ON THE INFLUENCE OF AGGREGATES ON THE MEMBRANE POTENTIALS AND THE OSMOTIC PRESSURE OF PROTEIN SOLUTIONS.

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I.

When a protein chloride solution is separated by a collodion membrane from a dilute HCl solution free from protein, under suitable experimental conditions an osmotic equilibrium is reached after about 6 hours. When at that time the hydrogen ion concentrations of the protein solution and the outside solution are measured, it is found that the hydrogen ion concentration of the outside solution is higher than that of the protein solution, while the chlorine ion concentration is higher in the protein solution than outside. This unequal concentration of the oppositely charged H and Cl ions inside and outside leads to a membrane potential and modifies the osmotic pressure of the protein solution. The values of both effects can be calculated from the differences in the pH (or pCl) inside and outside on the basis of Donnan's equation for membrane equilibria. The agreement between observed and calculated values on the basis of Donnan's equilibrium equation is excellent for the membrane potentials and equally good for the osmotic pressure, except that a slight difference in pH (in the second decimal) has a much greater influence on the calculated values of osmotic pressure than of the P.D. These facts were based on the writer's observations on solutions of gelatin and crystalline egg albumin and they were confirmed by Hitchcock's observations on solutions of edestin.

The writer has shown that if in a solution of gelatin chloride of a certain pH, part of the gelatin in solution is replaced by the

same weight of powdered gelatin (without changing the pH) the osmotic pressure of the solution is lowered, and this lowering of the osmotic pressure increases the more the more of the dissolved gelatin is replaced by powdered gelatin. This shows that only that part of the protein which is in true solution, \textit{i.e.} which does not exist in the form of larger aggregates, influences the osmotic pressure of a solution. The question arose whether or not powdered particles of gelatin would influence the membrane potential. To obtain an answer to this question the following experiment was made.

Powdered gelatin going through the meshes of sieve 30 but not through 60 was rendered isoelectric in the way previously described. Part of this isoelectric gelatin was melted and the melted and powdered isoelectric gelatin were mixed. The total weight of isoelectric gelatin in 100 cc. solution was always the same, but the proportion of powdered to dissolved gelatin varied as indicated in Table I. Thus when the weight of the powdered gelatin was 0.5 gm., the weight of the dissolved gelatin was about 0.3 gm.; when the weight of the powdered gelatin was 0.2 gm., that of the dissolved was 0.6 gm., etc. 100 cc. of the mixture contained 8 cc. of 0.1 N HCl, and the pH of the gelatin solution (at the equilibrium condition to be described) was between 3.2 and 3.3. At this pH the osmotic pressure of a gelatin solution is nearly a maximum. A 1 per cent solution of gelatin chloride has an osmotic pressure of about 450 mm. water at pH 3.4. Only a small part of this osmotic pressure is due to the osmotic pressure of the protein particles themselves; the rest of the observed osmotic pressure of gelatin chloride solutions of pH 3.4 is due to the excess concentration of the crystalloidal ions inside the collodion bag (in which the osmotic pressure of the gelatin solution is measured) over that of the outside aqueous solution free from gelatin, and this quantity is determined by the Donnan equilibrium.\footnote{Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1922, 232; \textit{J. Gen. Physiol.}, 1921–22, iv, 97.}

Collodion bags of a content of about 50 cc. were filled with these suspensions and closed with rubber stoppers perforated with glass tubes serving as manometers to measure the osmotic pressure.\footnote{Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1922, 169; \textit{J. Gen. Physiol.}, 1920–21, iii, 691.}
The bags were put over night at 21°C. into beakers containing each 350 cc. of 0.001 N HCl in water. The next day the osmotic pressure was read, the P.D. between the gelatin chloride solution and the outside aqueous solution free from gelatin was measured (with a Compton electrometer and indifferent saturated KCl-calomel electrodes), and the pH inside and outside was determined with the hydrogen electrode. Table I gives the results of these observations.

**Table I.**

| Powdered gelatin per 100 cc., g |  |  |  |  |  |  |  |
| Dissolved gelatin per 100 cc., g | 0.8 | 0.7 | 0.6 | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0 |
| Osmotic pressure | 85 | 132 | 181 | 230 | 268 | 310 | 342 | 346 | 398 |
| pH inside | 3.16 | 3.20 | 3.18 | 3.19 | 3.23 | 3.27 | 3.28 | 3.30 | 3.33 |
| pH outside | 2.82 | 2.85 | 2.83 | 2.85 | 2.82 | 2.84 | 2.87 | 2.85 | 2.87 |
| pH inside minus pH outside | 0.34 | 0.35 | 0.33 | 0.36 | 0.39 | 0.42 | 0.46 | 0.46 | 0.46 |
| P.D. calculated, millivolts | Between | 20.0 | 20.5 | 19.0 | 21.0 | 22.5 | 24.5 | 26.5 | 26.5 |
| Membrane potentials observed, millivolts | Between | 23.0 | 22.0 | 21.0 | 22.5 | 25.0 | 26.0 | 26.5 | 27.0 |
| No constant reading. | Between | 18.0 | 18.0 |

The table confirms the writer's former observation that the osmotic pressure of the gelatin solution diminishes the more the more of the dissolved gelatin is replaced by powdered gelatin. The latter obviously does not participate in the osmotic pressure.

The table shows furthermore that the P.D. observed at equilibrium between the gelatin solution and the outside aqueous solution varies much less than the osmotic pressure. It became necessary to ascertain whether or not this P.D. across the membrane which was measured with the aid of two indifferent electrodes (saturated KCl-calomel solution) was actually determined by the difference in the
hydrogen ion concentrations inside and outside, as we should expect if the membrane potentials are due to a membrane equilibrium. The pH inside and outside was therefore measured with the aid of the hydrogen electrode and the value 58 (pH inside minus pH outside) millivolts is called the calculated P.D. The reader will notice that the difference between the observed membrane potential (measured with indifferent electrodes) and the calculated P.D. is not more than 0.5 millivolt. This leaves no doubt that the observed P.D. is determined by the difference in the hydrogen ion concentration on the opposite sides of the collodion membrane and that this P.D. obeys Donnan's equilibrium equation.

These facts then show that the protein aggregates participate in the Donnan equilibrium almost to the same extent as do the isolated molecules or ions of gelatin, and this participation finds expression in the fact that the membrane potentials are lowered comparatively little when dissolved gelatin is replaced by powdered gelatin. The same particles, however, do not contribute to the osmotic pressure for the reason that their share in the excess of chlorine ions is contained inside the solid particles, where it serves to increase the swelling of the particles. The swelling of solid protein particles is, as Procter and Wilson have shown, due to the increase of osmotic pressure inside the particles caused by the Donnan equilibrium. In our experiment there exists inside of each particle of powdered gelatin a Donnan equilibrium whereby the concentration of Cl ions inside is greater than outside and this causes an osmotic pressure. Water will, therefore, diffuse into each granule until the cohesion pressure of the solid particles of gelatin equals the osmotic pressure inside the particles due to the Donnan equilibrium, and the particles will swell. When we therefore have a mixture of dissolved gelatin and powdered particles (micelles) we have two different osmotic pressures; namely, first, the osmotic pressure of the gelatin in true solution, and, second, the osmotic pressure inside each solid particle of gelatin. The former is measured by the hydrostatic pressure of the column of water required to equalize the rate of dif-
fusion in opposite directions through the membrane. This is the osmotic pressure of the protein solution in Table I. The osmotic pressure inside each particle of solid powdered gelatin results in swelling, i.e., an increase of the force of cohesion between the molecules of the gel particle, and this effect does not appear in the osmotic pressure of the solution. Only that part of the osmotic forces in a protein solution appears in the form of hydrostatic pressure which is directly or indirectly due to the isolated molecules of the protein; and this hydrostatic pressure is diminished when part of the protein in solution is replaced by aggregates or micelles of protein.

II.

When a solution of gelatin chloride containing solid granules of gelatin is separated by a collodion membrane from an aqueous solution (free from protein) two different equilibria are established; one across the membrane between the aqueous solution outside and the gelatin solution inside the membrane, and a second one between the solid granules of gelatin and the gelatin solution in which the granules are suspended. At first thought it might seem strange that when solid granules of isoelectric gelatin are suspended in a solution of gelatin and HCl, there should arise a difference in the distribution of the H and Cl ions inside the solid granules and the surrounding gelatin solution. Yet this is the case, as Table II shows, and the reason is easily understood. In the solid granules of gelatin the concentration of protein molecules is much higher than in the weak solutions of gelatin surrounding the granules, and if HCl is added the concentration of gelatin ions must be higher inside the solid gelatin granules than in the dilute gelatin solution in which the granules are suspended. It follows from Donnan’s theory that this difference in the concentration of protein ions inside the powdered particles and the solution must give rise to a Donnan equilibrium; as a consequence of which a P.D. must exist between the solid particles and the weaker gelatin solution.7

This consequence of the theory was confirmed by the following experiment. Mixtures of a solution of isoelectric gelatin and

powdered isoelectric gelatin were made so that 100 cc. always contained 0.8 gm. of isoelectric gelatin in all. The proportion of solid and liquid gelatin varied, however, in each case as indicated in Table II. In each 100 cc. of the mixture were contained 8 cc. of 0.1 N HCl. The mixtures were kept for 2 hours at 20°C. and frequently agitated to accelerate establishment of equilibrium between granules and solution. The solid powdered gelatin was then separated from the supernatant liquid by filtration.

### Table II.

**Donnan Equilibrium between Particles of Powdered Gelatin and Gelatin in Solution.**

<table>
<thead>
<tr>
<th>Powdered gelatin per 100 cc., gm</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved gelatin per 100 cc., gm</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>pH of powdered gelatin</td>
<td>3.30</td>
<td>3.26</td>
<td>3.28</td>
<td>3.24</td>
<td>3.29</td>
<td>3.28</td>
<td>3.25</td>
<td>3.24</td>
<td>3.26</td>
</tr>
<tr>
<td>pH of supernatant gelatin solution</td>
<td>2.99</td>
<td>2.97</td>
<td>2.90</td>
<td>2.88</td>
<td>2.83</td>
<td>2.77</td>
<td>2.72</td>
<td>2.69</td>
<td>2.62</td>
</tr>
<tr>
<td>pH solid minus pH liquid gelatin</td>
<td>0.33</td>
<td>0.36</td>
<td>0.40</td>
<td>0.41</td>
<td>0.51</td>
<td>0.52</td>
<td>0.61</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>P.D. calculated, millivolts</td>
<td>19.0</td>
<td>21.0</td>
<td>23.0</td>
<td>24.0</td>
<td>29.5</td>
<td>30.0</td>
<td>35.5</td>
<td>37.0</td>
<td></td>
</tr>
<tr>
<td>P.D. observed, millivolts</td>
<td>14.5</td>
<td>17.0</td>
<td>17.0</td>
<td>17.5</td>
<td>23.0</td>
<td>25.0</td>
<td>30.0</td>
<td>33.0</td>
<td></td>
</tr>
</tbody>
</table>

The solid gelatin was melted and poured into the vessels described on page 154 of a recent book by the writer, and the P.D. between the solid and liquid gelatin was measured. The values are found in Table II, showing that there exists a considerable P.D. between the gelatin granules and weak solutions of gelatin, and that this P.D. increases with the relative increase in the concentration of solid gelatin, as was to be expected.

Measurements of the pH in the solid and liquid gelatin showed that this P.D. was determined by the Donnan equilibrium.\(^8\) There exists, however, a discrepancy between calculated and observed values for the P.D., which requires further investigation. On the whole, however, the figures seem to prove that when a suspension of powdered protein in a weak gelatin solution is put inside a collodion bag, the latter dipped into an aqueous solution free from gelatin, two equilibria are established; namely, one between the solid

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\(^8\) The measurements of the P.D. between solid gelatin and solution are not as accurate as the measurements between liquids across a membrane.
gelatin and the solution of gelatin inside the collodion bag, and a second one between the gelatin solution inside the collodion bag and the outside aqueous solution free from gelatin.

This explains why the powdered particles of protein contained in a gelatin solution participate in the Donnan equilibrium and the membrane potential of the solution without adding to the osmotic pressure of the solution as measured by the hydrostatic pressure required to equalize the rate of diffusion of water in opposite directions through the membrane. The participation of the solid particles in the Donnan equilibrium leads to an osmotic pressure inside of each solid granule, but this osmotic pressure is measured in terms of cohesion pressure of the swollen particles.

This swelling of the solid particles increases the viscosity of the solution, and the writer has shown that this fact is a further support of the explanation of colloidal behavior on the basis of the theory of membrane equilibria. 3

SUMMARY AND CONCLUSIONS.

1. It is shown that when part of the gelatin in a solution of gelatin chloride is replaced by particles of powdered gelatin (without change of pH) the membrane potential of the solution is influenced comparatively little.

2. A measurement of the hydrogen ion concentration of the gelatin chloride solution and the outside aqueous solution with which the gelatin solution is in osmotic equilibrium, shows that the membrane potential can be calculated from this difference of hydrogen ion concentration with an accuracy of half a millivolt. This proves that the membrane potential is due to the establishment of a membrane equilibrium and that the powdered particles participate in this membrane equilibrium.

3. It is shown that a Donnan equilibrium is established between powdered particles of gelatin chloride and not too strong a solution of gelatin chloride. This is due to the fact that the powdered gelatin particles may be considered as a solid solution of gelatin with a higher concentration than that of the weak gelatin solution in which they are suspended. It follows from the theory of membrane
equilibria that this difference in concentration of protein ions must give rise to potential differences between the solid particles and the weaker gelatin solution.

4. The writer had shown previously that when the gelatin in a solution of gelatin chloride is replaced by powdered gelatin (without a change in pH), the osmotic pressure of the solution is lowered the more the more dissolved gelatin is replaced by powdered gelatin. It is therefore obvious that the powdered particles of gelatin do not participate in the osmotic pressure of the solution in spite of the fact that they participate in the establishment of the Donnan equilibrium and in the membrane potentials.

5. This paradoxical phenomenon finds its explanation in the fact that as a consequence of the participation of each particle in the Donnan equilibrium, a special osmotic pressure is set up in each individual particle of powdered gelatin which leads to a swelling of that particle, and this osmotic pressure is measured by the increase in the cohesion pressure of the powdered particles required to balance the osmotic pressure inside each particle.

6. In a mixture of protein in solution and powdered protein (or protein micelle) we have therefore two kinds of osmotic pressure, the hydrostatic pressure of the protein which is in true solution, and the cohesion pressure of the aggregates. Since only the former is noticeable in the hydrostatic pressure which serves as a measure of the osmotic pressure of a solution, it is clear why the osmotic pressure of a protein solution must be diminished when part of the protein in true solution is replaced by aggregates.