APPLICATION OF THE DIFFUSION HYPOTHESIS TO MEMBRANE POTENTIALS.

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In a paper on the acid-base equilibrium in two phase systems, the following case was suggested as an interesting one from the point of view of the Donnan equilibrium.

Two aqueous solutions, A and B, contain equal concentrations of undissociated lactic acid (HL), but different concentrations of Na-lactate (NaL). The solutions are separated by a layer of amyl alcohol in which there is a sufficient concentration of HL to be in equilibrium with both aqueous solutions, but in which the NaL is practically insoluble. The condition is approximately the one which would be obtained if amyl alcohol containing HL, in excess of the total base present, were shaken up several times alternately with two aqueous solutions containing originally two different concentrations of NaOH. The conditions of this equilibrium and the limitations of the mathematical treatment are fully described in the paper above.

In either solution (A or B) the greater part of the lactate ions (L) are derived from the Na lactate, and the hydrogen ion concentration (except in the very acid solutions) can be calculated from the equation

\[ H = \frac{HL}{NaL} \times \frac{K}{\gamma}; \text{ or } pH = \log \left( \frac{NaL}{HL} \right) + pK' \quad (pK' = 3.65) \]

At equilibrium, \( HL_A = HL_B \), therefore \( H_A \times L_A = H_B \times L_B \). Hence the solution with the lower concentration of NaL will have a lower concentration of L and a higher concentration of H. The conditions of the Donnan equilibrium in this respect are obviously fulfilled. The amyl alcohol is as effective a semipermeable membrane as can or-

\[ 1 \text{Murray, C. D., J. Biol. Chem., 1923, lvi, 569.} \]
DINARILY BE USED IN AN ACTUAL EXPERIMENT. IN THIS CASE IT IS RELATIVELY IMPERMEABLE TO Na IONS, WHICH HERE PLAY THE RÔLE OF THE PROTEIN IONS OFTEN USED IN SIMILAR STUDIES WITH COLLODION MEMBRANES. IT WILL BE NOTED THAT IN THIS CASE THE RELATIVE INDIFFUSIBILITY OF THE Na IONS IS A CONDITION IMPOSED BY THE MEMBRANE, WHEREAS IN EXPERIMENTS WITH PROTEIN IONS THESE IONS ARE INHERENTLY ALMOST NON-DIFFUSING, AND THEREFORE IN MANY CASES THE MEMBRANE HARDLY ALTERS THE NATURE OF THE SYSTEM.

\[ \text{Diagram} \]

\[ \text{Arrow shows direction of diffusion current when sign of P.D. is positive.} \]

\[ \text{FIG. 1.} \]

EXPERIMENTAL.

WITH THE SYSTEM DESCRIBED ABOVE SEVERAL SERIES OF EXPERIMENTS WERE PERFORMED TO DETERMINE THE POTENTIAL DIFFERENCES BETWEEN VARIOUS PAIRS OF SOLUTIONS, MADE UP SO THAT THE CONDITION OF EQUILIBRIUM DE-
scribed would be satisfied. The P.D. was measured by a potentiometer in the usual manner with the use of two saturated calomel electrodes.

The sign is taken as positive if the direction of the positive current is from left to right through the solutions (as drawn or described).

All the aqueous solutions had a concentration of 0.05 N HL, with varying amounts of NaL in addition. The pH of each solution was measured electrometrically.

Solution A, the most acid of the series and containing the least amount of NaL, was placed in the beaker on the left, and Solution B on the right, and the tips of the calomel electrodes were inserted. Through the top of the X-tube, which is shown in the drawing, amyl alcohol containing 0.025 N HL (equilibrium concentration previously determined) was introduced. Then at the top of the X-tube suction was applied until the levels of the two aqueous solutions nearly reached the ridge, leaving, therefore, a small portion of amyl alcohol as a junction or membrane.

At this point the P.D. was measured. This will be designated the alcohol junction P.D. Finally further suction was applied to allow the two aqueous solutions to come in direct contact by just surmounting the ridge and regulating the levels so that the less dense solution surmounted the ridge first. The aqueous junction P.D. was then quickly measured. By lowering and raising the levels again the measurements could be repeated several times with variations within 3 mv.

Introducing fresh portions of Solution A, fresh alcohol, and substituting successively Solutions C to I in place of B, a series of P.D. was obtained for the alcohol and aqueous junctions (Series 1). Solution C was then substituted for Solution A and measured against Solutions D to I (Series 2). Finally, Solution D was measured against Solutions E to I (Series 3).

The results are given in the table. Under Series 2 there are two columns, the first gives the observed P.D. figures in millivolts as measured, and the second gives the sum of the P.D. referred to Solution A. (Thus, A vs. C + C vs. I = 63.3). Series 3 is similarly treated. It will be seen that the P.D. is nearly additive.

In addition to the series tabulated, 0.01 N NaL was measured against 0.1 N NaL, thus eliminating the HL.

0.01 NaL vs. 0.1 NaL (alcohol junction) = 12 mv.
0.01 “ “ 0.1 “ (aqueous “ ) = −5 mv.
The results of the last mentioned experiment are indicated in Fig. 2 by the dotted slopes. It will be evident that a difference in pH of 1 in Series 1 is very nearly equivalent to a difference in pL of 1 in this last experiment. The other curves in the figure represent the P.D. obtained in Series 1, i.e. between Solution A of pH 2.50 and the solutions having the pH indicated by the abscissae. The steep slope indicates the theoretical P.D. if the system followed the Donnan conditions exactly.

**TABLE I.**

<table>
<thead>
<tr>
<th>Solution</th>
<th>pH</th>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.50</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.68</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.94</td>
<td>19.3</td>
<td>0.0</td>
<td>(19.3)</td>
</tr>
<tr>
<td>D</td>
<td>3.65</td>
<td>44.5</td>
<td>24.5</td>
<td>43.8</td>
</tr>
<tr>
<td>E</td>
<td>3.97</td>
<td>47.7</td>
<td>31.5</td>
<td>50.8</td>
</tr>
<tr>
<td>F</td>
<td>4.30</td>
<td>---</td>
<td>39.0</td>
<td>58.3</td>
</tr>
<tr>
<td>G</td>
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<td>H</td>
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<td>59.0</td>
<td>43.0</td>
<td>62.3</td>
</tr>
<tr>
<td>I</td>
<td>5.50</td>
<td>61.0</td>
<td>44.0</td>
<td>63.3</td>
</tr>
</tbody>
</table>

Alcohol junction.

<table>
<thead>
<tr>
<th>Solution</th>
<th>pH</th>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.50</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.68</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.94</td>
<td>6.0</td>
<td>0.0</td>
<td>(6.0)</td>
</tr>
<tr>
<td>D</td>
<td>3.65</td>
<td>3.0</td>
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<tr>
<td>E</td>
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<td>0.2</td>
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<tr>
<td>F</td>
<td>4.30</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>G</td>
<td>4.66</td>
<td>-5.2</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>H</td>
<td>5.08</td>
<td>-7.5</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>I</td>
<td>5.50</td>
<td>-13.0</td>
<td>-17.0</td>
<td>-11.0</td>
</tr>
</tbody>
</table>

Aqueous junction.

**THEORETICAL DISCUSSION.**

Let us first summarize the specific properties of the system with which we are dealing. Let \( x \) and \( y \) be any two solutions such as were described in the beginning. Then, within the limits set forth in the previous article

\[
\begin{align*}
H_x L_x &= H_y L_y \\
H_x \times L_x &= H_y \times L_y \\
pH_x - pH_z &= pL_x - pL_y
\end{align*}
\]
and in all but the most acid solutions, since

\[ \text{Na}_x = L_x \text{ and } \text{Na}_y = L_y \text{ (very nearly)} \]

then

\[ \text{pH}_y - \text{pH}_x = \text{pNa}_x - \text{pNa}_y \]

\[ \frac{H_x}{H_y} = \frac{L_y}{L_x} = \frac{\text{Na}_y}{\text{Na}_x} \]

or

\[ -\Delta \text{pH}_{y-x} = \Delta \text{pL}_{y-x} = \Delta \text{pNa}_{y-x} \]
In the experiment with the two concentrations of NaL, we have merely

$$\Delta p_{\text{Na}} = \Delta p_{\text{L}}$$

From these data we can write the classical approximate equations for two simple cases of diffusion potentials.²

A. When the concentration of H is negligible compared to the other ions, we have

$$E(x-v) = \frac{M_N(L) - M_{\text{Na}}(\text{Na})}{M_N(L) + M_{\text{Na}}(\text{Na})} \times 59 \log \frac{N_{\text{Na}}}{N_{\text{L}}},$$

in millivolts, where $M_{\text{Na}}$ and $M_N$ represent the ionic mobilities of the respective ions, and $(L)$ and $(\text{Na})$ represent the mean concentrations of these ions in the region of the diffusion boundary. The term $M_N(L)$ evidently represents the mean specific conductivity of the lactate ions, etc. This is the familiar equation for the diffusion potential between two concentrations of the same salt.

B. When the concentration or the mobility of Na is reduced to zero at the boundary due to an impermeable membrane, we have

$$E(x-v) = \frac{M_{\text{H}}(\text{H}) + M_N(L)}{M_{\text{H}}(\text{H}) + M_N(L)} \times 59 \log \frac{H_x}{H_y},$$

which is the same as the familiar equation for the diffusion potential which can be obtained between two solutions satisfying the Donnan equilibrium. Unfortunately, this equation can be simplified to

$$E(x-v) = 59 \log \frac{H_x}{H_y},$$

I say "unfortunately," because many who have used equation (3) have not emphasized the derivation which Donnan used. The distinction between the conditions of equation (1) and equation (2) is that:

In the first case, the positive and negative ions tend to diffuse in the same direction and the net e.m.f. depends on the difference in their transport numbers.

In the second case, the positive and negative ions tend to diffuse in opposite directions and the resulting e.m.f. depends on the sum of their transport numbers (the sum, of course, equals 1).

Interpretation of Experimental Results.

Now in Series 1, with the alcohol junction, between the first three or four solutions equation (2) holds as the limiting condition—we have a distinct Donnan P.D.

In the experiments with two concentrations of NaL (no HL), we have a case of a simple diffusion potential and applying equation (1) it will be noted from the sign that the term \( M_{Na}(Na) \) is greater than \( M_{L}(L) \) in the aqueous junction, whereas in the alcohol junction the term \( M_{L}(L) \) is the greater. Roughly, \( \frac{M_{Na}(Na)}{M_{L}(L)} = 1.2 \) in H\(_2\)O, = 0.66 in amyl alcohol, when \( Na_x = L_x \), and \( Na_y = L_y \). The results obtained between the last few solutions of Series 1, i.e. Series 3, follow the slopes indicated by the experiments with NaL solutions with the corresponding junctions and can evidently be interpreted along the lines suggested by equation (1).

But taking the alcohol junction P.D. of Series 1 over the whole range, as we measure Solution A against solutions of increasing NaL concentrations we are involved in a transition from the conditions of equation (2) (the Donnan P.D.) to those of equation (1) (the simple diffusion P.D.). Though the solubility of NaL in amyl alcohol is small, as the concentration of NaL in the aqueous solution increases, the specific conductivity due to NaL in the alcohol tends to outweigh the conductivity of the dissociated HL. As the conductivity of H ions in the boundary becomes relatively negligible we approach the conditions of equation (1).

In the case of the aqueous junctions there is at first in Series 1 a slight Donnan effect, (because the solutions are originally made up to satisfy a Donnan equilibrium as if they had been separated by an amyl alcohol membrane impermeable to Na) but obviously the increasing NaL concentrations outweigh the dissociated HL much sooner in these cases than in the cases with the alcohol junctions.
In themselves these experiments support the view advocated by Cremer\(^3\) and more recently by Prideaux and Crooks.\(^4\) They have convinced me that it would be more profitable to recognize the potentials occurring in such systems to be indications of a definite tendency of ions to diffuse from a region of high activity to one of lower activity. In all such cases, whether we have gelatin chloride in a colloid bag or NaL solution in contact with alcohol, the supposition is that (at equilibrium) the activities of the "undissociated" molecules

Fig. 3. These diagrams picture the diffusion process occurring in the various boundaries. The arrows in the end sections of each diagram represent roughly the concentrations in logarithmic units of H, Na, and L ions in the solutions corresponding to the letters. The bottom row, in this respect, is the same as the top row. The arrows in the central sections represent the relative tendencies to diffuse of the H, Na, and L ions. The arrow under each diagram represents the net p.d. and the direction in which the positive current tends to flow. The first diagram in the top row represents the conditions resulting from a previously established Donnan equilibrium. The central diagrams of both rows, if the H ion is neglected, represent simple diffusion conditions such as exist between two concentrations of the same salt. The other diagrams represent combinations of the two simple cases.

which can exist in both phases and migrate from one to the other must be equal in both phases so long as there is not a complete electrical circuit or a dissymmetry of membranes. This results, frequently, in the condition in which the activities of an individual ion differ in the two phases, although the activity product of the two ions which form the undissociated substance still remains equal in both phases.


Then, when the electrical circuit is completed, ions can move independently and the tendency is for the activities of individual ions to become equalized if the electrode P.D. is the same in the two phases (supposedly zero for saturated calomel electrodes). It is understood that, at equilibrium, what we have measured and called a diffusion potential is, under conditions where electrodes are not present, balanced by the electrostatic forces which hold oppositely charged ions together. These P.D. have usually called attention to the electrostatic condition, and the fact has been lost sight of that the source of the P.D., the source of the current (which we oppose with a battery or a charged electrometer) is due to the diffusing tendency of the ions, although this fact was well brought out by Donnan. Much has been written about "electrical double layers" and "charges" have been referred to as if they existed in aqueous solutions independently of ions. Some, too, have spoken of glass bulbs and collodion membranes acting "as hydrogen electrodes" merely because observed P.D. are proportional to the differences in pH on the two sides. This relation holds as long as the conditions of a true Donnan equilibrium are satisfied, but breaks down experimentally if the membrane becomes permeable to the ion which had been considered indiffusible, which happens usually when the concentration of this ion is large. Obviously everyone is referring to the same phenomenon, but I believe that the "kinetic" interpretation describes the processes in simpler terms and leads us more directly to an understanding of the discrepancies and anomalies in experimental results. The problem resolves itself to this general question:

A membrane, or a metal electrode, or an amalgam, or a piece of glass, all involve a second phase when in contact with a solution; what then are the thermodynamic properties of this second phase? At equilibrium what are the activities, the distribution coefficients, and the mobilities of the various components of the system in this second phase?

Incidentally, it might be mentioned that the experiments, as carried out, form an interesting example of "phase reversal." Changing from the alcohol to the aqueous junction in the last experiment listed (A vs. I) caused a change in P.D. from +61 mv. to -13 mv. It will be noted that in experiments with gelatin solutions removal of the collodion membrane causes no essential change in P.D. (in many cases)
because the gelatin ions are inherently non-diffusing. Many combinations of these experiments can be devised and membranes operated to simulate action currents and other bioelectric phenomena.

SUMMARY.

A system consisting of two aqueous solutions, containing equal concentrations of lactic acid, but different concentrations of Na lactate, separated by a layer of amyl alcohol has been described. This system exhibits electrical properties ranging (as the concentration of NaL is increased) from those characteristic of a simple Donnan equilibrium to those characteristic of simple diffusion.

The fact that the Donnan P.D. can be treated as a special case of a diffusion potential has been emphasized.

The experiments call attention to the effect of the thermodynamic properties of the membrane, and it is suggested that such properties as conductivities, ionic mobilities, and distribution coefficients in membranes of any sort should be investigated.

The experiments afford an interesting example of “phase reversal.”