small influence upon U_{K}/U_{A} is observed. For high concentrations U_{K}/U_{A} approaches the value 0.8 instead of 1 as in water. The reversal of potential beyond a certain concentration is not to be attributed to a reversal of skin permeability, but to the increasing influence of rapidly migrating anions as pointed out above.

Rein’s experiments with AlCl\textsubscript{3} can be explained in the same manner. The following values were obtained by subtracting the potentials measured in four series of experiments:

<table>
<thead>
<tr>
<th>AlCl\textsubscript{3}</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 0.001/0.001</td>
<td>+28</td>
</tr>
<tr>
<td>− 0.001 /0.01</td>
<td>−5</td>
</tr>
<tr>
<td>− 0.01 /0.1</td>
<td>−24</td>
</tr>
<tr>
<td>− 0.1 /1.0</td>
<td>−24</td>
</tr>
</tbody>
</table>
In very dilute solution the cation Al+++ alone carries electricity across cation-permeable membranes: however a concentration of 0.001 N is already sufficient to provoke a reversal of polarity due to penetration of Cl- ions which migrate much more rapidly than Al+++.

The conclusion that human skin is cation-permeable, is in agreement with the disturbances of neutrality observed by Rein and known as the "Bethe-Toropoff effect" (1914, 1915). If a direct current flows through a cation-selective membrane, a column of anions migrates towards the anode and a similar column of cations towards the cathode. But the anions are held back by the membrane and accumulate on the cathodic side, thus attracting H ions through the membrane from the anodic side. Thus an acid reaction is produced on the cathodic and an alkaline reaction on the anodic side. Under the anode the interior fluid of the skin becomes acid while under the cathode it becomes alkaline. The pain felt by the person subjected to the experiment was probably due to a change of pH in the intercellular fluid of the skin tissue.

Study of the Muscle Fibre with Respect to H Ion Selectivity

The fact that membranes with selective H ion permeability exist in living organs induced us to investigate the membrane of muscle fibre. For such experiments the buffer solutions ought to be applied directly to the membrane sheathing a single fibre, while the other solution should be applied to the interior of the fibre or to an injured spot. But as we cannot manipulate isolated fibres in our laboratory, we had to carry out the experiment with whole muscles. It is, however, practically impossible to bring a solution containing large ions, such as phosphate ions into direct contact with the majority of the fibres inside since diffusion through the interstitial fluid is much too slow. The potential thus observed will be principally a diffusion potential between buffer and interstitial fluid, while the membrane potential will not be modified. Assuming that CO2 would penetrate more rapidly inside the interstitial fluid and modify the pH in the immediate vicinity of at least a great part of the fibres, we exposed the uninjured part of the muscle alternately to air and a mixture of 80 per cent CO2 and 20 per cent oxygen. One electrode was connected by means of Ringer or Ringer + CO2 with this uninjured part, the other electrode with the injured part which was kept constantly in oxygen or air. The full magnitude of the potential difference for exclusive H ion selectivity can, however, by no means be expected because of the slow diffusion and shunting through the interstitial fluid.

The muscle (sartorius of temporaria) is cut off at the pelvis, keeping the insertion at the knee intact. It is then placed in a chamber with two compartments separated from each other by a partition with a hole in it. The muscle is pulled through the hole until one half of the muscle is in each compartment. The hole is then stopped
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up with vaseline. In this manner the uninjured and injured halves of the muscle can be exposed to different gases. Strings soaked in Ringer are laid on the muscle to establish contact with the electrodes.

The compartments were first filled with air until the injury potential remained constant (20 to 30 mv., the uninjured side being positive). Then the CO₂ mixture was let into the compartment containing the uninjured part. As soon as a constant value was obtained, air was brought back again. The

![Graph of Fig. 10](image)

**Fig. 10.** Effect of a mixture of 80 per cent CO₂ + 20 per cent O₂ (curve labelled CO₂) and air (curve labelled O₂) on the potential of frog sartorius. Curve 7, injured muscle; CO₂ applied to the non-injured part; the intact half in O₂. Curves 8 and 9, intact muscle.

carbonic acid caused in all experiments a fall in potential of 6 to 7 mv. on the uninjured side. When CO₂ was replaced by air (O₂ in the graph) the potential rose to its former value (e.g. curve 7, Fig. 10).

During the experiments the muscle lost its excitability. But even the unexcitable muscle continued to respond to carbonic acid and its potential rose again when carbonic acid was replaced by air.

Further experiments were carried on with uninjured muscles. The potential observed when both halves of the muscle were in air, was rather small. Introduction of CO₂ into one compartment brought the potential here to the negative side (curves 8 and 9, Fig. 10) and air made it rise again.

These results seem to be quite clear: the muscle membrane or sarcolemma is
specifically permeable to H ions. The sarcolemma shares this property with the basal membrane; it is furthermore stained by the same dyes as the latter and consists therefore of similar proteins.

This statement is not in agreement with the widely accepted view that the muscle membrane is selectively permeable only for K ions. We think, however, that the experimental evidence for this latter theory is by no means conclusive. One of the arguments is the swelling of normal muscle in KCl solution isotonic to physiological NaCl. It seems to prove that potassium permeates through the membrane while sodium does not. But as Verzar (1945) showed recently, muscles when poisoned with iodoacetic acid swell much more slowly in KCl. The swelling after application of KCl seems thus the consequence of a complicated biological response of the muscle and not a simple osmotic phenomenon.

As Buchthal and Lindhard (1936) found, the injury potential of an isolated muscle fibre increased from 27 to 52 mv., when the temperature rose by 12.5°. The potential dropped in the course of 8 minutes by cooling, while on heating the former value was restored in less than 1 minute. The behaviour of the muscle is thus very similar to that of the skin as described by Barnes (1939). This statement in connection with our observation on the selectivity for H ions of the sarcolemma leads us to the following conception of the injury potential of muscle: the concentration of metabolic products, especially carbonic acid, is greater within the respiring fibre than in the interstitial fluid from which the CO₂ is constantly diffusing out into the blood. Owing to the gradient of CO₂ and to the greater concentration of HCO₃⁻ ions in the serum a concentration difference of H ions will be established on both sides of the sarcolemma. A potential difference will thus arise across this membrane, the interstitial fluid being positive, the sarcoplasm negative. Since at the injured spot only a diffusion potential of small magnitude can arise (at the junction of the adjacent liquid and the sarcoplasm), we must assume that the major part of the observed injury potential is due to the membrane potential across the sarcolemma. Any variation of the pH inside the sarcoplasm must thus cause a variation of the resting potential; alkalinization must express itself by a shift of the potential in the negative, and acidification in the positive direction. It follows that the shift in the negative direction which is observed during the action current very probably indicates an alkalinization inside the fibre.

The Permeability of the Nerve Membrane

The selectivity constant of the external layer of frog sciatic can be deduced from concentration potentials measured by van Heuwerswyn (1936).

A 8/10 KCl solution was applied to two different spots of a nerve which had remained previously in isotonic glucose solution. An asymmetric potential between +6 and -6 mv. was observed. If one electrode remained unchanged and the 0.1 N
KCl solution on the other was replaced by 0.01 N KCl, the potential rose 20 mv. higher at the latter electrode. As can be calculated, a concentration ratio of 2:1 would cause a change of potential of about +6 to +8 mv. The outer layer is thus permeable for cations. If we admit for the ratio $U_{K}/U_{A}$ the same value as in water, i.e. 1:1, the selectivity constant is found to be $A = 0.02$ to 0.03. If 0.1 N NaCl and 0.001 N are successively applied, a potential near zero, as indeed was found by van Heuwerswyn can be expected, if the ratio $U_{Na}/U_{Cl}$ remains the same as in water; i.e., equal to 0.6. This is due to the fact that the cation selectivity is compensated by a greater migration velocity of the Cl ions.

A similar study was undertaken by van Heuwerswyn (1938) on the non-medullated nerve of lobster. After replacing 0.1 N NaCl by 0.001 N a potential difference of 77 mv. was produced which corresponds to a value of 11.5 mv. for a concentration ratio of 2:1. The selectivity constant must therefore be about $A = 0.03$. The outer layer is thus somewhat more selective for cations than in frog sciatic.

We are very much indebted to Dr. A. van der Wyk and Dr. Anderegg for their valuable advice and help.

**SUMMARY**

1. The electromotive forces which arise, if two electrolyte solutions are separated from each other by a layer of any kind, are discussed. A general equation is derived comprising the known equations for diffusion, partition, and membrane (Donnan) potentials as special cases.

2. A method is proposed to analyse membranes potentiometrically with respect to their cation or anion selectivity, their dissolving power for ions, and their influence on ion mobility (migration velocity).

3. The possibility of analysing a membrane composed of several layers of different permeability is discussed.

4. The investigation of the skin of the belly of *Rana temporaria* leads to the following results. It is composed of at least four layers of different permeability, one of which is specifically permeable to H ions and is very likely identical with the “basal membrane” situated between the stratum germinativum and the corium. The major part of the resting potential of the skin is located across this membrane and is due to the difference of H$^+$ concentrations on both sides of the membrane.

5. Experiments on muscle show that the sarcolemma is specifically permeable to H ions. The injury potential of the muscle is attributed to the difference of H$^+$ concentration inside and outside the fibre.

**BIBLIOGRAPHY**


Loeb, J., and Beutner, R., Science, 1911, 34, 884.
Mansfeld, G., Arch. ges. Physiol., 1910, 131, 457.
Michaelis, L., Naturwissenschaften, 1926, 14, 33.
Van Heuwerswyn, J., Arch. internat. physiol., 1936, 43, 316; 1938, 47, 76.