

THE SWELLING AND OSMOTIC PRESSURE OF GELATIN IN SALT SOLUTIONS.

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Since the time of Hofmeister a very large number of experimental results relating to the swelling of gelatin under various conditions have been collected. The first step toward a simplification of this mass of contradictory and seemingly irrational data was made by Procter,¹ who showed, by the application of Donnan's equilibrium theory, that the swelling of gelatin in acid was due to the osmotic pressure of the excess concentration of the ions of the acid in the gelatin over that in the surrounding liquid. Jacques Loeb then took up the problem and in a long series of experiments,² that may well be called classical, was able to show that not only the swelling in acid but the osmotic pressure, viscosity, membrane potentials, and the depressing effect of salts on these properties, both of gelatin and of other proteins, could all be logically arranged, predicted, and in most cases calculated from the same theory. Jacques Loeb's experiments were done in rather dilute solutions, both of the protein and of the electrolytes, and under these conditions the slight discrepancies from the theory were so small as to be almost within the experimental error. He recognized, however, that in very high salt concentrations and under other experimental conditions effects were noted which were unexpected from the viewpoint of the theory. It is with these "second order" effects that the present paper has to do.

Hofmeister stated that the swelling of gelatin was increased by various salts to different extents, and this result has been found in many

¹ Procter, H. R., *J. Chem. Soc.*, 1914, cv, 313. Procter, H. R., and Wilson, J. A., *J. Chem. Soc.*, 1916, cix, 307.

² Loeb, J., *Proteins and the theory of colloidal behavior*, New York and London, 2nd edition, 1924.

other cases by subsequent workers. This effect is noticeable only in high concentrations. To quote from Jacques Loeb:

“It is hardly necessary to point out that the effects observed by Hofmeister have nothing to do with the production of swelling by acid and alkali and with the depression of such swelling by salts, since the swelling of gelatin caused by acid and alkali is already completely annihilated by concentrations of salts of less than $m/2$. Further, salts cause in such cases only a depression, not an increase in swelling.”

This statement is a necessary consequence of the Donnan theory if it is assumed that non-diffusible ions can be formed only by acid or alkali. It was found by the writers,³ however, that the ions of some salts can also unite with the protein to form a non-diffusible ion and can therefore give rise to an equilibrium just as do acids or alkalies. It was also found in the case of $ZnCl_2$ that the osmotic pressure, swelling, and membrane potentials of gelatin were increased as in the case of acid, only to a very much smaller extent. It seemed possible, therefore, that the effect of strong salt solutions on gelatin might also be due to the production of a Donnan equilibrium. If this were true, salts must necessarily increase the osmotic pressure as well as the swelling of gelatin. The statements in the literature,⁴ however, are to the effect that salts only depress the osmotic pressure but do not increase it. It is evident that unless the experiments were done near the isoelectric point of gelatin, the depressing effect of the salt on the osmotic pressure due to the acid or alkali would be so great as to completely mask the effect of the salt itself. A number of osmotic pressure experiments on 10 per cent isoelectric gelatin at $37^\circ C$. were therefore made with a series of salts. At the same time swelling experiments at $0^\circ C$. were performed. It was found in every case that a considerable increase in swelling was obtained accompanied by a proportional change in osmotic pressure. The parallelism between osmotic pressure and swelling exists, therefore, in the case of salts just as in the case of acid or alkali. It was also found that a non-diffusible ion was formed with one of the salt ions and that a Donnan equilibrium was set up; but the osmotic pressure calculated from this equilibrium was

³ Northrop, J. H., and Kunitz, M., *a*, *J. Gen. Physiol.*, 1924–25, vii, 25; *b*, 1925–26, ix, 351.

⁴ Lillie, R. S., *Am. J. Physiol.*, 1907–08, xx, 127.

found to be much smaller than the observed, except in the case of aluminium chloride. With this salt the observed and calculated pressures agreed very well. AlCl_3 therefore sets up a Donnan equilibrium as Jacques Loeb⁵ had suggested. In general, however, as Jacques Loeb stated, the effect of neutral salts on the osmotic pressure of gelatin is qualitatively different from that of acid or alkali and can be shown with at least a fair degree of probability to be due to an increase in the osmotic pressure of the gelatin itself rather than to a difference in the ion concentration. The swelling in salt solutions is therefore an osmotic phenomenon just as in the case of acids, except that the increase in osmotic pressure is not due to the ions of the salt but to an increase in the osmotic pressure of the protein.

EXPERIMENTAL PROCEDURE.

The osmotic pressure measurements were made in a rocking osmometer at 37°C. as already described.^{3, b} Equilibrium under these conditions is usually reached in 24 hours and then remains constant for at least 30 days. The experiments were usually left for 48 hours before the reading was taken. The membrane potential measurements and the analytical procedure were also the same as in the previous experiments.

Swelling.—For the swelling experiments isoelectric gelatin was prepared as usual and dried with acetone. It was then sifted and the grains passing through a 30 but not a 60 sieve were used. 15 gm. of this gelatin was suspended in the solution, the total volume made up to 250 cc. and stirred for 2 hours at 0°C.⁶ The solution was then filtered off rapidly with suction and the volume of filtrate measured. The difference between this figure and 250 is the volume of gelatin. There is a small error due to water held by capillarity between the gelatin particles, but with powerful suction and a large funnel, so that the layer of gelatin is less than 1 cm. deep, this error is small. With the same sample of gelatin the results could usually be duplicated to within 2 per cent. The filtrate was always tested for dissolved gelatin. Traces only were found except in very high salt concentrations where the gelatin commences to be completely dissolved.

⁵ Loeb, J., *J. Gen. Physiol.*, 1921–22, iv, 741.

⁶ The swelling of gelatin, as emphasized by Arisz (Arisz, L., *Kolloidchem. Beihefte*, 1915, vii, 51), takes place in two stages. There is at first a rather rapid increase in volume which soon reaches an apparent maximum. If readings are taken at long time intervals it will be seen, however, that there is a continual, although very slow, increase. It is only the first rapid swelling that is considered here and under the experimental conditions used the maximum is reached in about 1 hour.

In some experiments where the swelling was followed through a number of conditions, the particles were allowed to settle in a graduate for 2 minutes and the volume noted. This method is subject to a number of errors and can only be used as a rough approximation.

Comparison of the Effect of Salts on the Osmotic Pressure and Swelling of Gelatin at pH 4.7.

The results of the experiments are shown in Figs. 1, 2, and 3. In the lower part of Fig. 1 the osmotic pressure of 10 per cent gelatin at 37°C. has been plotted against the logarithms of the salt concentrations in the gelatin expressed as equivalents per 1000 gm. of water. The upper part of the figure represents the volume of swollen gelatin expressed as per cent of the original volume of dry gelatin. Fig. 2 shows the same experiments except that the abscissæ are equivalents per 1000 gm. water instead of the logarithms of this figure. Fig. 3 gives the results with various sodium salts. It is evident that the curves for swelling and for osmotic pressure are very similar in every case. Those salts which dissolve the particles in high concentrations cause a large increase in osmotic pressure, while those which cause a decrease in the volume of gelatin also cause a decrease in osmotic pressure.

These experiments, therefore, agree qualitatively with the assumption that the swelling of gelatin under these conditions is due to the increase of osmotic pressure in the gelatin, just as Procter and Wilson showed in the case of acid swelling. If this is true, the bulk modulus calculated from these figures should be constant. Before this calculation can be made, it is necessary to know the effect of the concentration of gelatin on the osmotic pressure. The osmotic pressure of various concentrations of gelatin in distilled water and in 1 M CaCl₂ was therefore determined at 37° and at 50°C. The result of this experiment is shown in Fig. 4. The osmotic pressure in both cases increases slightly more rapidly than the concentration, but over a short range may be considered proportional to it. It may be noted that the osmotic pressure has a negative temperature coefficient in high concentrations, as would be expected from the high heat of solution of gelatin (Katz⁷). The relative increase in the osmotic pressure pro-

⁷ Katz, J. R., *Kolloidchem. Beihefte*, 1917-18, ix, 1.

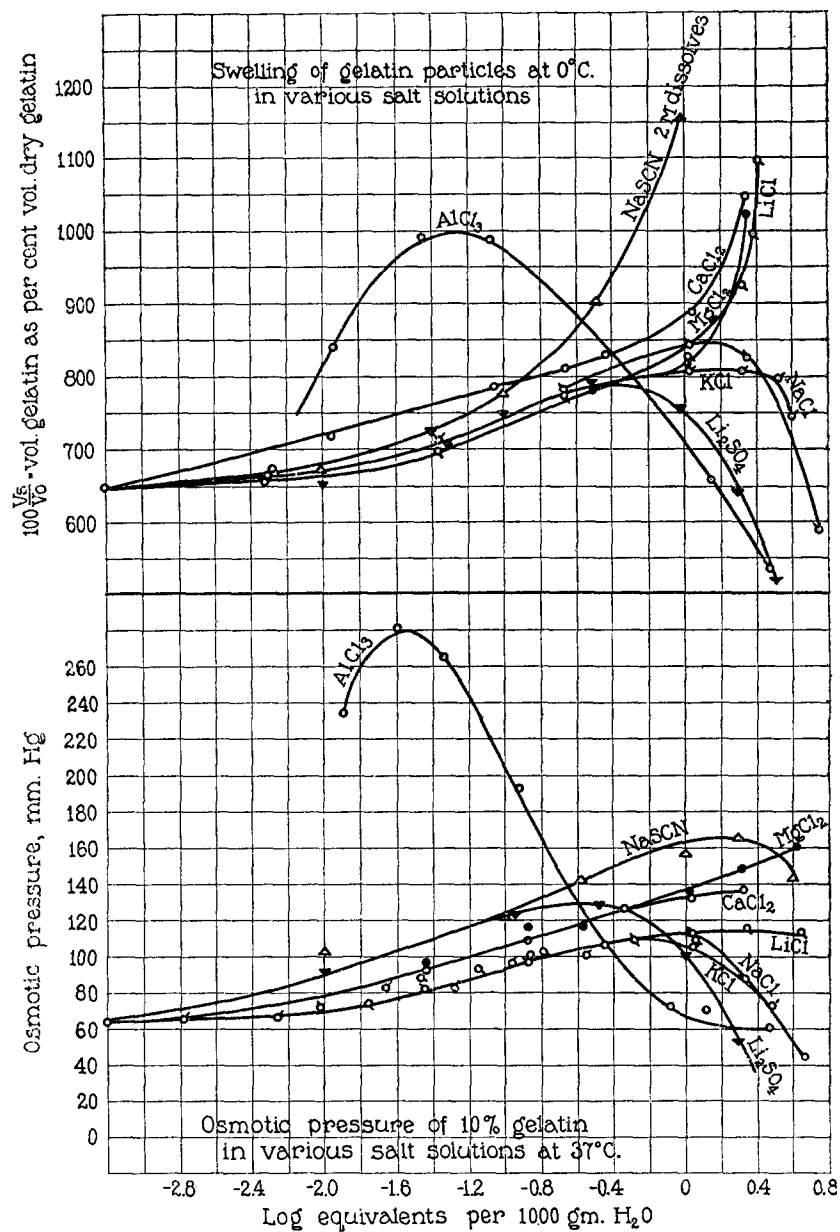


FIG. 1. Comparison of osmotic pressure and swelling of gelatin in various salt solutions.

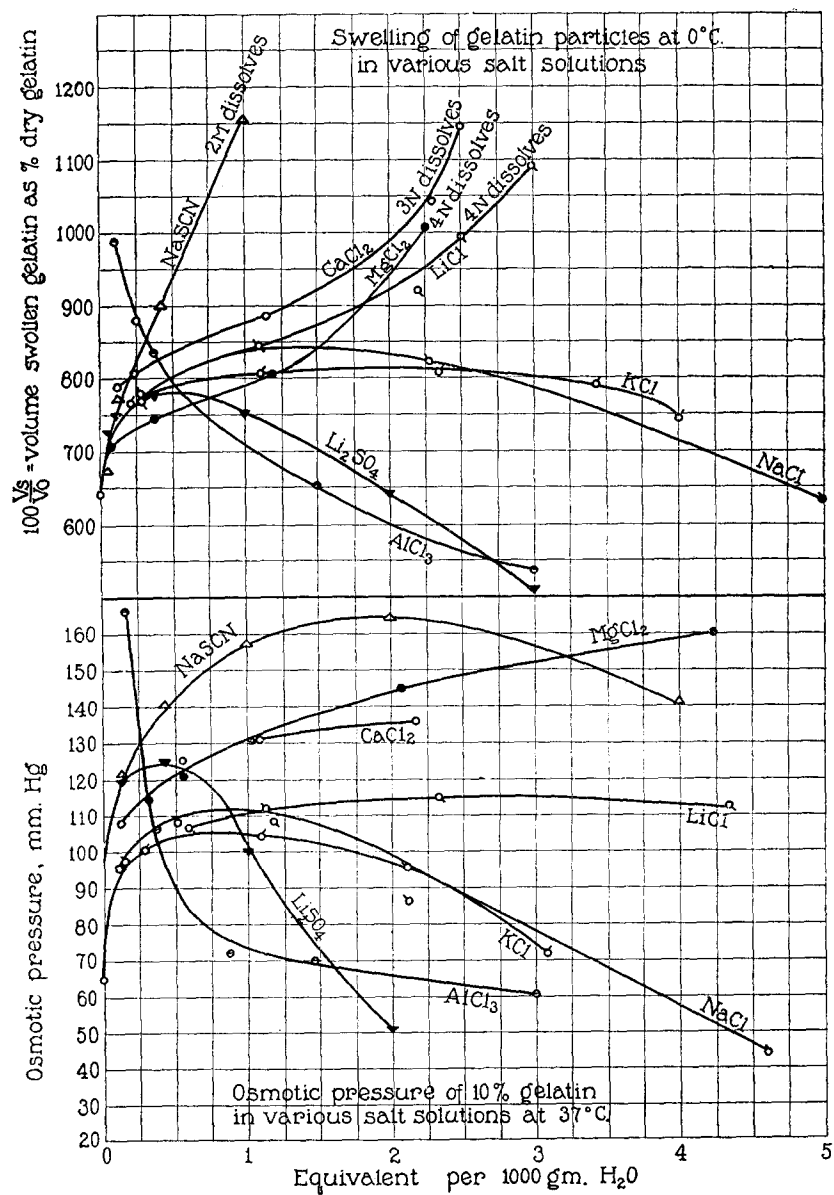


FIG. 2. Comparison of osmotic pressure and swelling of gelatin in various salt solutions.

duced by the salt is shown in Table I. It is less in low than in high concentrations of gelatin, and is nearly independent of the temperature.

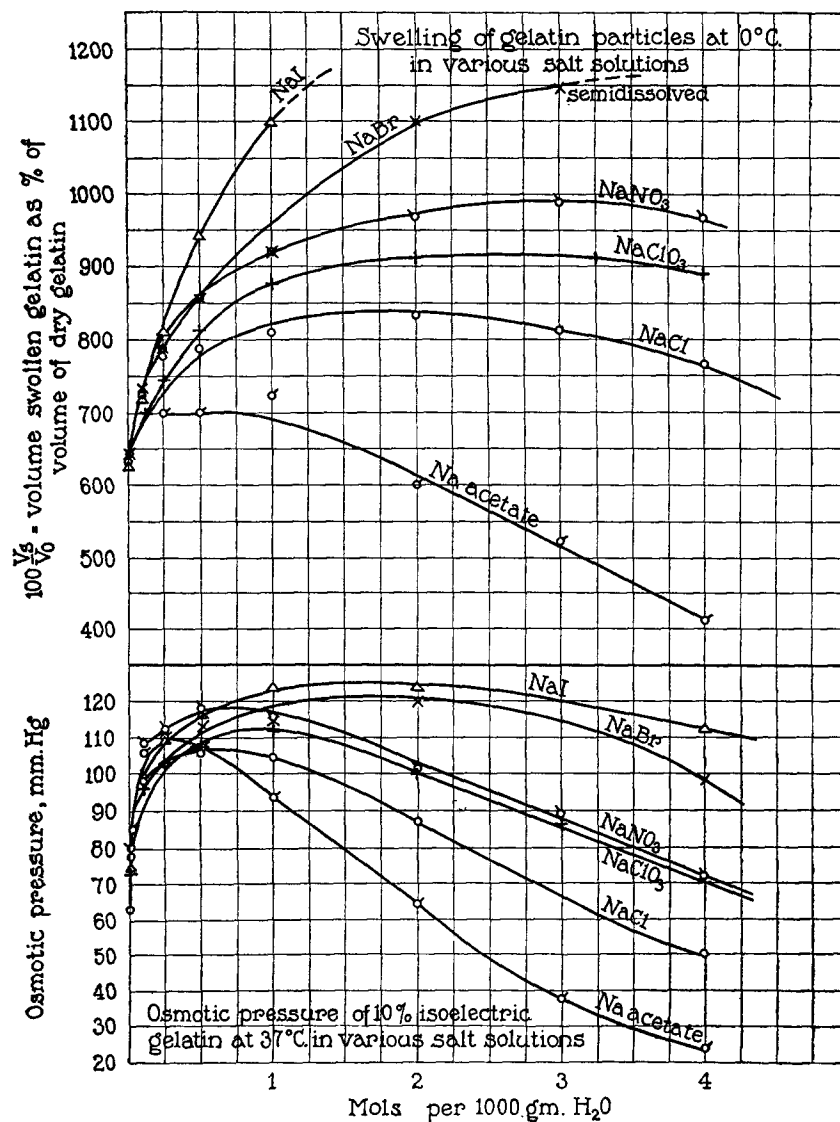


FIG. 3. Comparison of osmotic pressure and swelling of gelatin in various salt solutions.

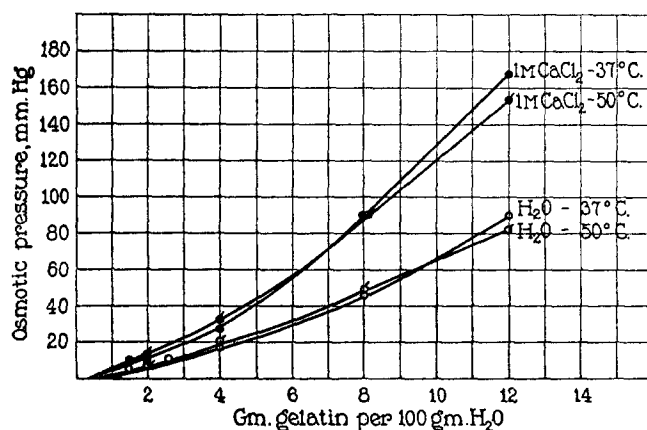


FIG. 4. Osmotic pressure of various concentrations of gelatin in H₂O and in 1 M CaCl₂ at 37° and 50°C.

TABLE I.

Ratio of Osmotic Pressure of Various Concentrations of Gelatin in H₂O and in 1 M CaCl₂.

Concentration of gelatin, gm. per 100 cc.	Osmotic pressure of gelatin in CaCl ₂ Osmotic pressure in H ₂ O	
	37°C.	50°C.
gm.		
2	1.18	1.37
4	1.55	1.60
8	2.00	1.87
12	1.85	1.87

If we assume that the pressure in the swollen particles is proportional to the osmotic pressure of gelatin of the same concentration in the same solution, then the pressure, P , in the particles can be expressed as follows:

Let

V_s = volume of swollen gelatin.

V_0 = volume of dry gelatin = 1.0.

1.3 = specific gravity of dry gelatin.

$1.3V_0$ = weight of dry gelatin.

$\frac{1.3V_0}{V_s}$ = gm. of gelatin per cc. of swollen gelatin.

P_0 = osmotic pressure of solution of gelatin containing 0.10 gm. per cc. of solution.

P = pressure in gelatin particle.

Then

$$P = 13CP_0 \frac{V_0}{V_s}$$

The bulk modulus, K (by definition) = $\frac{P \cdot V_0}{V_s - V_0}$.

Therefore

$$\frac{K}{C} = \frac{13 P_0 V_0^2}{V_s (V_s - V_0)}$$

where C is the proportionality factor connecting the osmotic pressure with the swelling pressure. Unpublished experiments indicate that

TABLE II.

Ratio of Osmotic Pressure to Swelling of Gelatin in Various Salts; i.e., Values of

$$\frac{K}{C} = \frac{13 P_0 V_0^2}{V_s (V_s - V_0)}$$

Equivalents per liter.	NaCl	NaSCN	KCl	LiCl	Li ₂ SO ₄	CaCl ₂	MgCl ₂	NaBr	NaI	NaNO ₃	NaClO ₃	Na acetate.
0	26	26	26	26	26	26	26	26	26	26	26	26
0.01	25	32	25	26	32	24	32	28	31	32	30	32
0.10	25	31	25	26	32	30	33	28	31	28	31	33
0.25								26	26	28	29	35
0.50								23	20	25	25	34
1.00	24	17	24	24	27	26	32	21	15	19	22	28
2.00	21		22	21	21	21	25	14.7	Partly dissolved.	17	18	29
3.00	19		16	14		0	0	13	Clear solution.	13	15	23
4.00	17							Partly dissolved.	“ “	11	13.5	26

$C = 1$, but for the present it is simply considered a constant and will therefore affect only the numerical value of K and not its variation with P and V .

In the case of $AlCl_3$ the calculation is very uncertain since the osmotic pressure does not vary in any simple way with the concentration. As will be shown below, this is due to the fact that the osmotic pressure in $AlCl_3$ is a Donnan pressure caused by the ions of the salt itself. $AlCl_3$ has therefore been omitted from this calculation.

The values of $\frac{K}{C}$ calculated in this way are given in Table II. The table shows that this value is approximately constant up to about 1.0

m salt concentration and is equal to 25 mm. Hg. If the pressure in the gelatin particles, therefore, is equal to the osmotic pressure of the same concentration of gelatin (*i.e.* if $C = 1$) then it requires a pressure of 25 mm. Hg to increase the volume by an amount equal to the original volume.

In higher salt concentrations the bulk modulus decreases rapidly in some salt solutions and remains nearly constant in others. A decrease in the bulk modulus indicates that a smaller force is required to expand the gelatin, and may be due to a change in the nature of the gelatin or simply to the fact that the elastic limit has been exceeded. If the latter were the case, it would be expected that if the bulk modulus were compared at equal degrees of swelling, the figures would be the

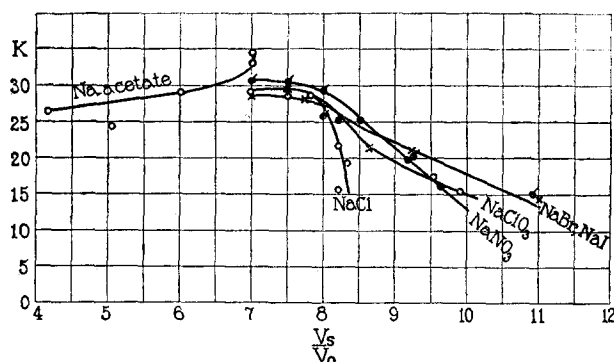


FIG. 5. Comparison of K for different salts at different degrees of swelling.

same for various salts. In Fig. 5 the bulk modulus for the Na salts has been plotted against the relative volume of the swollen gelatin. The curves are all similar and indicate that under the conditions of this experiment the elastic limit is exceeded when the volume has increased more than about 8 times, no matter what salt causes the swelling. In the case of acid swelling, however, as Procter and Wilson showed, this is not true since they were able to assume the bulk modulus constant over a much wider range of swelling.

If this decrease in the values of K is really due to the effect of salt on the elasticity of the gelatin, it might be expected that the salts would affect the modulus of linear elasticity in the same way as they do the bulk modulus. Blocks of gelatin were therefore prepared and

the elastic modulus measured by noting the decrease in length on the application of a weight. The modulus of elasticity E (Young's modulus) was then calculated by the usual formula. The results of these experiments are shown in Tables III and IV. Table III shows the relation between the modulus of elasticity and the concentration of gelatin. When the gelatin concentration is changed by allowing the gelatin to swell, the modulus of elasticity is nearly proportional to the concentration, but when blocks made from gelatin solutions of varying strengths are measured, the modulus changes much more rapidly

TABLE III.

Effect of Time, Temperature, and Concentration on E.

Block of dry isoelectric gelatin left at 0°C. in water for 24 hours. Cylinders cut and put in water. E and concentration measured.

Time at 0°C.	Concentration, gm. gelatin per 100 cc.	E gm. per mm. ²	$\frac{E}{\text{Concentration}}$
<i>hrs.</i>			
0.10	33	31	0.94
0.40	27	21.3	0.79
1.40	25	19.2	0.77
2.40	21	17.1	0.81
Temperature raised over $\frac{1}{2}$ hr. to 18°C.	15	11.1	0.74
Solution of gelatin of noted concentration made at 37°C. Cooled to 0°C. for $\frac{1}{2}$ hr. and cylinders cut out.			
	14.6	7.0	0.48
	10.0	3.6	0.38
	5.0	1.5	0.30

than the concentration, as found by Sheppard and Sweet.⁸ The fact that the modulus is proportional to the concentration of gelatin when the concentration is changed by swelling shows that the elastic modulus of the gelatin itself remains constant independent of the amount of water held in the block. When the gelatin has been previously melted, this is not true as might be expected since the melting destroys the structure. The elastic modulus is decreased by heating to 18°C. even after correcting for the concentration change. The effect of the

⁸ Sheppard, S. E., and Sweet, S. S., *J. Am. Chem. Soc.*, 1921, xliii, 539.

salt solutions on the modulus of elasticity is shown in Table IV. The figures vary in the same way as the bulk modulus calculated from osmotic pressure and swelling experiments, and after correcting for the concentration change are nearly proportional to the bulk modulus. They are, however, much larger. Measurement of the elasticity in various concentrations of NaBr also gave figures varying in the same way as the bulk modulus. It must be remembered that the elasticity measurement refers to the block of swollen gelatin as a whole, whereas the bulk modulus refers only to the gelatin itself and does not include

TABLE IV.

Value of Modulus of Elasticity, E, for Gelatin in Presence of 2 M Salt Solutions.

Method of preparing block.	Salt.				
	0	NaBr	NaNO ₃	Na acetate.	NaCl
10 per cent solution of gelatin made up in salt solutions at 37°C. Cooled to 0° for ½ hr. and cylinders cut out. <i>E</i> .	3.6	1.0	1.0	3.9	2.5
	4.1	1.6	1.3	4.6	3.4
	6.0			5.0	
Block of dry isoelectric gelatin swollen in water at 0° for 24 hrs. Cylinders cut out and left in solution at 0° for 3 days. <i>E'</i>	27	10	12	32	21
	36	15			29
Relative concentration of gelatin, <i>C</i>	1	0.57	0.65	1.0	0.72
$\frac{E'}{C}$, average.....	31	22	19	32	35
Bulk modulus, <i>K</i> , from osmotic pressure and swelling, gm. per mm. ²	0.34	0.19	0.22	0.37	0.25

the water. For this reason also it is impossible to calculate the bulk modulus itself from the modulus of elasticity, as can be done in the case of ordinary solids. The bulk modulus, however, for ordinary solids, is a linear function of the modulus of elasticity; hence the figures should be proportional, although no significance can be attached to their numerical values.

It was stated above that the decrease in the bulk modulus was probably due to the fact that the elastic limit was exceeded in the

case of salt swelling, while in acid swelling this was not true. It might be expected, therefore, that the same effect would be noticed in the elasticity measurements. The results shown in Table V confirm this prediction. The gelatin swollen in NaBr loses its elasticity very rapidly, while the gelatin swollen in acid returns nearly to its original length even after 24 hours.

A more direct experiment is to compare the behavior of the particles after removal of the electrolyte. If the particle is elastic it should return to the volume reached in water alone, while if the elastic limit

TABLE V.

Effect of Time on Elasticity of Gelatin in HCl or NaBr.

Dry isoelectric gelatin allowed to swell in H₂O for 7 days. Cylinders cut out, left in electrolyte solution for 24 hours at 0°C., and placed under weight, in solution, at 0°C. After the time intervals noted, the length while under the weight was measured. The weight was then removed and the length again measured after 3 minutes, by which time it had become constant. The weight was then replaced and left in position during the next time interval. The measurement was then repeated.

Time.	0.001 M HCl. Weight, 19 cm. Hg.		1.0 M NaBr. Weight, 16 cm. Hg.	
	Length without weight.	Length with weight.	Length without weight.	Length with weight.
<i>hrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
0	4.0	3.2	4.4	3.2
0.5	4.0	3.2	4.3	3.2
1.0	3.95	3.2	3.8	3.0

has been exceeded the block will not return to its original volume when the expanding pressure has been removed. The result of such an experiment is shown in Fig. 6. Removal of the acid (by neutralization and washing) from gelatin swollen in acid results in immediate shrinking to the original volume whereas gelatin swollen in NaBr decreases in size but does not reach the size of the gelatin swollen in water. Scarth⁹ found that acid did not affect the elasticity of swollen gelatin.

It would be possible, of course, to account for this experiment

⁹ Scarth, G. W., *J. Phys. Chem.*, 1925, xxix, 1009.

equally well by assuming that the pressure caused by the salt was not reversible but remained even after the salt had been removed. It would be expected from this point of view that the osmotic pressure increase caused by salt would also be irreversible. This is, however,

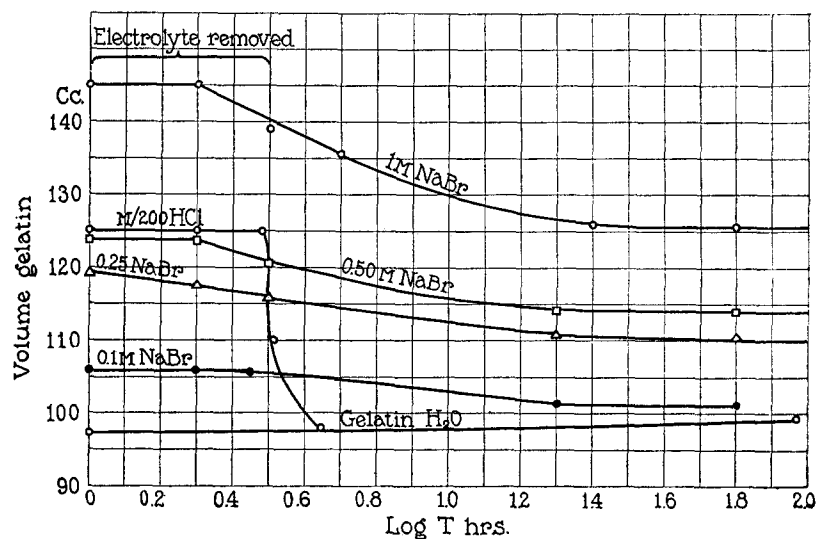


FIG. 6. Effect of removal of electrolyte on volume of gelatin swollen in NaBr or HCl.

TABLE VI.

Reversibility in Osmotic Pressure.

10 per cent gelatin, 37°C.

Salt.....	Outside solution replaced at intervals with 4 M glycerol.					Outside solution replaced with water.		
	0	LiCl		CaCl ₂		0	CaCl ₂	
	Osmotic pressure, mm. Hg.	Equiv. per liter.	Osmotic pressure, mm. Hg.	Equiv. per liter.	Osmotic pressure, mm. Hg.	Osmotic pressure, mm. Hg.	Equiv. per liter.	Osmotic pressure, mm. Hg.
<i>hrs.</i>								
0	86	4	112	4	115	63	4	135
24	86	0.035	110	0.063	128		1.24	119
72	85	0.004	81	0.008	88	64	0.22	106
96	85	0.0005	85	0.003	80	65	0.007	65

not the case, as Table VI shows.¹⁰ The osmotic pressure effect is completely reversible, since removal of the salt causes the pressure to return to the value obtained in pure water.

This experiment may be varied by making up two solutions of gelatin, a concentrated one in concentrated salt and a dilute one in dilute salt, and then diluting the concentrated solution until it is identical in composition with the dilute. Table VII shows that under these conditions also the increase in osmotic pressure is reversible.

TABLE VII.

Osmotic Pressure of Gelatin Previously Treated with NaSCN.

I. 10 per cent isoelectric gelatin in 0.1 N NaSCN. 20°C. 3 hours. Diluted to 0.005 N NaSCN, 0.5 per cent gelatin.

II. 0.5 per cent gelatin in 0.005 N NaSCN. 20°C. 3 hours. Both solutions put in osmometer at 25°C.

	I.			II.		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
Osmotic pressure after 24 hrs., mm. H ₂ O. . . .	27	29	32	25	37	35

It is still possible that this difference between the osmotic pressure and the swelling experiments is due to the fact that the swelling experiments were done at 0°C., while the osmotic pressure experiments were made at 37°C. Repetition of the swelling experiments at 25°C., however, gave the same result as at 0°C., except that the results are complicated by the solubility of the gelatin.

Effect of Sugar on Gelatin Particles Swollen in NaBr.

If the swelling of gelatin is primarily due to osmotic pressure, it should be possible to decrease it by increasing the osmotic pressure of the surrounding liquid. If swollen gelatin particles, therefore, are washed with strong sugar solutions, it would be expected that water would be withdrawn from the gelatin at first owing to the osmotic

¹⁰ Glycerol was used in one experiment in order to avoid the great temporary increase in osmotic pressure caused by diluting the outside solution with water. The glycerol itself caused an increase in the osmotic pressure of the gelatin, as would be expected, since gelatin swells more in glycerol solutions than in water (Arisz, L., *Kolloidchem. Beihefte*, 1915, vii, 51).

pressure of the sugar. Later, as the sugar diffuses into the particle, the osmotic pressure due to the sugar would be equal inside and outside the particle and the particle should return to its original volume or beyond, since sugar alone causes some swelling of gelatin. This is the experimental result, as is shown in Fig. 7. The swelling of gelatin has frequently been assumed to be hydration. It would be possible on this basis to account for the decrease by supposing that the sugar removed the water from the gelatin, but it appears difficult if not impossible from this point of view to account for the subsequent return to the original volume.

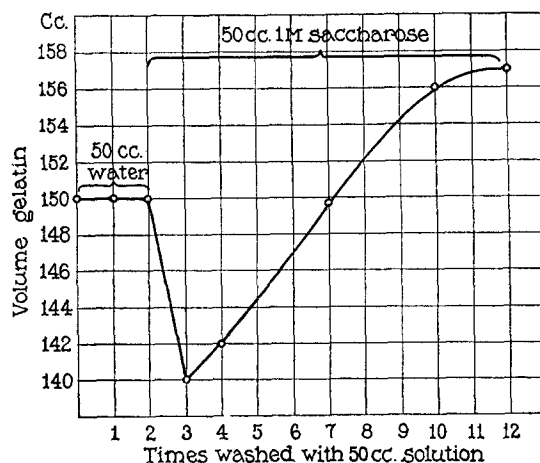


FIG. 7. Effect of washing gelatin swollen in 1 M NaBr with 1 M saccharose.

The Mechanism of the Effect of Salts on the Osmotic Pressure of Gelatin.

The foregoing experiments show that the swelling of gelatin is primarily due to the change in osmotic pressure caused by the salt but modified somewhat by the effect of the salt on the elasticity of the gelatin. The problem of the swelling therefore is essentially the same as that of osmotic pressure. The increase in osmotic pressure evidently may be due to a difference in the concentration of the ions of the salt inside and outside of the gelatin, due possibly to a Donnan equilibrium or to a change in the osmotic pressure of the gelatin

itself. It was stated above that some of the salt ions were found to be combined with the gelatin and that therefore there was a Donnan equilibrium established even in the case of neutral salts. It was found, however, that this effect was entirely too small to account for the observed osmotic pressure differences except in the case of aluminium chloride.

TABLE VIII.
Calculation of Osmotic Pressure Due to Donnan Equilibrium.

	KCl	AlCl ₃
Mol salt outside.....	0.256	0.00394
Mol salt inside, total.....	0.277	0.00875
P.D., millivolts.....	-0.4	17.0
Ratio $\frac{\alpha\text{Cl}^- \text{ inside}}{\alpha\text{Cl}^- \text{ outside}}$	0.984	1.94
Ratio $\frac{\alpha\text{Al}^{+++} \text{ outside}}{\alpha\text{Al}^{+++} \text{ inside}}$		7.30
Calculated mol Cl ⁻ inside.....	0.252	0.023
Calculated mol cation inside.....	0.260	0.00054
Total K ⁺ + Cl ⁻ or Al ⁺⁺⁺ + Cl ⁻ inside, calculated.....	0.512	0.02354
Total K ⁺ + Cl ⁻ or Al ⁺⁺⁺ + Cl ⁻ outside.....	0.512	0.01576
Calculated K _i ⁺ + Cl _i ⁻ - (K _o ⁺ + Cl _o ⁻) or Al _i ⁺⁺⁺ + Cl _i ⁻ - (Al _o ⁺⁺⁺ + Cl _o ⁻).....	0	0.0078
Total (K _i + Cl _i) - (K _o + Cl _o) or (Al _i ⁺⁺⁺ + Cl _i) - (Al _o ⁺⁺⁺ + Cl _o).....	0.042	0.0193
$\frac{\Delta v}{\Delta \infty}$	0.82	0.78
Calculated osmotic pressure due to electrolyte:		
1. From total analytical concentration, mm. Hg.....	800	360
2. Assuming osmotic pressure \propto total ion concentration.....	0	150
3. Assuming osmotic pressure $\propto \frac{\Delta v}{\Delta \infty}$ ion concentration.....	0	116
Total observed osmotic pressure.....	100	141
Osmotic pressure corrected for protein.....	36	111

An example of such a calculation is shown in Table VIII and Fig. 8. The data were obtained as already described. The calculated figures, in the case of aluminium, obtained from the difference in salt concentration predicted by the Donnan equation and corrected for the apparent dissociation of the salt by means of the conductivity ratios, agree very well with the experimental values after correcting for the pressure due to the protein itself. The figures for KCl cal-

culated on the same basis, however, are very much too small. The other salts give the same result as KCl although the calculated Donnan pressure in the case of CaCl_2 is quite appreciable. The calculation of the Donnan pressure, especially in the case of KCl, depends on very small potential differences and is very sensitive to errors in the analytical data. If the osmotic pressure were due to a Donnan equilibrium in spite of the negative results of the calculation, it should be possible to notice the characteristic depressing effect of another salt of higher

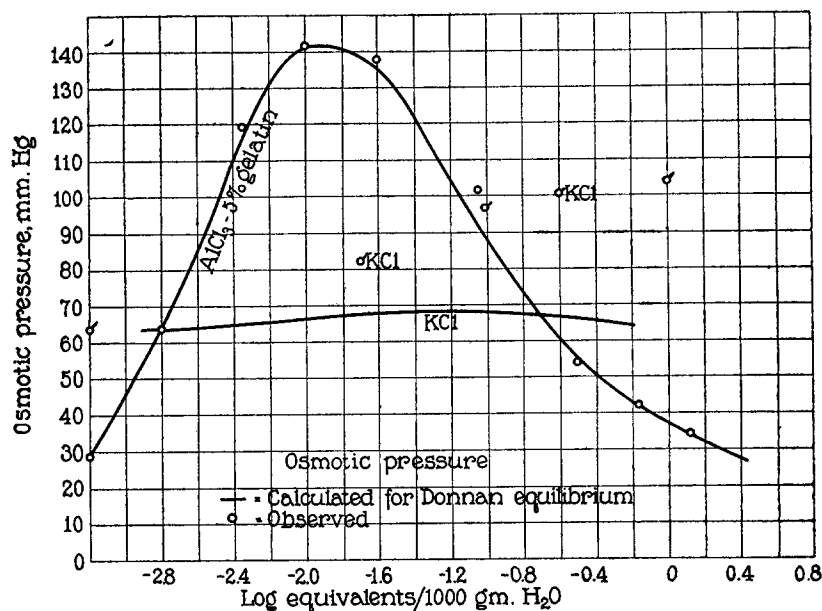


FIG. 8. Observed osmotic pressure and osmotic pressure calculated from Donnan equilibrium.

valence. Since the swelling and osmotic pressure effects are parallel, this effect should be noted in the case of swelling as well as osmotic pressure. Increasing concentrations of MgCl_2 or CaCl_2 were therefore added to gelatin swollen in HCl, in AlCl_3 , and in KCl. The results of the experiments are shown in Fig. 9. The swelling in HCl and in AlCl_3 is depressed markedly, as would be predicted from Donnan's theory, while the swelling in KCl is depressed very slightly, in fact only about to the extent of the calculated Donnan pressure in this

salt. It may also be noted that in very high MgCl_2 concentrations the swelling increases again in the HCl and KCl samples, while it does not do so in the AlCl_3 . The "salt effect" may evidently be superimposed on the Donnan swelling in the case of acid, but not with AlCl_3 .

It will be noted from the table, however, that the total salt concentration in both cases is higher in the gelatin solution than in the outside, and that if the osmotic pressure is calculated simply from these figures without taking into account the Donnan equilibrium, the result

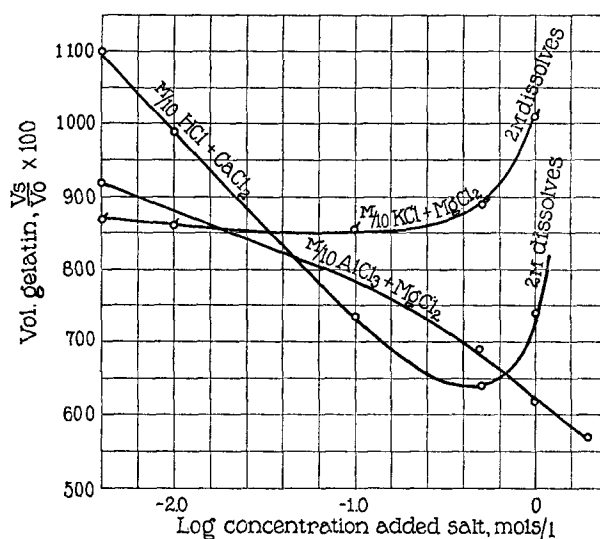


FIG. 9. Effect of addition of MgCl_2 or CaCl_2 on swelling of gelatin in KCl, HCl, or AlCl_3 .

is much too high. There is reason to suppose therefore that the Donnan effect is correctly calculated and that it is too small to account for the observed osmotic pressure.

According to the present theory of osmotic pressure, it is defined by an equation of the general form (Hückel),¹¹

$$P = RT gC$$

¹¹ Hückel, E., *Ergebn. exakt. Naturwissensch.*, 1924, iii, 199.

where g is the "osmotic coefficient" and C is the number of molecules¹² (ions) per liter. R is a constant and T the absolute temperature. It follows from this that an isothermal change in the osmotic pressure of a solution can be due either to a change in the osmotic coefficient or to a change in the number of molecules. (It is evident, however, that the liquefying effect of strong salt solutions on gelatin cannot be analogous to the increase in the solubility of a salt in presence of high electrolyte concentration, since as this latter is due to a decrease in the activity coefficient it must necessarily also cause a decrease in osmotic pressure, whereas in this case there is an increase in osmotic pressure.)

The experiments already described show that, when the Donnan equilibrium is taken into account, there is practically no difference in ion concentration. In any case, the swelling of gelatin in pure water cannot be due to a difference in ion concentration and it appears probable, therefore, that the salts increase the osmotic pressure of the gelatin itself. This conclusion is borne out by Stiasny's¹³ experiments, in which it was found that the addition of NaSCN to gelatin caused the same change in mutarotation as when the gel was transformed into a sol by heat. Stiasny also found that gelatin in the presence of NaSCN would pass through a membrane which was impermeable to gelatin in water. It might be supposed that this decrease in the particle size was due to an actual hydrolysis of the gelatin, but formol titration of the gelatin after the addition of 2 M LiCl or CaCl₂ showed no change. The number of reacting carboxyl groups is therefore not affected, a conclusion which was also reached by Stiasny.

SUMMARY.

1. The swelling and the osmotic pressure of gelatin at pH 4.7 have been measured in the presence of a number of salts.
2. The effect of the salts on the swelling is closely paralleled by the effect on the osmotic pressure, and the bulk modulus of the gelatin

¹² If the molecular weight is defined by this equation then evidently, as far as osmotic pressure is concerned, the particles in the solution may be considered as molecules no matter what their size and whether or not they may be increased or decreased. This has been pointed out by Einstein, Svedberg, and others. (Cf. Svedberg, T., *Colloid chemistry*, New York, 1924, 92.)

¹³ Stiasny, E., *Kolloid-Z.*, 1924, xxxv, 353.

particles calculated from these figures is constant up to an increase in volume of about 800 per cent. As soon as any of the salts increase the swelling beyond this point, the bulk modulus decreases. This is interpreted as showing that the elastic limit has been exceeded.

3. Gelatin swollen in acid returns to its original volume after removal of the acid, while gelatin swollen in salt solution does not do so. This is the expected result if, as stated above, the elastic limit had been exceeded in the salt solution.

4. The modulus of elasticity of gelatin swollen in salt solutions varies in the same way as the bulk modulus calculated from the osmotic pressure and the swelling.

5. The increase in osmotic pressure caused by the salt is reversible on removal of the salt.

6. The observed osmotic pressure is much greater than the osmotic pressure calculated from the Donnan equilibrium except in the case of AlCl_3 , where the calculated and observed pressures agree quite closely.

7. The increase in swelling in salt solutions is due to an increase in osmotic pressure. This increase is probably due to a change in the osmotic pressure of the gelatin itself rather than to a difference in ion concentration.