

## SYNERESIS AND SWELLING OF GELATIN.

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The name syneresis was given by Graham<sup>1</sup> in 1864 to the phenomenon of the breaking up of jellies on long standing or when disturbed. The jelly then, instead of consisting of one homogeneous mass, becomes segregated into solid lumps surrounded by a thin liquid. Syneresis is quite common in jellies or, as they are called, gels of gelatin, agar, starch, etc. It is possible that the separation of serum from clotted blood and the splitting of soured milk into curd and whey may also be classified under the same term. Various theories have been suggested as to the possible cause of syneresis.

Thus Ostwald<sup>2</sup> considers syneresis as simply a separation of phases similar to the separation of phases in a critical fluid mixture. Lloyd<sup>3</sup> explains syneresis of isoelectric gelatin as being due to the absence of soluble gelatin salts, which otherwise keep the network extended by their osmotic pressure. As it will be seen from further discussion, Miss Lloyd's hypothesis is partly true with respect to the absence of syneresis in gelatin in the presence of electrolytes, although there is apparently some confusion as to the meaning of "soluble gelatin salts." With the exception of some recent work by Liepatoff<sup>4</sup> on *geranine* and Mukoyama<sup>5</sup> on *viscose*, very little quantitative work has been done on syneresis in general and of gelatin in particular.

The writer approached the problem of syneresis in gelatin through

<sup>1</sup> Graham, T., Chemical and physical researches (collected papers), Edinburgh 1876, 619.

<sup>2</sup> Ostwald, W., An introduction to theoretical and applied colloid chemistry, New York, 1917, 93.

<sup>3</sup> Lloyd, D. J., *Biochem. J.*, 1920, xiv, 165.

<sup>4</sup> Liepatoff, S., *Kolloid.-Z.*, 1927, xliii, 396.

<sup>5</sup> Mukoyama, T., *Kolloid.-Z.*, 1927, xlii, 79.

the study of the swelling of blocks of dilute gelatin when immersed in water. All of the former studies on swelling of gelatin have dealt with the swelling of dry gelatin or of concentrated gels. In all these cases there is always a gain of water and an increase in size of the gelatin block even at low temperatures. Quite different results are obtained when blocks of isoelectric gelatin of concentrations of less than 10 per cent are immersed in cold water. Instead of gaining water the blocks of gelatin lose water, and the lower the concentration of gelatin the greater is the amount of water lost.

Table I shows the values of swelling at 5°C. of gelatin gels of various concentrations when immersed in M/1000 acetate buffer pH 4.7. The

TABLE I.

*Swelling of Blocks of Gels of Various Gelatin Content at 5°C. in M/1000 Acetate Buffer pH 4.7.*

Electrolyte-free isoelectric gelatin has been used in making up the gels. Gels set for 24 hrs. at 5°C. before they were placed in the buffer solution.

Concentration of gelatin in gm. per 100 cc. solution.....	3.0	4.0	5.0	6.0	8.0	10.0	12.0	14.0	16.0	20.0
Original weight of block of gel (after setting).....	1.610	1.600	1.505	1.790	1.890	1.847	2.020	2.120	2.090	2.250
Final weight of block at equilibrium (after 20-25 days).....	1.150	1.140	1.130	1.435	1.730	1.845	2.200	2.470	2.570	3.050
Per cent change in weight.	-29	-29	-25	-20	-8	0	+9	+17	+23	+36

values are those obtained at equilibrium, which is reached after 20 to 25 days. It is seen that while at concentrations above 10 per cent the gelatin blocks gain in weight, at the lower concentrations there is a continuous drop in weight.

The theories of swelling as developed by Procter, Wilson, Loeb, Northrop and the writer deal with the process of positive swelling of gelatin. These may be summarized as follows:

1. Swelling is mainly a process of osmosis due to a greater concentration of mobile molecules in the block over that of the outside solution in which it is immersed.

2. The molecules causing osmosis may be diffusible ions, as in the case of swelling due to acids, alkalies and some salts, or non-diffusible molecules or groups of molecules as in the swelling of isoelectric gelatin.

3. A block of solid gel behaves like a true elastic body. The swelling is regulated by the elasticity of the gelatin block. At equilibrium the elastic pressure is equal and opposite to the osmotic pressure.

4. When a gelatin sol sets to a gel it is under no elastic strain as long as it is not immersed in water. As soon as it is put into water or salt solution the osmotic forces begin to act against the elasticity of the block causing a strain in the block due to an increase in the bulk. A strain is also brought about in a block of gelatin when water is removed from it by evaporation.

The tendency of a block of a dilute gel to shrink when placed in distilled water indicates that there is still a possible strain in the gel while it sets, which is contrary to the apparent non-strained condition of gels of higher concentration, as demonstrated by Northrop.<sup>6</sup> With the object of finding a possible explanation of this peculiar behavior of dilute gels the writer undertook a detailed study of the "negative swelling" of gelatin under various conditions.

#### *Experimental Procedure.*

All the experiments as well as the weighings were done in a refrigerator room kept at a temperature of about 5°C.

Solutions containing various amounts of isoelectric gelatin in distilled water of pH 4.7 or in salt solutions were heated to 50°C. and coated on weighed microscopic slides (1.5 cc. per slide), or 2 cc. were poured into moulds, consisting of short Pyrex glass tubing of about 15 mm. diameter, which were mounted on a clean paraffin block. The gelatin was allowed to set in the refrigerator for about 20 hours. Care was taken to prevent evaporation by keeping the slides in "moist chambers" or stoppering the tubes with rubber stoppers. After the period assigned for setting, the slides or the blocks, on removal from the tubes, were weighed and put into 150 cc. M/1000 acetate buffer pH 4.7 or into other solutions as described later. After various intervals of time the slides or blocks were dried with filter paper and weighed. In drying blocks of dilute gelatin it was found more convenient to use a clean towel instead of filter paper.

The fluid originally used as the outside solution was distilled water. This was brought to pH 4.7 by means of acetic acid.\* But it was soon found that the pH of the water increased slightly after a few days, with the result that the gelatin began

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<sup>6</sup> Northrop, J. H., *J. Gen. Physiol.*, 1926-27, x, 901.

\* Distilled water made up with acetic acid to pH 4.7 is designated throughout this paper as H<sub>2</sub>O pH 4.7.

to gain in weight instead of reaching a constant value. Hence distilled water was replaced by  $m/1000$  acetate buffer pH 4.7.

Fig. 1 shows the effect of two concentrations of acetate buffer pH 4.7 as well as of  $H_2O$  on the negative swelling of 4 per cent isoelectric gelatin coated on slides. During the first few days there was a loss of water in all the gelatin blocks; but afterwards those that were kept in

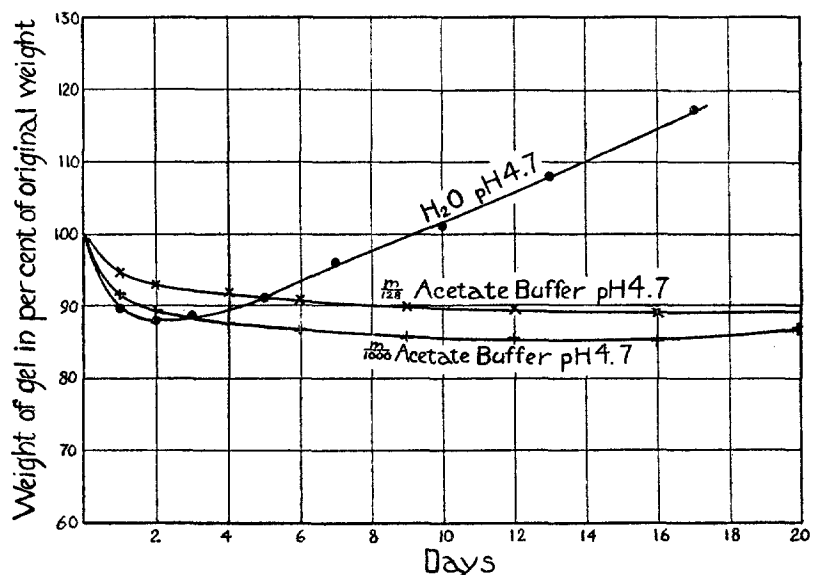


FIG. 1. Effect of a slight change with time in the pH of the outside solution on the change in weight of 4 per cent gels at  $5^\circ C$ . The pH of the distilled water became slightly higher than 4.7 on standing.

$H_2O$  began to gain weight continuously, while the others that were put in buffer solution kept on losing weight until the 10th day when an approximate equilibrium was established. After the 20th day the block in  $m/1000$  buffer began to gain slightly in weight but on renewal of the buffer the equilibrium weight was reached again. The experiment also shows that there is less loss in weight of gelatin with the increase in concentration of salt in the surrounding medium. This is simply due to the fact that salts generally increase the swelling of isoelectric gelatin up to a concentration of  $m/2$ .<sup>7</sup>

<sup>7</sup> Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1925-28, viii, 317.

*Is the Negative Swelling Due to Solution of the Gelatin?*

A qualitative test on the protein content of the surrounding medium showed that only a trace of the gelatin goes into solution. Quantitative experiments were carried out as follows:

Blocks of 4 per cent of isoelectric gelatin were allowed to remain in large volumes of  $m/1000$  acetate buffer pH 4.7 until the loss in weight became constant. The blocks were then removed, put into weighed evaporating dishes, weighed and then dried in an electric oven at  $100^{\circ}\text{C}$ . for 24 hours. In all cases the dry weight measurements checked with the weight of gelatin as calculated from the loss of

TABLE II.

*Effect of Duration of Setting on the Negative Swelling of 5 Per Cent Isoelectric Gelatin in  $m/1000$  Acetate Buffer pH 4.7.*

Time of setting.....	0	16 hrs.	40 hrs.	68 hrs.	92 hrs.	6 days	8 days
Weight of gelatin after being in moist chamber, in per cent of original weight.....	100	99.4	100	101	98.5	95	100
Final weight of gelatin in $m/1000$ acetate buffer at equilibrium.....	87.0	91.0	90.0	86.0	91.0	88.4	90.0

water. Thus it is clear that while some of the gelatin may go into solution, the amount is too insignificant to affect the results. The negative swelling is due to loss of pure solvent.

*Effect of Duration of Setting on the Negative Swelling of Gels.*

A number of weighed slides were coated with 5 per cent isoelectric gelatin. The slides were then reweighed and placed over water in rubber-stoppered glass tubes. Care was taken that the gelatin was not in contact with the water. After various intervals of time the slides were removed, weighed and put into  $m/1000$  buffer pH 4.7. The results are given in Table II.

It is seen that the duration of setting has very little effect on the final loss of water from the gel. In addition the experiment shows that the amount of water lost from the gelatin while kept in a closed moist chamber is very small during the first few days.

*Effect of Drying of Gels.*

A series of slides were coated with 5 per cent isoelectric gelatin solution and then placed for various lengths of time in loosely covered Petri dishes the cover of which had a padding of moist filter paper. This was done in order to prevent too rapid drying of the gels. The slides were afterwards put into  $m/1000$  buffer pH 4.7. Table III shows that the final loss of water is independent of the loss of water due to evaporation. If more water was lost by evaporation than would have been the case had the block been immersed in water, then the gelatin gains water when placed in liquid. In other words, the equilibrium value can be obtained from two directions.

TABLE III.

*Effect of Drying on the Equilibrium of Negative Swelling of 5 Per Cent Isoelectric Gelatin in  $m/1000$  Acetate Buffer pH 4.7.*

Time of drying, in hrs.....	4	16	44	68	92	116
Weight of gelatin after drying, in per cent of original weight.....	99.0	97.0	93.0	90.0	87.0	83.0
Final weight of gelatin in $m/1000$ acetate buffer.....	87.0	87.5	87.5	87.5	88.0	87.0

*Effect of Volume of Outside Solution or Size of Block on the Equilibrium Value of Negative Swelling.*

A 4 per cent solution of isoelectric gelatin was allowed to set for several days in a stoppered flask. No fluid was observed on the surface of the gelatin except for a few drops of condensed vapor on the sides of the flask. A lump of about 10 gm. of gel was then removed by means of a spatula and placed in a weighed porcelain Gooch crucible and its weight determined. The gel was then mashed up by means of the spatula. Fluid immediately began to ooze from the gelatin. Also fluid appeared in the cavity in the stock of gelatin in the flask. The crucible was left to drain overnight on a Gooch funnel. The weight of the gelatin next morning was found to be 82 per cent of the original. The crucible was then placed in a beaker with enough  $m/1000$  acetate buffer to reach the edge of the crucible (about 50 cc.). After several

hours the weight of the gelatin became about 80 per cent and next day it reached the value of 77 per cent, which then became constant. Its dry weight was then determined and was found to check with the weight as calculated from the loss of water. The amount of water lost here by the 4 per cent gel through "syneresis" was identical with the amount of water lost by a solid block of 4 per cent gel weighing 1.5 gm. and immersed in 150 cc. of liquid with several changes of the outside solution. In this last case it took about 10 days to reach the equilibrium state. In another experiment two solid blocks of 4 per cent gel each weighing about 3.5 gm. were placed in stoppered Pyrex tubes, to one of which was added about 5 cc. of H<sub>2</sub>O pH 4.7, while the other tube had no water at all. But the pressure of the block of gelatin on the glass was enough to start the diffusion of fluid from the gelatin with the result that the loss in weight of both blocks was identical, each losing 17 per cent in weight after 3 days.

In complete absence of water the process of syneresis is very slow due to the slow rate of diffusion of water through the dry surface of the solid gelatin. A trace of water placed on the surface through actual addition or through pressure is enough to start a rapid diffusion of the water from the solid gelatin. A similar occurrence takes place when a water-permeable collodion bag is filled with water and a moderate pressure applied to it. The rate of diffusion of water from the bag is much greater when the bag is immersed in water than when the bag is placed in a moist chamber after it has been dried carefully with a towel. In an actual experiment the writer found that while it took only about 1 minute for a definite volume of water under a pressure of 10 cm. mercury to diffuse out from a collodion bag when immersed in water, it required about 20 minutes for the same volume of water under the same pressure to diffuse out from the same bag after it was dried outside with a towel and placed in a closed tube *over* water. When the bag was immersed in water again the diffusion became as rapid as before.

#### *The Mechanism of Syneresis.*

The experiments thus far described prove that syneresis of gelatin can be conveniently studied by measuring the loss of water in dilute gelatin blocks when kept in dilute buffer pH 4.7 at low temperature,

since the same quantity of water is lost finally whether the block is immersed in a large volume of water or when syneresis is initiated through mechanical forces such as shaking, pressure, etc. Fig. 2 shows the rate of gaining or losing water by isoelectric gels of various gelatin content when kept in  $M/1000$  acetate buffer pH 4.7 at  $5^{\circ}\text{C}$ . The equilibrium values of the concentrations of gelatin in the various gels were plotted separately in Fig. 3 against the original concentra-

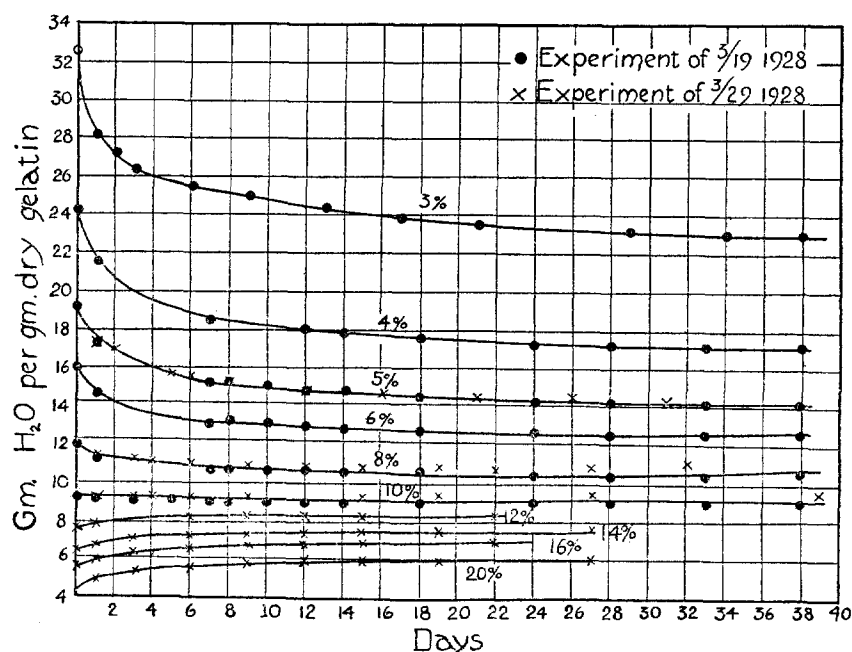


FIG. 2. Swelling of various concentrations of isoelectric gelatin blocks in  $M/1000$  acetate buffer pH 4.7 at  $5^{\circ}\text{C}$ . The gelatin was allowed to set for 24 hours at  $5^{\circ}\text{C}$ . before placing it in the buffer solution.

tions. It is seen that all the points lie on a smooth curve, no matter whether there has been positive or negative swelling, thus suggesting that there is a common mechanism to both forms of swelling. The theoretical relation between the original and final concentrations of a swelling gel has been developed by Northrop<sup>8</sup> for concentrations of

<sup>8</sup> Northrop,<sup>6</sup> p. 898.



gelatin above 10 per cent. His theory is based on the assumption that a block of gelatin behaves like a perfect elastic body, namely that in accordance with Hooke's law any strain in the block is proportional to the stress producing it. A block of freshly set gel is under no strain

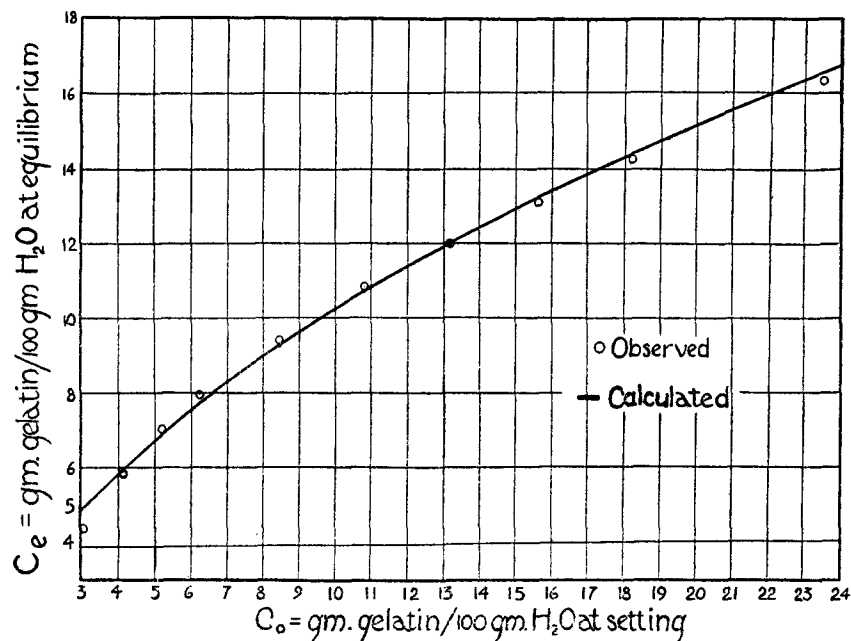


FIG. 3. Relation between the original and the final concentrations of the gelatin in swelling of gels. The dots are the experimental values. The smooth curve represents the theoretical relation:

$$C_e = -\frac{K_e - 140}{26.6} + \sqrt{\left(\frac{K_e - 140}{26.6}\right)^2 + \frac{K_e}{13.3} C_o}$$

where  $K_e = 195$ .

but as soon as it is placed in water the block swells under the influence of a certain swelling pressure  $P$  and a strain is produced in the block. At equilibrium we have

$$P = K_e \frac{V_e - V_o}{V_o} \quad (1)$$

Where  $K_e$  is bulk modulus of elasticity of the block and  $V_o$  and  $V_e$  are cubic centimeters of water per gram of dry gelatin at setting and at equilibrium respectively.

The swelling pressures of gels of higher than 10 per cent have been

TABLE IV.

*Application of Northrop's Formulas for Swelling of Gels.*

$$\frac{1330}{V_e} - 140 = K_e \frac{V_e - V_o}{V_o} \text{ and } P = \frac{1330}{V_e} - 140$$

$K_e$  = Bulk modulus of elasticity in mm. Hg of pressure.

$V_o$  = cc. H<sub>2</sub>O per gm. gelatin at setting.

$V_e$  = cc. H<sub>2</sub>O per gm. gelatin at equilibrium.

$P$  = Swelling pressure in mm. Hg.

Concentration at setting of gelatin in gm. per 100 cc. solution	$V_o$	$V_e$	$P$	$K_e = \frac{P \times V_o}{V_e - V_o}$	$C_o = \frac{100}{V_o}$ gm. gel. per 100 cc. H <sub>2</sub> O	$C_e = -\frac{K_e - 140}{26.6} + \sqrt{\left(\frac{K_e - 140}{26.6}\right)^2 + \frac{K_e}{13.3} C_o}$	$C_e$ observed $= \frac{100}{V}$
3.0	32.5	23.0	-82.2	281	3.07	4.95	4.35
4.0	24.2	17.0	-61.8	208	4.13	5.98	5.88
5.0	19.2	14.2	-47.0	180	5.18	6.90	7.04
6.0	16.0	12.6	-34.5	163	6.25	7.72	7.94
8.0	11.80	10.65	-16.0	164	8.47	9.26	9.40
10.0	9.25	9.20	+4.5		10.80	10.70	10.86
12.0	7.60	8.35	+19.5	198	13.15	11.98	11.98
14.0	6.42	7.60	+35.0	191	15.6	13.20	13.15
16.0	5.50	7.00	+50.0	183	18.2	14.40	14.30
20.0	4.26	6.10	+78.0	181	23.5	16.60	16.40
Average.....				195			

measured directly and found that they can be expressed empirically as the following function of  $V_e$ , namely,

$$P = \frac{1330}{V_e} - 140 \quad (2)$$

Combining the two equations we get the following relation between  $V_o$  and  $V_e$ :

$$\frac{1330}{V_e} - 140 = K_e \frac{V_e - V_o}{V_o} \quad (3)$$

As shown by Northrop, this relation holds well for the swelling of blocks of gels of a gelatin content of more than 10 gm. per 100 cc. H<sub>2</sub>O. The same formula was applied by the writer to the cases of negative swelling of gels of lower than 10 per cent with the following results.

Table IV gives the values of  $K_e$  as calculated from the known values of  $V_e$  and  $V_o$ . It is seen that  $K_e$  is practically constant, varying as much in the cases of negative swelling as in the cases of positive swelling. Equation (3) can be easily used to find the values of the equilibrium concentrations of the gelatin in the gels in grams per 100 cc. H<sub>2</sub>O for the various concentrations used, namely,

$$C_e = -\frac{K_e - 140}{26.6} + \sqrt{\left(\frac{K_e - 140}{26.6}\right)^2 + \frac{K_e}{13.3} C_o}$$

where

$$C_e = \frac{100}{V_e} \text{ and } C_o = \frac{100}{V_o}$$

The calculated values of  $C_e$  as well as the observed ones are given in the last two columns of Table IV. They are practically identical with the exception of the lowest concentration. This is also shown clearly on Fig. 3 where the calculated values of  $C_e$  are plotted on the smooth curve.

Thus it is evident that the same laws which hold for swelling of gels of a gelatin content higher than 10 gm. per 100 cc. H<sub>2</sub>O hold also for the process of giving off water by gels of a lower gelatin content. The active force  $P$  in both processes can be calculated by means of equation (1). The values for  $P$  are positive in the high concentrations and negative in the lower concentrations, all lying on one straight line which crosses the zero axis at a concentration of gelatin of about 10 per cent (see Fig. 4).

#### *What is Swelling Pressure?*

It has been assumed by Northrop and the writer<sup>9</sup> that the active force  $P$ , *i.e.*, the swelling pressure in gels, is brought about by the

<sup>9</sup> Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1926-27, x, 162.

osmotic pressure of the soluble ingredient in the gel, and is numerically equal to it. But the fact that the swelling pressure becomes negative in gels of a gelatin content of less than 10 per cent indicates that osmotic pressure is not the only factor which causes swelling, that there is also another force which evidently works in the opposite

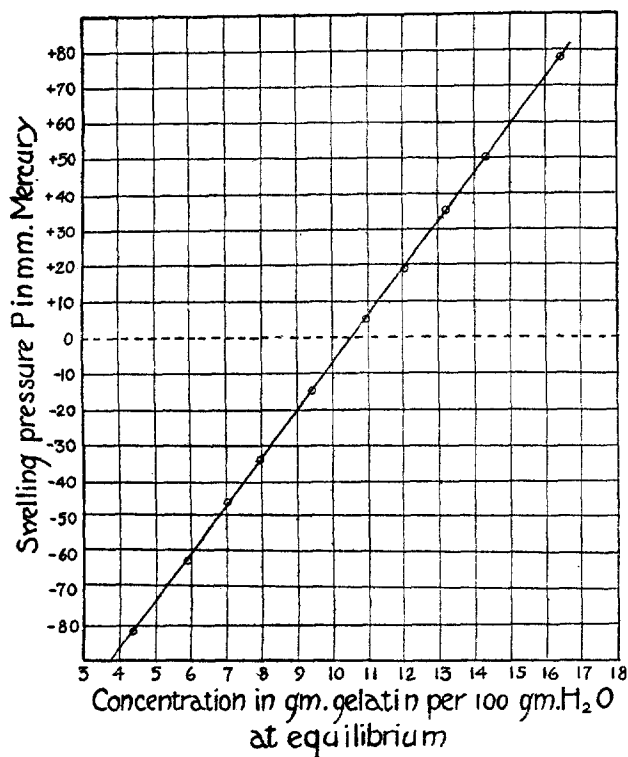


FIG. 4. Swelling pressure of gels of various gelatin content pH 4.7 at 5°C., from the relation  $P = 13.3 C_e - 140$ .

direction to that of the osmotic force, and that the observed swelling pressure is the resultant of the two oppositely acting forces. It will be shown in the following discussion that the force that causes shrinking of dilute gels is connected with the micellar structure of gelatin solutions and is brought about by the strain prevalent in the micellæ before the gelatin has set to a gel.

Loeb<sup>10</sup> first established the theory that the increase of hydration of gelatin in solution due to a change in pH, as shown by viscosity measurements, is brought about by the swelling of the micellæ in the gelatin solution. The micellæ swell because of a higher osmotic pressure inside of the micellæ than outside due to a difference in the ion distribution in accordance with the law of "Donnan equilibrium." The writer<sup>11</sup> has further developed Loeb's idea of hydration by osmosis by applying it to the case of hydration of gelatin at its isoelectric point, where in the absence of diffusible ions no Donnan equilibrium occurs. It was, namely, shown that the hydration of isoelectric gelatin in solution, as demonstrated by viscosity and osmotic pressure measurements, is caused by the fact that each micella in gelatin consists of an insoluble shell containing a definite amount of a soluble ingredient of gelatin. The latter exerts an osmotic pressure on the micella and brings about an inflow of water into it until the osmotic pressure in the micella is balanced by the total osmotic pressure of the solution acting against it and its elastic pressure. The equilibrium state can be expressed as  $P_i - P_o = Eq$  where  $P_i$  and  $P_o$  are the osmotic pressures inside and outside of the micella, respectively,  $E$  is a constant proportional to the bulk modulus of elasticity of the micella, and  $q$  is the amount of water of hydration per gram of gelatin. At low concentration of gelatin the outside osmotic pressure is small, hence the micellæ take up individually relatively large amounts of water. But as the total concentration increases the opposing outside osmotic pressure increases and the micellæ swell less, with the result that  $q$ , *i.e.* the amount of hydration per gram of gelatin gradually becomes less and less. Thus, although the micellæ are at equilibrium with the outside solution, they are still under a strain exerted by a pressure equal to  $Eq$ , the magnitude of which decreases with the increase in the total concentration of gelatin.

*The Theory of the Rôle of the Micellæ in Swelling of Gelatin.*

The hydrated micellæ in a gelatin solution are kinetically free and are able to exert osmotic pressure as any other particles or molecules.

<sup>10</sup> Loeb, J., *Proteins and the theory of colloidal behavior*, New York and London, 2nd edition, 1924, 270.

<sup>11</sup> Kunitz, M., *J. Gen. Physiol.*, 1926-27, x, 811.

The first effect of cooling of a gelatin solution is a decrease in the kinetic energy of the micellæ. Their motion becomes sluggish with more chance to adhere to each other, until they begin to form fibrils. Gradually the fibrils interlace and form a network occluding the rest of the gelatin solution. In the absence of electrolytes most of the soluble gelatin becomes insoluble or precipitates out as soon as the temperature has fallen low enough to make the solution supersaturated with respect to it. The micellæ which were under a strain before the gelatin began to set, due to the osmotic pressure of the soluble ingredient inside of the micellæ, now shrink and lose water owing to the precipitation of the soluble gelatin inside of the micellæ. This loss of water probably takes place even before the gelatin becomes solid, but as soon as it solidifies the shrinkage of the micellæ becomes slower because of the resistance to the diffusion of the liberated water offered by the dry surface of the gel, as mentioned before. But as soon as the gel is brought in contact with water a rapid diffusion of water from the block of gel takes place. Each individual micella shrinks, with the result that the whole network contracts and it expels not only the water that was inside of the micellæ but also a great deal of water that was held by capillarity between the micellæ. The shrinkage of the micellæ continues until it is balanced by the elastic resistance of the block as a whole.

*Effect of Concentration of Gelatin.*

As stated before, the strain due to the inner osmotic pressure in each individual micella in a gelatin solution becomes less as the total concentration of the gelatin increases. Hence when the stress on the micella is removed by the "precipitation" of the soluble gelatin there is less contraction in the micellar network of concentrated gels than in the dilute ones, and less syneresis. There is also another factor which makes the syneresis decrease with the increase in the concentration of the gel, namely the osmotic pressure of the gel as a whole. It was shown by Northrop and the writer<sup>12</sup> that even at a temperature as low as 0°C. there is still enough soluble material left in a solid gel to produce osmotic pressure. The amount of soluble material naturally

<sup>12</sup> Northrop and Kunitz,<sup>9</sup> p. 174.

increases with the increase in total concentration of gelatin. Hence concentrated gels not only do not lose water but actually gain water. Thus syneresis and swelling are caused by two opposite forces acting against the elasticity of the solid block of gel, namely the elastic strain in the micellæ and the osmotic pressure of the block as a whole. At low concentration the first one is prevalent, at higher concentrations the second force becomes more important; while at a concentration of

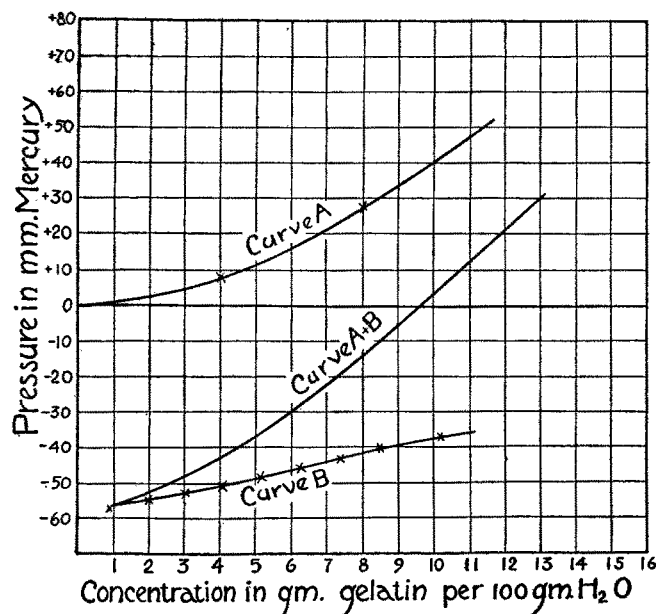


FIG. 5. Curves: *A* for osmotic pressure of isoelectric gelatin solutions at 25°C., and *B* for the stress on the micellæ at 35°C. The resultant curve *A + B* represents the hypothetical curve for swelling pressure of gels.

about 10 per cent the two forces balance each other and the block of gel neither swells nor loses water. The observed swelling pressure *P* is then the algebraic sum of the two forces and it is balanced by the elastic pressure of the block of gel.

There seems to be no way to determine directly either the osmotic pressure of a gel or the elastic strain in the micellæ of a gelatin solution at its setting point. Still, an idea of the character of the curve

representing the sum of the two pressures at various concentrations of gelatin may be obtained by plotting the observed values for osmotic pressure of various concentrations of gelatin solutions at a temperature above the setting point, and also the values for the elastic strain in the micellæ as obtained from measurements of viscosity of gelatin solutions. Fig. 5 shows the curve for osmotic pressure at 25°C.<sup>13</sup> plotted as positive, and the curve for  $Eq$  (strain in the micellæ) at 35°C.<sup>14</sup> as

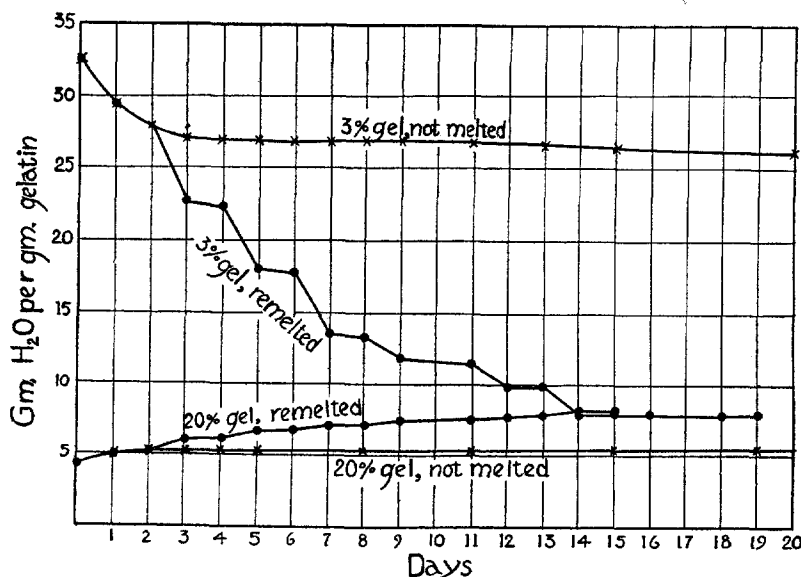


FIG. 6. Effect of reheating of gels on the absorption or liberation of water at 5°C.

negative values of  $P$ . The third curve represents the algebraic sum of the two pressures. It resembles closely the actual curve for swelling pressure as shown in Fig. 4.

#### *Effect of Reheating of Gels.*

The limiting factor in swelling or in shrinking of gels is the elasticity of the block. When the block of gel is heated the elastic strain caused by the swelling is released. Hence when the gel is cooled again and

<sup>13</sup> Northrop and Kunitz,<sup>9</sup> p. 166.

<sup>14</sup> Kunitz,<sup>11</sup> p. 820.



put back into water the gel continues swelling until the swelling pressure is balanced by the new elastic pressure. This has been shown by Northrop<sup>15</sup> to hold true for positive swelling. The writer tried the effect of reheating on two gels, one of which was of 3 per cent gelatin content and the other of 20 per cent content. For this purpose the gelatin solutions were coated on slides, allowed to set for 24 hours, weighed and put into  $m/1000$  acetate buffer pH 4.7. Every 3rd day the gels were heated carefully over a small alcohol flame until melted. They were then allowed to set for 30 minutes, weighed and put back into the solution. The results are given in Fig. 6. The smooth curves are those of blocks which have not been reheated, while the broken curves are those of the reheated blocks. It is to be noticed that the 3 per cent gel keeps on losing water while the 20 per cent continues gaining water until the concentration reaches about 12 per cent in both cases, where no further change takes place on reheating.

*Effect of pH of the Gelatin on the Loss of Water by Gels.*

When blocks of isoelectric gelatin are placed in acid or alkali solution they swell enormously due to the setting up of a Donnan equilibrium. This takes place even with very dilute gels. The same thing happens when the gels are made up of gelatin solutions containing acid or alkali. When put into water or acid or alkali the gel swells. It has also been observed by Jordis<sup>16</sup> and noticed by the writer that gels containing electrolytes are quite stable even in dilute solution, and no syneresis occurs. Quite different results are obtained when the acid or alkali is removed by dialysis.

*Experiment.*—A series of solutions of 3 per cent gelatin were made containing various amounts of HCl or NaOH. The solutions were heated to 50°C., then poured into tubes (2 cc. in each) and allowed to set in a refrigerating room for 24 hours. The blocks were afterwards removed from the tubes, weighed and put into 150 cc.  $m/30$  acetate buffer pH 4.7 where they were kept for several days until the weight became constant. The gels were then transferred to  $m/1000$  acetate buffer pH 4.7 and kept there until new equilibrium had been

<sup>15</sup> Northrop.<sup>6</sup>

<sup>16</sup> Jordis, E., *Z. Electrochem.*, 1902, viii, 677.

established. The  $m/1000$  buffer was renewed several times during the experiment.

The results are given in Fig. 7. It is seen that gels which had a pH other than that of the isoelectric point of gelatin lose more water than gels made of isoelectric gelatin. This shrinking takes place only after the acid or alkali has been removed by neutralization and dialysis. The greater shrinking of gels which contained at setting some acid or

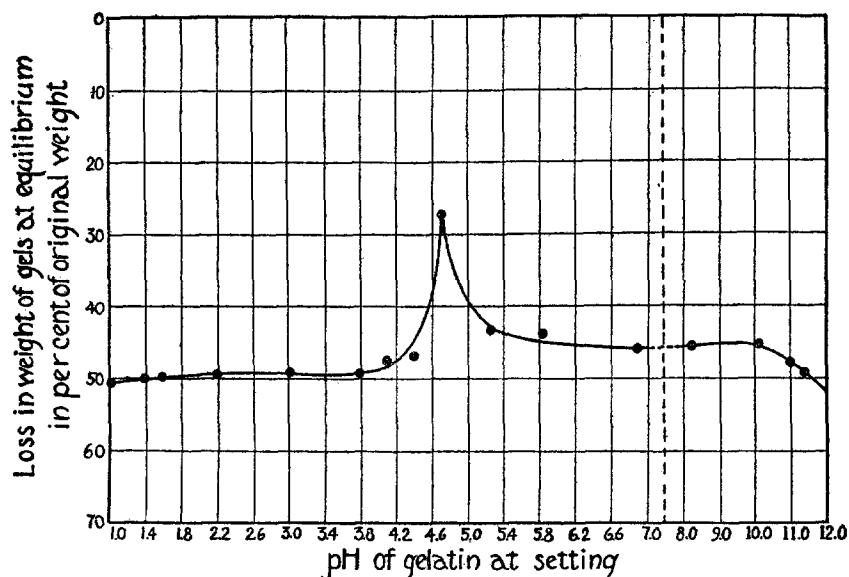


FIG. 7. Effect of pH on syneresis of 3 per cent gel at 5°C.

alkali in solution over those which were electrolyte-free takes place only in gels of low gelatin content. The difference disappears in case of gels of higher concentrations. This is shown in Table V. At a concentration above 6 per cent the loss in water is identical for all gels no matter whether they originally contained acid or alkali or were made up of pure isoelectric gelatin. The peculiar effect of the pH of the original solutions on the loss of water by the gels after the gel was brought back to the isoelectric point becomes clear in view of the theory developed here.

*The Theory of Setting of Gels Containing Acid or Alkali.*

In dilute solutions of gelatin containing moderate amounts of acid or alkali the micellæ are much more swollen than at the isoelectric point due to a greater concentration of diffusible ions inside than outside of the micellæ. Cooling and setting does not change this unequal distribution of ions. Hence, until the ions are removed by dialysis the micellæ do not lose water and no syneresis takes place. But as soon as the acid or alkali is removed by neutralization and dialysis the micellæ shrink much more than in the case of originally isoelectric

TABLE V.

*Swelling of Various Concentrations of Gelatin of Various pH in M/30 Acetate Buffer pH 4.7 at 5°C.*

Expressed as per cent of original weight.

Concentration of gelatin in gm. per 100 cc. solution.....		3	4	5	6	8	10
1928							
2/15	pH 9.0. 15 days in M/30 acetate	75	84		99	110	118
2/24	" 4.7. 15 " " " "	95	96		103	108	114
3/10	" 4.7. 2 " " " "	97	98	100	101	106	110
3/10	" 2.0. 2 " " " "	83	90	95	101	106	111
3/10	" 4.7 2 " " " " then 8 days in H <sub>2</sub> O pH 4.7	79	85	87	88	95	102
3/10	" 2.0 2 days in M/30 acetate then 8 days in H <sub>2</sub> O pH 4.7	57	70	77	85	93.3	101

gels. With increase in the concentration of gelatin the pH effect on the swelling of the micellæ in a gelatin solution diminishes rapidly, as shown by viscosity measurements,<sup>17</sup> with the result that the pH effect on syneresis in the solid gels is also diminished with increase in concentration of the gelatin. There is also another factor in the effect of acid or alkali on syneresis, namely the solubility effect which is shown also by salts.

*The Effect of Salts on Syneresis.*

It is generally known that salts affect the solubility of gelatin and the rate of setting (Levites<sup>18</sup>). At temperatures above the setting point of

<sup>17</sup> Kunitz,<sup>11</sup> p. 832.

<sup>18</sup> Levites, S. J., *Z. Chem. u. Ind. Kolloid.*, 1907, ii, 161.

solutions of gelatin salts split the micellæ into smaller units thus giving rise to higher osmotic pressures (Northrop and Kunitz<sup>19</sup>). There is very little action of the salts on the soluble fraction of gelatin, which is in solution even in the absence of salts. The total volume of the micellæ is not changed to any considerable extent, and the viscosity of the gelatin solutions is only slightly increased by addition of salt. As a salt-free dilute solution of isoelectric gelatin is allowed to set the soluble fraction both inside and outside of the micellæ gradually

TABLE VI.

*Effect of NaCl on Swelling of 3 Per Cent Gelatin Gels.*

Measured after 24 hours in solution.

Weight in per cent of original weight of block.

Concentration of NaCl pH 4.7.....	0 (H <sub>2</sub> O)	M/256	M/128	M/64	M/32	M/16	M/8	M/4
I. 3 per cent salt-free isoelectric gelatin blocks put into salt solutions	88.6	94.0	95.4		100	102	104	108
II. 3 per cent isoelectric gelatin blocks made up in varying concentrations of NaCl and put into the corresponding salt solutions	86.6	88.5	87.4	89.4	91.2	93.2	94.6	98.4
III. 3 per cent isoelectric gelatin blocks made up in various concentrations of NaCl and put into H <sub>2</sub> O pH 4.7	90.0	86.0	84.0	82.0	80.0	77.0	73.0	
IV. 3 per cent isoelectric gelatin blocks made up in various concentrations of NaCl. Kept in M/1000 acetate buffer pH 4.7 until equilibrium was established, and then weighed	71.0		60.0	56.0	55.0	54.0		50.0

“precipitates” out during the setting. As stated before, the micellæ lose some of their water of hydration during setting, and the block of gel begins to lose weight almost immediately after it sets if the conditions for diffusion of the water are favorable.

In the presence of salts the setting is slower, first because of the finer state of the micellæ, and secondly because the salts prevent the

<sup>19</sup> Northrop and Kunitz,<sup>7</sup> p. 332.

precipitation of the "soluble" ingredient of gelatin, as shown by higher swelling of isoelectric gelatin in the presence of salts. The micellæ lose much less water during setting than in the absence of salts. It is possible that the micellæ even take up water during setting in the presence of salts because of the reduction of the outside osmotic pressure due to the immobilization of the micellæ.

When the salt-containing gel is put in H<sub>2</sub>O pH 4.7 or dilute buffer of the same pH, the salt dialyzes out and the soluble gelatin both inside and outside precipitates out as in case of originally salt-free gels. The stress on the micellæ due to the greater concentration of soluble

TABLE VII.

*Effect of M/8 NaCl on Swelling of Concentrated Gels of Isoelectric Gelatin at 5°C.*

Gels were made up in M/8 NaCl, allowed to set for 24 hrs. and then put into M/1000 acetate buffer pH 4.7. Buffer changed several times.

Concentration of gelatin in gm. per 100 cc. solution.....	10	12	14	16	20
Cc. H <sub>2</sub> O per gm. of gelatin at setting.....	9.20	7.55	6.37	5.48	4.23
" " " " " " equilibrium.....	9.20	8.30	7.50	6.90	6.00
Per cent increase.....	0	+10.0	+17.6	+26.0	+42.0
Same gels but without salt					
Cc. H <sub>2</sub> O per gm. gelatin at setting.....	9.25	7.60	6.42	5.50	4.26
" " " " " " equilibrium.....	9.38	8.35	7.60	7.00	6.10
Per cent increase.....	+1.5	+10.0	+18.5	+27.5	+43.0

gelatin inside than outside is removed and the micellæ shrink, thus bringing about a greater loss of water due to syneresis in salt-containing gels than in salt-free gels of pH 4.7, as shown in Table VI. It is interesting to observe that the salt-containing gel, which unlike the salt-free gel is not turbid in appearance, does not assume any turbidity even after the salt is dialyzed out. On the other hand when a milky white salt-free gel is placed in salt solution or acid the gel swells but the turbidity persists, thus proving the turbidity is caused by the structure of the micellæ before setting. The turbidity is apparently caused only by coarse micellæ. The splitting of micellæ into finer elements by salts prevents turbidity formation.

*Is the Effect of Salt on Syneresis Due to a Change in the Bulk Modulus of Elasticity of the Block?*

A possible explanation of the peculiar effect of dilute salt solutions on syneresis is that the elasticity of the gel is affected by the presence of salt in it and that even after removal of the salt from the gelatin by dialysis the elasticity of the block still remains altered. The change expected would be a decrease in the bulk modulus so that a smaller

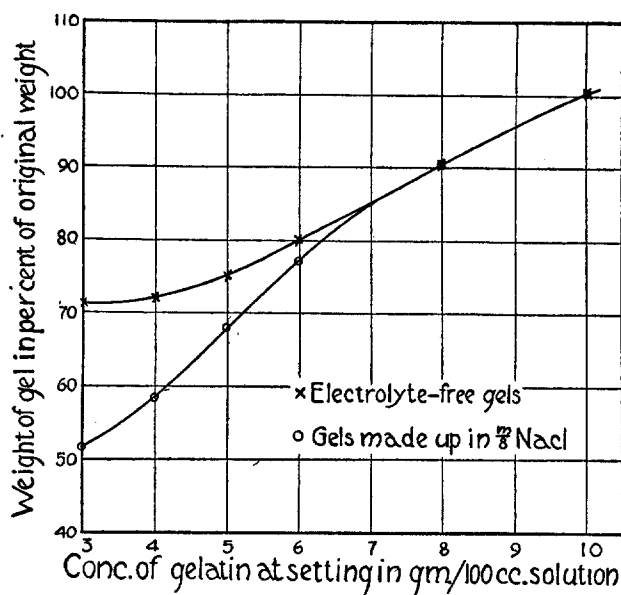


FIG. 8. Effect of salt on syneresis of various concentrations of gelatin blocks in  $M/1000$  acetate buffer pH 4.7 at  $5^{\circ}C$ .

force is required to compress the block. But if salts in low concentrations affect the elasticity of a gel then it is to be expected in gels of high gelatin content, that those which originally contained salt and from which the salt was afterwards removed would swell more than gels made up of ordinary isoelectric gelatin. Table VII shows that this is not the case. The swelling of gels of a gelatin content of more than 10 per cent is identical whether the gels originally contained salt or not. The effect of salt is only on the shrinking of gels of low concen-

trations, and it disappears at a concentration of about 8 per cent, as shown in Fig. 8, and is best explainable by the effect of salts on the solubility of the gelatin in the micellæ.

*The Solubility Effect of Dilute Acid or Alkali on Syneresis.*

Acids and alkalies in addition to their pH effect on gelatin have also an enormous effect on the solubility of gelatin. This explains why the curve for syneresis effect of pH does not give a maximum point similar to the pH viscosity curves. A continuous increase in acid or alkali concentration has the same effect on the solubility of gelatin as addition of large amounts of salt and at higher concentrations of acid or alkali the solubility effect prevails.

SUMMARY.

1. When solid blocks of isoelectric gelatin are placed in cold distilled water or dilute buffer of pH 4.7, only those of a gelatin content of more than 10 per cent swell, while those of a lower gelatin content not only do not swell but actually lose water.

2. The final quantity of water lost by blocks of dilute gelatin is the same whether the block is immersed in a large volume of water or whether syneresis has been initiated in the gel through mechanical forces such as shaking, pressure, etc., even in the absence of any outside liquid, thus showing that syneresis is identical with the process of negative swelling of dilute gels when placed in cold water, and may be used as a convenient term for it.

3. Acid- or alkali-containing gels give rise to greater syneresis than isoelectric gels, after the acid or alkali has been removed by dialysis.

4. Salt-containing gels show greater syneresis than salt-free gels of the same pH, after the salt has been washed away.

5. The acid and alkali and also the salt effect on syneresis of gels disappears at a gelatin concentration above 8 per cent.

6. The striking similarity in the behavior of gels with respect to syneresis and of gelatin solutions with respect to viscosity suggests the probability that both are due to the same mechanism, namely the mechanism of hydration of the micellæ in gelatin by means of osmosis as brought about either by diffusible ions, as in the presence of acid or

alkali, or by the soluble gelatin present in the micellæ. The greater the pressures that caused swelling of the micellæ while the gelatin was in the sol state, the greater is the loss of water from the gels when the pressures are removed.

7. A quantitative study of the loss of water by dilute gels of various gelatin content shows that the same laws which have been found by Northrop to hold for the swelling of gels of high concentrations apply also to the process of losing water by dilute gels, *i.e.* to the process of syneresis. The general behavior is well represented by the equations:

$$P_1 - P_2 = K_e \frac{V_e - V_o}{V_e}$$

and

$$P_1 - P_2 = \frac{1330}{V_e} - 140$$

where  $P_1$  = osmotic pressure of the soluble gelatin in the gel,  $P_2$  = stress on the micellæ in the gelatin solution before setting,  $K_e$  = bulk modulus of elasticity,  $V_o$  = volume of water per gram of dry gelatin at setting and  $V_e$  = volume of water per gram of gelatin at equilibrium.

The writer wishes to express his indebtedness to Dr. John H. Northrop for very valuable suggestions and advice.