There are two distinct methods which are used to study the volume changes which take place when a protein is placed in a solution. The first and most common method measures the volume changes of the solid alone and is commonly known as swelling. The second measures the total volume change which takes place upon mixing the two components—that is, the volume of the solution plus the volume of the solid protein minus the volume of the resulting system. The results of the first method have been thought to indicate the degree of water absorption, while the second supposedly indicates the degree of hydration.

A knowledge of the latter might possibly lead to a better understanding of the processes of the former, and, consequently, to a better understanding of the phenomenon of swelling. Up to the present time, very few measurements have been made on the total volume changes occurring during the swelling of proteins, and the results which have been published do not substantiate each other. Therefore a study of the volume changes accompanying the swelling of gelatin in solutions of varying hydrogen ion concentration has been made in an attempt to correct and correlate the data already present in the literature.

Measurements have been made to determine the influence of pH on the contractions accompanying the swelling of gelatin. It was found that a definite minimum in contraction occurred at the isoelectric point, the contraction increasing with increase or decrease in the hydrogen ion concentration. The maximum and minimum in the contraction curve below the isoelectric point occurs at a pH corresponding to the maximum and minimum in the swelling curve given by Loeb. The maximum on the basic side is shifted toward the isoelectric point. No second minimum occurs at the higher pH values.

The contraction-time curve has the same general shape as an absorption-velocity curve. When the contraction per gram of water imbibed is plotted against the time a straight line is obtained. The results indicate that the contraction per gram of the original gel is independent of the amount of gelatin used.
CONTRACTIONS ACCOMPANYING THE SWELLING OF GELATIN IN SOLUTIONS OF VARYING pH

by

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CONTRACTIONS ACCOMPANYING THE SWELLING OF GELATIN
IN SOLUTIONS OF VARYING pH

The diversified views and inconsistent reports of the various investigators, as well as the heterologous nature of the theories proposed to explain the results, is sufficiently vindicative to warrant further study on the volume changes of protein systems.

There are two distinct methods which are used to study the volume changes which take place when a protein is placed in a solution. The first and most common method measures the volume changes of the solid alone which is commonly known as swelling. The second measures the total volume change which takes place upon mixing the two components—that is, the volume of the solution plus the volume of the solid protein minus the volume of the resulting system. The results of the first method have been thought to indicate the degree of water absorption, while the second supposedly indicates the degree of hydration.

A knowledge of the latter might possibly lead to a better understanding of the processes of the former, and, consequently, to a better understanding of the phenomenon of swelling. Up to the present time, very few measurements have been made on the total volume changes occurring during the swelling of proteins, and the results which have been published do not substantiate each other. Therefore a study of
the volume changes accompanying the swelling of gelatin in solutions of varying hydrogen ion concentration has been made in an attempt to correct and correlate the data already present in the literature.

History

That the growth, metabolism, and condition of an organism is in some way closely related to its water content has long been recognized. This has led to considerable study of swelling. Fischer (6-12) was among the first to realize the importance of swelling in connection with biological study and published extensive data on the influence of salts, acids and bases of various normality on the degree of swelling. From the results of his experiments he proposed that edema and nephritis were caused by unnatural degrees of swelling brought about by unusual acid content of the affected organism. His explanation was at once criticized (5, 13, 21) and from the controversy there arose a prodigious amount of experimenting and theorizing on swelling.

Both swelling and the accompanying contraction are dependent upon a number of factors. These are:

Temperature. An increase in temperature is usually accompanied by an increase in swelling (1,35,43,49,58,59,64) and a decrease in the contraction (46,52-57,57,75). That the swelling of gelatin is increased upon increasing the
temperature is diametrically opposite to what is required by
the principle of Le Chatelier, inasmuch as gelatin emits
considerable heat in the process of absorption.

**Thickness.** There is considerable controversy as to
whether or not the thickness and shape of the pieces of
gelatin affect the swelling (36,71,72). On the basis of ex-
perimental results, Pauli (62) and Hofmeister (22) have
developed equations which relate the swelling to the thick-
ness of the plates of gelatin used. Northrop and Kunitz,
on the other hand, have conducted experiments in which they
varied both the thickness and the shape of the gelatin and
found that the resulting swelling was constant within exper-
imental errors—the same for any given weight of gel.

**pH, Acids and Bases.** Possibly the greatest influence
on swelling is the hydrogen ion concentration (3,18,23-29,
34,38,40,41,43,45,48,64-67,70,73). It is generally accepted
that the swelling value is a minimum at the isoelectric
point, increasing on either addition of acid or base, to a
maximum at pH's of about 3 and 11. The maximum on the basic
side is never so high as that on the acid side, probably due
to an increased solution at the higher hydrogen ion concen-
trations. After reaching the maximum value on the acid side
the swelling decreases to a minimum and then increases again
as the hydrogen ion concentration is increased (16). In the
basic range the swelling rises more gradually from that at
the isoelectric point up to a pH of about 7 at which point the curve flattens into a plateau in the pH range of about 7 to 6.5 after which it rises abruptly to a maximum at a pH of about 11, falling off continuously with increasing pH. There are a few investigators (63,69,60) who report two minimum points—one at the isoelectric point and the other at approximately pH 8.

Marie and Marinesco (46) have obtained similar results for the influence of the hydrogen ion concentration on the volume contraction. Neville, Thies, and K'Burg (52), and Svedberg (75) report the opposite effect—that is, when the contraction is plotted against the pH a maximum contraction is obtained at the isoelectric point while increasing or decreasing the pH causes a decrease in the contraction.

**Effect of Salts and Other Added Substances.** In general the addition of neutral salts to a gel-water system decreases the swelling in either the acid or basic range and causes an increase near the neutral point (14,30,33,35, 47,48,56,57,74,78,79). The swelling is approximately the same for all ions of the same valence type (13,34); however, there is a characteristic difference observed even among the ions of the same valence type. The ions have been arranged by Hofmeister (22) according to their influence upon the swelling. Loeb finds that if the pH of the solutions is the same, the swelling produced by ions of the same valence type will be practically equal, at a relatively low concentration
of the ion (41,44). As a rule non-electrolytes have little influence on the swelling of gelatin (19,33,51).

Svedberg (75) has found that neutral salts, weak acids and alkalis, and non-electrolytes have little effect upon the total contraction. On the other hand, in their work on astringent action of various substances on hide, Neville, Thies, and K'Burg (52) found that when fully hydrated hides were placed in salt solutions the system expanded. In other words the salts used decreased the forces causing the contraction.

**Original Concentration.** The greater the water content of the gel at the time of setting the greater will be the swelling after the gel is dried and placed in solution (4, 71,72). A possible explanation for this is given by D. J. Lloyd (39), who believes that the water present at the time of setting reduces the cohesive forces between the gelatin micelles with the result that the same osmotic pressure produces a greater swelling. With gels of the same concentration at the time of setting, the greater the water content after drying the less is the swelling inasmuch as all gelatin gels tend to swell to an equilibrium concentration of approximately ten percent (31).

As would be expected, the greater the water content of the gel the less is the total contraction (75).

**Volume of Solution.** A number of investigators (15, 58,59) have found that the volume of the solution greatly
Figure 1

capillary

meter stick

rubber stopper

120 cc bottle

bolts

submersion fluid

Cu gauze container
influences the resulting swelling but when ash-free samples of gelatin are used this effect is not obtained (2, 31, 36, 39, 55).

Marie and Marinesco (46) observed that only the first traces of water produced any marked contraction. They state, "Under these conditions contraction, \( dv \), reported per gram of the dry colloid, diminished rapidly with increase in dilution. For example, in one series of measurements at pH 5.2, we obtained a contraction in volume per gram of 29 cubic mm. with the addition of three centi-grams of water; upon increasing the dilution much more, \( dv \) tends toward a lower limit corresponding to 0.35 grams of water per gram of colloid. The solvent introduced no longer produces an increase in contraction and acts as tho the micellae had arrived at a sort of saturation of hydration."

Experimental Procedure

In order to measure the total volume change which takes place when gelatin is introduced into a solution, it is necessary to have some form of apparatus such that the gel and solution will be at a constant temperature throughout the experiment and also such that the volume of gel and solution can be determined just previous to the introduction of the gel to the solution. These conditions were satisfied by the use of a simple dilatometer, Figure 1, similar to that described by Hampton (20).
This consisted of a 120 cc. bottle to which was fitted a rubber stopper carrying a capillary of approximately one square millimeter in cross-sectional area. The rubber stopper and capillary were held in place by means of two metal strips bored with holes to fit the bolts which were used to clamp them in place as indicated.

Mercury was first used as the submersion fluid, and the gel was held under the mercury by means of small wax discs attached to the stopper by means of a glass rod. This was found to be unsatisfactory inasmuch as the air absorbed in the gel is slowly displaced by the mercury and, due to the high surface tension of the mercury, cannot escape to the surface but is trapped, along with the gel, under the disc and, upon releasing the gel, rises to the surface increasing in volume as it rises and causing a corresponding rise of the liquid level in the capillary. A constant error from this source could not be relied upon.

It was thought best to use a liquid of a lower surface tension and encase the gelatin in some relatively open container, thus eliminating any possibility of encasing the air. This was accomplished by placing the gel in small copper gauze containers and suspending them in carbon tetrachloride by means of #35 copper wire threaded thru the capillary. After equilibrium was reached the level of the liquid in the capillary was noted. The container was drawn up into the
aqueous phase and the difference in the length of the wire extending from the capillary was noted. From these readings the contraction is calculated.

All readings were made at 10⁰ C. Eastman Kodak purified gelatin was used in nearly all of the experiments. For the determinations of the effect of time and weight of gelatin on the contraction, the gelatin was set in eight inch test tubes, removed from these, sliced into uniform pieces, and dried. This was done to obtain pieces of nearly the same cross-sectional area and thickness in order to obviate any differences in contraction which might arise from that source.

The effect of time, weight of gelatin, and pH on the contraction of gelatin was determined. The solutions of acid and base were made up from standard solutions of HCl and NaOH and the pH was determined, electrometrically, before and after swelling.

Experimental Results

The contraction per gram of the original gelatin occurring when gelatin is allowed to remain in water for varying intervals of time is given in Table 1 and plotted, in Figure 2. Inasmuch as the curve becomes nearly horizontal after a time interval of about eight hours the acid and basic determinations were made in this time interval. Because of the similarity between this curve and an adsorption-
Figure 2

Contraction per gram HgO imbibed A
Contraction per gram dry colloid in cu. mm. B

Figure 3

Log of contraction per gram dry colloid in cu. mm.
velocity curve, the log of the contraction per gram of the original colloid is plotted against the log of the time and the resulting curve is a straight line, Figure 3. The contraction per gram of water imbibed is plotted in Figure 2 and results in a straight horizontal line.

Table 1

The effect of time on the contraction.

Volume of water used -- 90 cc

Weight of gelatin used -- approximately 0.5g

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Contraction cubic mm per g of original gel.</th>
<th>Contraction cubic mm. per g of water imbibed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>12.06</td>
<td>6.81</td>
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<tr>
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<td>24.0</td>
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<td>4.48</td>
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</table>

The contractions occurring when varying amounts of gelatin were brought into contact with the same volume of solution are given in Table 2 and are plotted in Figure 4. With the small amounts of gelatin the contraction was small and consequently the errors in reading the differences in height of the liquid in the capillary were relatively large.
Figure 4

Cu. mm. contraction per gram dry gelatin

Figure 5

Cu. mm. contraction per gram dry gelatin
Table 2
The effect of the weight of gelatin on contraction.

The volume of water used -- 90cc

<table>
<thead>
<tr>
<th>Grams gel.</th>
<th>Contraction cubic mm. per g. of original gel</th>
<th>Contraction cubic mm. per g. water imbibed</th>
<th>Grams water imbibed per g. original gel</th>
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<td>23.43</td>
<td>9.59</td>
<td>2.44</td>
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</table>

The variations of the contraction with the pH are given in Table 3 and plotted in Figure 5. The values given for the contraction per gram of the water absorbed and for the grams of water absorbed per gram of the original gelatin are questionable for, in the pH range where maximum swelling occurs and at the higher pH values where solution effect was large, small amounts of gelatin were lost in handling.
Table 3

The influence of the pH on the contraction.

<table>
<thead>
<tr>
<th>Grams gel</th>
<th>Initial pH</th>
<th>Final pH</th>
<th>Contraction cu. mm per g original gel</th>
<th>Contraction cu. mm per g water imbibed</th>
<th>g water imbibed per g of original gel</th>
</tr>
</thead>
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<tr>
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</tr>
</tbody>
</table>

Discussion of Results

Pauli (61) has ascribed the hydration of hydrophilic colloids to the formation of a compressed water sheath about the colloidal micelle. Irregardless of whether the water is held about the micelle by gravitational (6) or electrostatic (60) forces, there will be a resulting increase in the density of the water in the immediate
Figure 6

Cu. mm. contraction for 50 grams of gelatin

Figure 7

Cu. mm. contraction per g. dry gelatin
neighborhood of the micelle. This will result in a decrease in the total volume of the system. Accepting this viewpoint the more highly hydrated the system becomes the greater will be the contraction.

Neville, Thies, and K'Burg (52) found that maximum hydration occurred at the isoelectric point and decreased with the addition of either acid or base, Figure 6. They gave no explanation for the decrease in hydration at the higher and lower pH's but stated that it was probably due to a decrease in the compression of the water sheath about the micelle. An examination of their curves reveals that they have entirely ignored any determination of contraction between the pH values of approximately 4 and 6. Although the article consists mainly of a discussion of the properties of gelatin at its isoelectric point, all points lie decidedly to either side of pH 4.7. An examination of Figure 5 reveals that maximum contraction on the acid side occurs near a pH value of 3.7, corresponding to the first point given on their curves below the isoelectric point, and that the contraction is well on its way to the maximum, on the basic side, at a pH of about 5.6, corresponding to the first point given on their curves above the isoelectric point. Plotting the values obtained from Figure 5 corresponding to the points indicated by Neville, Thies, and K'Burg, a curve which resembles theirs is obtained, Figure 7.
Svedberg (75) obtained similar results, working at a temperature above the setting point of the gelatin, Figure 8. As reason for the decrease in hydration with the addition of acid and base he states: "If the density increase is due to the formation of a shell of highly compressed water around the gelatin particle, we should expect the density to vary with the amount of acid in solution since there is a contest set up between the gelatin particles and the acid for possession of the water."

Contrary to Svedberg's interpretation, Figure 5 shows a minimum at the isoelectric point, and an increase in acid and base concentration produces an increase in the contraction up to a maximum. The curve has the characteristic shape of the swelling curve given by Loeb (43). A maximum on the acid side of the isoelectric point falls at the same pH value on both the contraction and the swelling curves, pH of about 3.4. Both curves show a minimum at a pH of about 2.1. On the basic side the maxima do not occur at the same point, the maximum in the contraction curve occurring at a pH value somewhat nearer the isoelectric point than that for the swelling curve. This is probably due to the increased hydrolysis of the gel at the higher pH values.

When gelatin is placed in acid or basic solutions of varying concentration, two things occur, according to Kuntzel (32). These two things are: an increase in the
Figure 8

Cu. mm. contraction per g. dry gelatin

![Graph showing contraction per g. dry gelatin with NaOH and HCl curves.]

Figure 9

A. Resultant Swelling  B. Swelling due to charge  C. Swelling due to solution

![Graph showing swelling with pH scale.]
charge at the surface of the gelatin micelle, and an increase in the solubility of the gelatin.

Consider, first, the effect of these two phenomena on the swelling. If the increased swelling in acid and basic solution were due entirely to the first phenomenon, both curves would be symmetrical to the axes drawn through the points of maximum swelling which would also represent maximum charge. However, this is not what is obtained, the curves being skewed away from the isoelectric point. Kuntzel regards the skewness as being caused by the second phenomenon—that is, a decrease in the cohesive forces between the gelatin particles or an increase in the solubility of the gelatin. This is summed up in Figure 9. The dotted curve A A' represents the swelling due to increased charge, or acid and base absorption. The dotted curve B B' represents the swelling due to the decrease in the cohesive forces between the micellae. The unbroken curve represents the sum of the other curves and approximates the swelling curve.

The influence of the pH on the contraction may be explained in a similar manner if, in addition to the other two, a third factor, pore size, is included. Gelatin is composed of micellae and the interstices will form a large number of small pores. As the charge and solubility increase the micellae will be forced further and further apart and the pore size will increase up to a maximum and then decrease due
Figure 10

E'AB' Swelling Curve
FEAB Contraction Curve
to the decrease in the charge with subsequent decrease in hydration. The compressive force due to surface tension will vary according to the equation $P = \frac{2\sigma}{r}$, where $P$ is the pressure exerted by the surface tension $\sigma$ when the radius of the pore is $r$. In Figure 10 the relation between the swelling and contraction curves is represented. At the point A, both the swelling and the contraction are at a minimum. With the addition of acid, the charge on the micellae is increased and consequently hydration is greater; but at the same time swelling takes place and the gel micellae are forced apart. The increased pore size, caused by swelling, produces a decrease in the internal pressure of the aqueous phase, and therefore the density increase, due to hydration, is partially compensated by the decrease in the compressive force of the surface tension. Near the points E, E', the charge and hydration are at a maximum. At these points the pores have attained a maximum size, and for this reason the contraction curve is considerably lower than the swelling curve. As the concentration of acid is increased, the charge on the micellae is gradually neutralized and hydration decreases. At the same time swelling decreases, the pore size becomes smaller, and consequently the internal pressure of the aqueous phase increases, partially offsetting the decrease in volume due to the decreased hydration. The swelling curve again approaches the con-
Figure 11

Contraction pH Curve of Marie and Marinesco

Cu. mm. contraction per g. dry gelatin
traction curve. At the point, F, a minimum charge and hydration are obtained, and a minimum is observed in the contraction curve. This minimum is not as low as the minimum obtained at the isoelectric point inasmuch as the charge at this pH is not zero. Upon further addition of acid, the charge again increases with an accompanying increase in hydration. However, at the higher acid concentration, the increase in contraction is not as great because of the increasing influence of solution and hydrolysis.

The same reasoning can be applied to the basic side of the curve. B and B' represent maximum charge and hydration, but at the higher pH's the influence of solution and hydrolysis is greater than on the acid side. This causes the contraction to diminish much more rapidly than the swelling.

Marie and Marinesco (46) obtained a contraction curve, Figure 11, similar to Figure 5 but did not continue their measurements above and below the points of maximum contraction.
Measurements have been made to determine the influence of pH on the contractions accompanying the swelling of gelatin. It was found that a definite minimum in contraction occurred at the isoelectric point, the contraction increasing with increase or decrease in the hydrogen ion concentration. The maximum and minimum in the contraction curve below the isoelectric point occurs at a pH corresponding to the maximum and minimum in the swelling curve given by Loeb. On the basic side of the isoelectric point the maximum is displaced toward the isoelectric point—probably due to the hydrolysis at the higher pH values. No second minimum occurs at the higher pH's—at least not in the pH range studied—but the curve descends until at a pH of about 13 there is no contraction.

Measurements were also made to determine the contractions occurring at different intervals of time and to determine the influence of varying the amount of gelatin placed in the same volume of solution. The contraction-time curve has the same general shape as an absorption-velocity curve. When the contraction per gram of water imbibed is plotted against the time a straight line is obtained. The results indicate that the contraction per gram of the original gel is independent of the amount of gel used.


64. Pleass, W. B. Absorption of Water by Gelatin VII Influence of Temperature on the Swelling in acid Solutions and the Combination of Gelatin with hydrochloric, Nitric or sulfuric acid. Biochem. J. 24:1943-8, 1931.


80. Wilson, J. A.