THE SWELLING PRESSURE OF GELATIN AND THE MECHANISM OF SWELLING IN WATER AND NEUTRAL SALT SOLUTIONS.

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(Accepted for publication, June 15, 1926.)

The swelling of gelatin when placed in aqueous solution may be readily separated into three types. The swelling in acid or in alkali and the effect of neutral salts on this swelling have been shown by the work of Procter and Wilson, and Loeb to be due to the osmotic pressure of the ions of the electrolyte, in accordance with the Donnan equilibrium. The initial swelling of dry gelatin in water—which evidently is not connected with the Donnan equilibrium—has been carefully studied by Katz, who was able to show that the heat effects, volume, pressure, and vapor pressure changes were strictly analogous to those observed in the formation of concentrated solutions of many substances, and that the system as a whole behaved as an ideal concentrated solution. The large amount of heat liberated indicates strongly that a reaction occurs between the water and gelatin, resulting, presumably, in the formation of a gelatin hydrate. When sufficient water has been added, however, to reduce the gelatin concentration to less than 50 per cent, the heat effects become very small and yet the gelatin may swell, under favorable conditions, until the concentration of gelatin is 5 per cent or less. It appears improbable that this water is all combined with the gelatin in the form of a hydrate, and it seems necessary to consider this as a third distinct type. It is this type of swelling which is discussed in the present paper.

The influence of salts on this tertiary swelling was found by the

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writers to be closely parallel to the effect of salts on the osmotic pressure of gelatin solutions, and it was further shown that these effects could not be accounted for on the basis of a Donnan equilibrium. It was suggested that this swelling was a purely osmotic phenomenon and that the effect of salts was due primarily to their influence on the osmotic pressure. It is evident that the problem would be greatly simplified if the pressure with which the water was drawn into the solid gelatin could be measured instead of merely the rate or extent of swelling. This "swelling pressure," although analogous to osmotic pressure of a gelatin solution, cannot be measured in the same way, since if solid gelatin is enclosed in a rigid membrane the pressure will be exerted on the walls of the membrane. It was found possible however, by placing the gelatin outside a porcelain thimble coated with collodion, to measure this swelling pressure. The measurements show that the pressure measured in this way is but little less than the osmotic pressure of the same gelatin when liquid, and they corroborate the idea that the swelling in water and neutral salt solutions is due to the osmotic pressure of the solution held in the meshes of the gel.

Experimental Method.

Gelatin.—Isoelectric gelatin was prepared as described by Loeb and all measurements were made at pH 4.7.

The method of making the measurements is shown in Fig. 1. The Chamberland filter was coated with collodion by pouring collodion slowly on the surface while the thimble was rotated mechanically. The tube was partially filled with gelatin of the desired concentration and the thimble and manometer tube, previously filled with water, inserted as shown in the figure. The tube was then placed in a water bath at the desired temperature and the pressure measured after equilibrium was reached. A number of experiments were made to determine whether the thickness of the gelatin layer or the previous treatment of the system had any influence on the final reading. The results of some of these experiments are shown in Table I. The equilibrium pressure is evidently independent of the thickness of the gelatin layer, and the temperature effects are reversible. It was also found that the final pressure was independent of the initial pressure; i.e., it is a true equilibrium value. The results, when the tube was completely filled with gelatin were much more regular, and this method was used in most of the experiments. The figures are the averages of 4 to 8 measurements and are reliable to about 5

Fig. 1. Apparatus for measuring swelling pressure of gelatin.

Fig. 2. Osmotic or swelling pressure of various concentrations of gelatin at different temperatures.
SWELLING PRESSURE OF GELATIN

per cent. In working with the thin coatings of gelatin it was occasionally noticed that the water evaporated from the gelatin and condensed on the surface of the tube even when the manometer showed high negative pressure on the water in the thimble. This result is exceedingly difficult to account for except on the basis of some temperature difference, and is perhaps similar to von Schroeder’s anomalous findings. The pressure of the gelatin when liquid was determined in a rocking osmometer as previously described.  

Effect of Temperature and Concentration of Gelatin on the Swelling Pressure of Gelatin.

The result of a series of experiments in which the pressure of various concentrations of gelatin as measured over a range of temperatures is shown in Fig. 2. The solid line indicates that the gelatin was solid and the dotted line indicates liquid gelatin. It will be seen that in high concentrations of gelatin the pressure increases with decreasing temperature to give a maximum near 37°C., and then decreases quite sharply, as the gelatin solidifies, to become nearly independent of the temperature below 15°C. The negative temperature coefficient in the high concentrations is due presumably to the large positive heat of solution and so does not occur in the low concentrations. The drop

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TABLE I.

Effect of Thickness of Gelatin Layer and of Previous Treatment on Swelling Pressure of 10 Per Cent Gelatin at 25°C.

<table>
<thead>
<tr>
<th>Gelatin on thimble.</th>
<th>Other conditions.</th>
<th>Pressure.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td></td>
<td>mm Hg.</td>
</tr>
<tr>
<td>25</td>
<td>Suspended in closed tube in air.</td>
<td>49</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>195</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td>250</td>
<td>Tube filled with gelatin.</td>
<td>45</td>
</tr>
<tr>
<td>250</td>
<td>At 0°C. for 10 min. before placing at 25°C.</td>
<td>48</td>
</tr>
<tr>
<td>250</td>
<td>0° “ 18 hrs. “ 25°”</td>
<td>46</td>
</tr>
<tr>
<td>250</td>
<td>37° “ 25°”</td>
<td>47</td>
</tr>
</tbody>
</table>

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in the pressure between 30° and 20°C. occurs also in the low concentrations where the gelatin does not solidify, and in these cases approaches zero. In these dilute solutions a precipitate forms and hence no osmotic pressure would be expected. If gelatin is considered as a single chemical substance, these results are evidently exceedingly difficult to understand. The drop in the pressure below 35°C. would result if an insoluble substance is separating out, but if the solution contained only this substance the pressure must become independent of the initial concentration as soon as the solid phase appears. This

\[ \text{FIG. 3. Osmotic or swelling pressure of gelatin of different concentrations as per cent of value at 37°C.} \]

is not the case. The experiment is analogous to Sörensen's results on the solubility of the globulins, in that the osmotic pressure (concentration) increases with the total amount of substance added even in the presence of the solid phase. This is the expected result if it is considered that gelatin is a mixture of (at least) two substances, one of which is easily soluble over the range of temperature studied, and the other of which is readily soluble above 35°C., but much less soluble below 15°C. The drop in the pressure between 35° and 15°C. is due

\[ \text{Sörensen, S. P. L., Proteins, The Fleischmann Laboratories, New York, 1925.} \]
then to the separation of the insoluble fraction. If the concentration is sufficiently high a gel results; if not, particles are formed containing the soluble material and there is no osmotic pressure of the solution as a whole. The osmotic pressure as per cent of the value at 37°C. is plotted in Fig. 3 and as a function of the concentration of gelatin in Fig. 4. The percentage drop in the osmotic pressure is nearly constant in this range except for the very low concentrations. It will be noticed that the osmotic pressure increases more rapidly than the concentration, a result which can be predicted from the viscosity curves (Kunitz⁸) and which is due to the fact that a considerable quantity of the water is contained in the particles. (This effect will be discussed quantitatively in a subsequent paper.) The pressure also increases with the total concentration even when the gelatin is solid. This shows, as stated above, that the system cannot be considered as a saturated solution of one substance.

It should be possible, according to these results, to separate gelatin into two fractions: a soluble one, having high osmotic pressure, low

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viscosity, and no tendency to gel formation, and an insoluble one, having a limited solubility at low temperatures, a low osmotic pressure, and undergoing little or no swelling. It might also be expected, since the soluble fraction must be held in the meshes of the insoluble one, that isolation of the former would be much more easily accomplished than the separation of the insoluble from its accompanying soluble component. It was found possible after a number of attempts to separate gelatin into two fractions having nearly the properties described above. This was done by combining the temperature effect on the solubility with alcohol precipitation. The fractionation was carried out as follows:

**Preparation of Insoluble Fraction.**

10 liters of 5 per cent isoelectric gelatin at 35°C. and 7.5 liters of 95 per cent alcohol added; 18 hours at 20°C., filtered with folded paper. Filtrate = No. 1.

Precipitate: Made up to 8 liters with water, heated to 35°C., and 2 liters of alcohol added. Cooled to 20°C. and filtered. Filtrate rejected.

Precipitate: Made up to 8 liters, etc. as above, and repeated 4 times.

Precipitate: Made up to 2 liters with water, heated to 35°C., and 1.6 liters of alcohol added. Cooled to 30°C. Jelly-like residue settled. Supernatant liquid decanted and rejected.

Residue: Made up to 2 liters, etc. as above, and repeated 5 times.

Final residue: Made up to 2 liters with water at 35°C., 800 cc. alcohol added, and cooled to 10°C. Precipitate filtered and dried with alcohol and ether. 42 gm. marked “insoluble fraction.”

**Preparation of Soluble Fraction.**

Filtrate No. 1: 24 hours at 0°C. and filtered at 0°C. Filtrate rejected.

Precipitate: Made up to 400 cc. with water, heated to 30°C., 400 cc. of alcohol added, and cooled to 20°C. Filtered. Precipitate rejected.

Filtrate: 18 hours at 0°C. Filtered at 0°C. Filtrate rejected.

Precipitate: Made up to 200 cc., heated to 30°C., and 200 cc. of alcohol added. No precipitate on cooling to 20°C. Cooled to 0°C. 18 hours. Filtered at 0°C. Precipitate dried with alcohol and ether. 25 gm. marked “soluble fraction.”

No evidence is at hand to show that either of these fractions is a chemical individual and on the contrary there is reason to suppose that the insoluble fraction still contains a considerable quantity of the soluble since it still swells, and further purification caused a still further
Fig. 5. Effect of increasing amounts of soluble fraction on swelling of insoluble fraction. 5 cc. H₂O, 0.4 gm. insoluble + noted amounts of soluble fraction at 15°C.

Fig. 6. Osmotic pressure of various concentrations of gelatin and of the soluble and insoluble fractions.
decrease in the osmotic pressure. Owing to the difficulty of obtaining sufficient material with which to work, however, the process was stopped at this stage.

![Graph showing viscosity of various concentrations of gelatin and of the soluble and insoluble fractions.](image)

**Fig. 7. Viscosity of various concentrations of gelatin and of the soluble and insoluble fractions.**

**Comparison of the Properties of the Two Fractions and of Gelatin.**

In accordance with the mechanism of swelling outlined above it would be expected that the insoluble fraction should swell very little while the soluble fraction should dissolve. Mixtures of the two should swell the more the greater the percentage of soluble material. Fig. 5 shows that this is actually the case. The swelling of the in-
soluble fraction itself is probably due to the fact that it still contains an appreciable quantity of the soluble material, although it could of course be assumed that a different mechanism was responsible for this swelling.

Figs. 6 and 7 show the results of osmotic pressure and viscosity measurements. The viscosity of the insoluble fraction is much greater than that of gelatin while the osmotic pressure is less. The osmotic pressure of the soluble fraction is greater and the viscosity less, as would be expected. Since the insoluble fraction swells less than gelatin, it might be expected that the viscosity would be less instead of greater. It must be remembered, however, that although the individual particles swell less, the number of particles per gm. is greater in the insoluble fraction than in gelatin, since the latter also contains the soluble material. Since the viscosity, according to Einstein, depends on the total volume occupied by the particles, the higher viscosity of the insoluble fraction is due to the fact that the increase in the number of particles capable of swelling more than makes up for the difference in the swelling of the individual particles. As was stated above, the form of the osmotic pressure curves can be quantitatively accounted for by the absorption of water in the particles. Qualitatively it can
be predicted that the substance having the highest viscosity should have the greatest curvature in the osmotic pressure-concentration curves and, as the figures show, this is the case. The marked difference in the viscosity of the soluble and insoluble fractions was ascribed above to the fact that the insoluble fraction in solution consisted largely of particles capable of swelling, while the soluble fraction did not. If this is the case, it would be expected that the insoluble fraction should show a marked pH-viscosity effect, while the soluble fraction should show little or none, since, according to Loeb, the increase in viscosity on addition of acid is due to the swelling of such particles by the Donnan equilibrium set up in them. Fig. 8 shows that this is the experimental result. On the other hand, it would be expected that the effect of acid on the osmotic pressure would be about the same, since in this case the collodion sac is the particle and is evidently present in both cases. This expectation is also fulfilled as is shown in Fig. 9.
The titration curves of the two fractions, and the speed with which they are digested by pepsin, do not differ significantly from those of ordinary gelatin.

The Effect of Temperature and Salts on the Osmotic Pressure.

It was suggested above that the increase of pressure occurring when gelatin solutions or gels are raised from 25° to 37°C. was due to the solution of the substance forming the network and corresponding to the insoluble fraction. It would be expected therefore that the temperature would have a still greater effect on the osmotic pressure of the insoluble fraction and a smaller effect on the soluble fraction. This is the result, as shown in Table II. The insoluble fraction still exerts pressure below 25°C. which indicates, as stated above, that it still contains some soluble material.

As is well known, strong solutions of some neutral salts liquefy gelatin and, as was shown in a preceding paper, also increase the osmotic pressure. The action of these salts on gelatin at 15°C., and the effect of raising the temperature to 37°C. are therefore similar except that the increase of osmotic pressure due to the salt is greater than that due to increasing the temperature. A solution of gelatin at 37°C. contains particles capable of swelling, as is shown by the pH-viscosity effect. The osmotic pressure of such a solution could be increased in two ways:

First, the salt might increase the swelling of the particles. Since

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Soluble fraction Pressure in mm. Hg.</th>
<th>Insoluble fraction Pressure in mm. Hg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>50 liquid.</td>
<td>10 liquid.</td>
</tr>
<tr>
<td>25</td>
<td>48 &quot;</td>
<td>6.7 solid.</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>5.3</td>
</tr>
</tbody>
</table>
in this process water is removed, while the number of particles remains
the same, the result is an increase in the mol fraction, and a resulting
increase in the osmotic pressure. Second, the salt might cause these
particles to break up into smaller ones, thereby increasing the number
of particles and hence the osmotic pressure. On the basis of the first
assumption it would be expected that the salt should also increase the
viscosity and further that the effect of temperature, in the presence of
salt, would not differ very much from that in the absence of salt. On

TABLE III.

Effect of Salts on Viscosity and Osmotic Pressure of 10 Per Cent Gelatin.

<table>
<thead>
<tr>
<th>Concentration of salt</th>
<th>0</th>
<th>2 M NaSCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative viscosity, ( \frac{\eta}{\eta_{\text{salt}}} ), at 37°C</td>
<td>19.3</td>
<td>18.10</td>
</tr>
<tr>
<td>Osmotic pressure, mm. Hg, at 37°C</td>
<td>60</td>
<td>122</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; &quot; 25°C</td>
<td>44</td>
<td>113</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; &quot; 15°C</td>
<td>20</td>
<td>108</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; &quot; 5°C</td>
<td>20</td>
<td>102</td>
</tr>
</tbody>
</table>

TABLE IV.

Effect of NaSCN on the Osmotic Pressure of 5 Per Cent Soluble and Insoluble Fractions of Gelatin at 37°C.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Soluble</th>
<th>Insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of NaSCN inside and outside</td>
<td>0</td>
<td>2M</td>
</tr>
<tr>
<td>Osmotic pressure, mm. Hg</td>
<td>50</td>
<td>61</td>
</tr>
</tbody>
</table>

the second assumption there would be expected no change or a decrease
in the viscosity and the temperature effect on the osmotic pressure
in the presence of salt should be much less than when no salt is present.
The experiments in Table III show that the latter results are obtained.
2 M NaSCN has very little effect on the viscosity of 10 per cent gelatin
and the osmotic pressure decreases only slightly in the range from 37°C
to 3°C. The optical properties of the solution also bear out this as-
sumption, since a solution of gelatin at 37°C shows a very marked Tyndall cone, whereas the same solution in 2 M NaSCN shows only a faint cone.

It has already been shown that the effect of temperature is largely on the insoluble component and it remains to be seen whether the effect of salt is also on this component or on the soluble fraction. Table IV gives the effect of NaSCN on the osmotic pressure of a 5 per cent solution of the soluble and insoluble fractions at 37°C. It is evident that there is a very large increase in the osmotic pressure of the insoluble fraction and only a small increase in that of the soluble one. The salt therefore affects the same constituent as does raising the temperature.

DISCUSSION.

The preceding experiments appear to the writers to furnish a basis for a simple and satisfactory picture of the mechanism of the swelling of isoelectric gelatin in water and in neutral salt solutions. The block of gelatin is a network consisting of threads of a substance insoluble in cold water and holding in its meshes a solution of a substance soluble in water. (For the sake of simplicity only two substances are considered, although in reality there are probably a series of substances whose properties grade from very easily soluble to insoluble.) The process of manufacture of gelatin would be expected to lead to the formation of just such a series of compounds, since it is formed by the hydrolysis of an insoluble substance, collagen. Such a reaction would be expected to produce a series of split products ranging in complexity from the amino-acids up to unchanged collagen. In the further process of purification and washing, all substances which can diffuse through the network of insoluble material are removed while those that cannot are held back. When the block is immersed in water the internal solution exerts osmotic pressure and water is taken in until the osmotic pressure is equalized by the elastic force of the network. A slow subsequent swelling will occur, however, owing to the fatigue of this elastic force. A quantitative expression for the kinetics of swelling has been derived on this basis and will be discussed in a subsequent paper.

Raising the temperature causes an increase in the concentration of
the soluble material and hence in the osmotic pressure of the solution and at the same time causes a decrease in the amount of insoluble material forming the fibers. The swelling, therefore, increases very rapidly with the temperature until above 35°C. the network dissolves and the block disintegrates. The insoluble material, however, still exists in the solution in the form of particles capable of swelling, as shown by the pH-viscosity effect, and containing a large amount of water, as shown by the form of the osmotic pressure-concentration curves and the viscosity curves. The addition of neutral salts to the solution acts in the same way as increasing the temperature, except that the material forming the network is broken up into smaller particles. In very high salt concentrations the reverse effect occurs and the gelatin eventually precipitates. The various peculiar hysteresis effects which have been noted in connection with the swelling of gelatin are referable to the effect of the conditions on the elasticity of the network, and are similar to those observed in any elastic body. Cooling the solution results in the reverse process. The particles or molecules of the insoluble material precipitate in the form of a network—if the concentration is high enough—enclosing the solution of the soluble material. If the concentration is low, this network separates in the form of small clots which still contain nearly all of the soluble fraction. It seems quite possible that the formation of this peculiar structure rather than of an ordinary precipitate is due to the presence of the soluble material which acts as a protective colloid, and that a pure preparation of the insoluble material would form a normal precipitate. A very similar condition occurs in the solution and repurification of casein; addition of small amounts of acid or alkali to isoelectric casein results in a normal saturated solution, as Cohn has shown. Precipitation of such a solution by back titration, however, gives a milky colloidal solution which may form a gel, so long as any of the casein remains in solution. When the isoelectric point is reached, where all the casein is insoluble, a normal precipitate is again formed.

9 This is perhaps analogous to the prevention of agglutination in strong salt solutions. In the case of bacteria it was found that a marked decrease in the "cohesive force" of the particles was noted in these high salt concentrations. Northrop, J. H., and De Kruif, P. H., J. Gen. Physiol., 1921-22, iv, 639.

This mechanism also accounts for the fact that gelatin and similar substances swell only in those solvents in which they are soluble, and it predicts that the greater the solubility the greater the swelling. The swelling which occurs during the early stages of the action of enzymes on gelatin also would be predicted since the hydrolysis increases the concentration of soluble material and hence the osmotic pressure. The swelling caused by enzymes is similar to the effect of salts or higher temperatures except that it is not reversible.

The alternative hypothesis that the effect of temperature or salts is to increase the hydration of the particles and hence the osmotic pressure seems less probable, since it requires that the hydration increase with increasing temperature. Since the hydration of gelatin liberates heat this would be contrary to Le Chatelier's principle. It also fails to predict the increased swelling obtained on adding the soluble fraction to the insoluble, since the osmotic pressure and viscosity curves show that the soluble fraction is less hydrated than the insoluble.

It is evident that the structure of gelatin outlined above is quite similar to that proposed by Hardy\(^1\) and now widely accepted, except that at least two substances are postulated. The whole mechanism agrees in detail with the theory clearly presented by Duclaux\(^2\) in connection with the swelling of rubber.

**SUMMARY.**

1. A method is described for measuring the swelling pressure of solid gelatin.
2. It was found that this pressure increases rapidly between 15° and 37°C., and that the percentage change is nearly independent of the concentration of gelatin.
3. It is suggested that this pressure is due to the osmotic pressure of a soluble constituent of the gelatin held in the network of insoluble fibers, and that gelatin probably consists of a mixture of at least two substances or groups of substances, one of which is soluble in cold water, does not form a gel, and has a low viscosity and a high osmotic pressure.

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pressure. The second is insoluble in cold water, forms a gel in very low concentration, and swells much less than ordinary gelatin.

4. Two fractions, having approximately the above properties, were isolated from gelatin by alcohol precipitation at different temperatures.

5. Increasing the temperature and adding neutral salts greatly increase the pressure of the insoluble fraction and have little effect on that of the soluble fraction.

6. Adding increasing amounts of the soluble fraction to the insoluble one results in greater and greater swelling.

7. These results are considered as evidence for the idea that the swelling of gelatin in water or salt solutions is an osmotic phenomenon, and that gelatin consists of a network of an insoluble substance enclosing a solution of a soluble constituent.