CLXXVIII. THE ABSORPTION OF WATER BY GELATIN.

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DURING the last half-century a large number of papers has been published on the absorption of water by gelatin and the influence on this process of various physical and chemical factors. A review of the literature has recently been given by one of us [Jordan Lloyd, 1926]. Previous work has shown that acids, alkalis, salts, temperature and the relative masses ·of the gelatin and the water [Jordan Lloyd, 1920; Kuntzel, 1926] all affect the final water content of a gelatin jelly in equilibrium with its surrounding fluid. The correlation between one factor and another is still, however, a fruitful subject for investigation, and this paper provides such a study in the case of a simple system in which all the diffusible ions are univalent. This system is gelatin in the presence of hydrochloric acid, sodium hydroxide, and sodium chloride, over a temperature range from 0° to 24°, a p_H range of 0·5 to 12, and a concentration of sodium chloride from zero to 2·0 mol. per litre. A uniform relation of 100 cc. of fluid to every 0·1 g. of gelatin was used throughout the experiments.

Throughout this paper the term "gel" is used to denote undispersed gelatin, and "jelly" the characteristic 2-phased rigid solution of gelatin in water.

MATERIAL.

The gelatin used was prepared from Coignet's " Gold Label " leaf gelatin. Twelve sheets were soaked in 3 litres of 0·2 M sodium chloride, adjusted to p_H 3 by the addition of hydrochloric acid; the gelatin was kept in gentle movement, and after 3 or 4 hours the reaction of the fluid was tested and brought back to p_H 3 by the addition of more acid, this process being repeated until equilibrium had been attained. At this stage the whole of the fluid was poured off and replaced by a fresh solution of sodium chloride at p_H 3. This remained on the gelatin overnight, after which a test for calcium always gave a negative result. The gelatin was then dialysed against a stream of distilled water until free from chlorides. The whole preparation lasted a week. During

the last stages of washing the gelatin became white and opaque. The dialysed pieces were dried on coarse net at room temperature. The ash content of the purified material varied from 0·05 to 0·02 % of the dry weight at 100°. This method of purification, which is a modification of one used by Smith [1922], is not so rapid, or so complete, as electrodialysis, but requires no special apparatus. The sodium chloride in the early stages of washing acts as a solvent for any calcium phosphate present and, in addition, inhibits undue swelling of the gelatin in the acid liquors.

EXPERIMENTAL RESULTS.

Water absorption under varying conditions was studied by weighing the swollen pieces of gelatin after the attainment of equilibrium with the surrounding fluid, usually after 3 days. The weights of the swollen gelatin are expressed in the final records as a percentage on the dry weight at 100°. The values obtained are not absolute equilibrium figures, since gelatin goes on slowly absorbing water for an indefinite period [Jordan Lloyd, 1920], but they represent the end of the first rapid series of changes due to the influence of the electrolytes on the physical state of the gelatin, the later slow changes being mostly due to hydrolysis. The p_H value of the fluids in equilibrium with the gelatin were all obtained electrometrically. No measurements were made of the possible fixation of sodium chloride by the gelatin.

Three variables influencing water absorption were examined—p_H, temperature (t) and salt content (M). The experimental results are summarised in the curves shown in the figures.

In reviewing the curves shown in the different figures, it should be borne in mind that gelatin is a substance which undergoes a reversible change with change of temperature between two structurally distinct forms known as gelatin A and B [Smith, 1919]. These differ in rotatory power and in solubility, A being nearly insoluble in distilled water, while B has a considerable solubility. In simple gelatin-water systems the whole of the gelatin is present as the A form at temperatures below 15°, and as the B form at temperatures above 35°. The curves shown at 0° and 12° should, assuming for the moment that the presence of the electrolytes does not appreciably affect the proportions of the two forms, indicate systems containing only gelatin A, those at 18° and 24° systems containing mixtures of A and B, although at 18° the proportion of B may be assumed to be very small, while at 24° it will be roughly 50 %. A comparison of the curves in the different figures does actually show very similar behaviour of the systems at 0°, 12° and 18° and a marked contrast at 24°.

The influence of p_H (hydrochloric acid or sodium hydroxide) on the swelling of gelatin at 18° (gelatin A) is shown in Fig. 1. In the absence of inorganic salts the swelling curve has a minimum at p_H 5·0, which may therefore be taken as the isoelectric point, and has maxima in the acid range at p_H 2·6 and in the alkaline range at p_H 9·8. On the alkaline side of the isoelectric
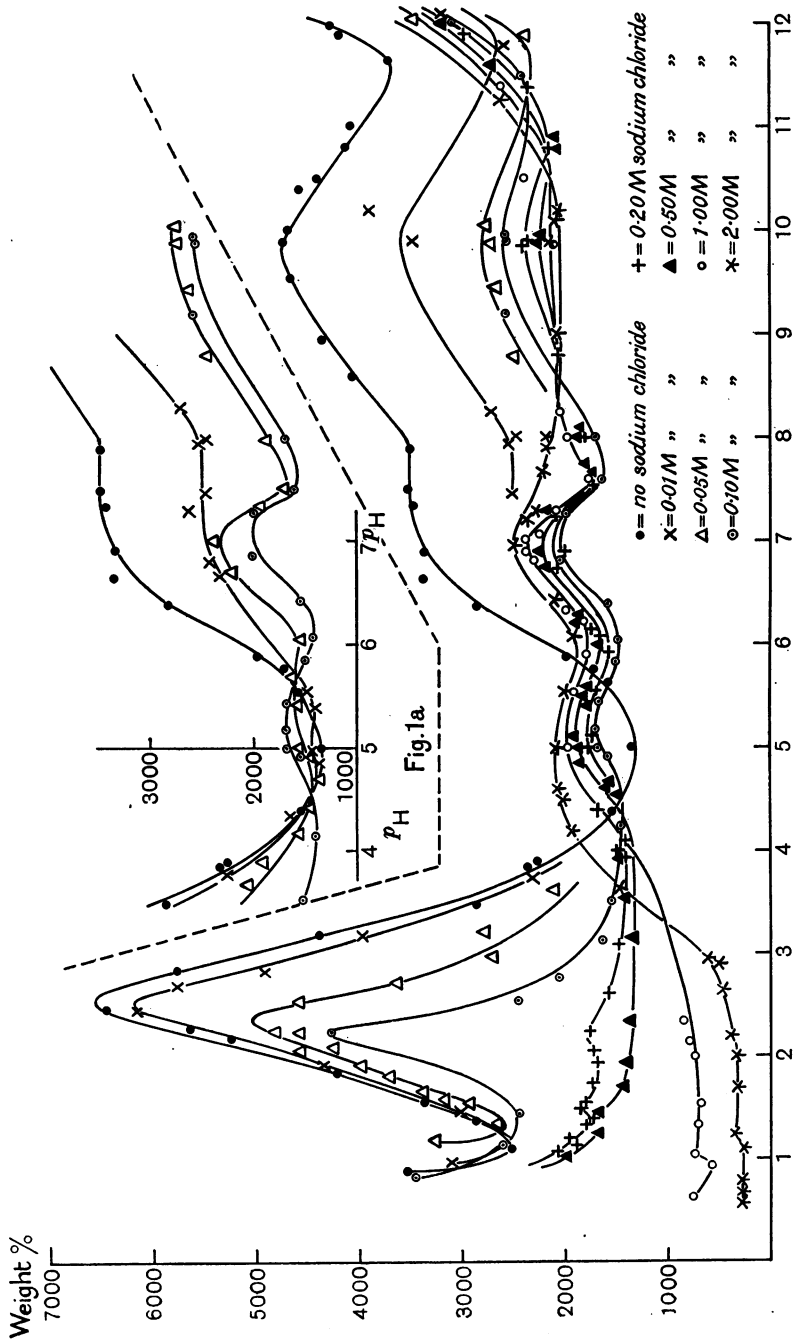
Fig. 1. p_H varying, t constant at 18°, M constant over a series of concentrations from zero to 2·0 M.

point, but close to absolute neutrality (between p_H 7·5 and 7·9), there is an additional broadly defined maximum in the curve of alkaline swelling. At $p_H < 1·1$ or $> 11·6$ the gelatin dissolves in the external fluid.

It will be convenient to consider separately the behaviour of gelatin in the four zones of hydrogen ion concentration centring respectively on p_H 2·6, 9·8, 5·0 and 7·0. These will be designated as the acid, alkaline, isoelectric and neutral zones.

In acid systems the addition of sodium chloride suppresses the swelling due to the acid, and with increasing concentration ($> 0·5$ M) actually leads to a coagulation of the gelatin (Figs. 1, 7).

The acid swelling zone (p_H 1·5 to 3·5).

The swelling of gelatin gels in acid solutions was first shown by Procter [1914] to be an example of Donnan's well-known phenomenon of membrane equilibrium. The combination of the weak base gelatin with part of the acid present to form soluble, ionisable and hydrolysable salts leads to the production of a non-diffusible cation and one or more diffusible anions and hence to an unequal distribution of the diffusible ions across the gel surface with an excess of diffusible ions inside the gel, the presence of which gives rise to an internal osmotic pressure leading to the absorption of water by the gel. Loeb [1922] has also shown that, between certain limits, the repressive action of metallic salts on the osmotic pressures of gelatin solutions with acid reactions is due to their influence on the membrane equilibrium. Loeb equates the internal osmotic pressure of a gelatin-hydrochloric acid-sodium chloride system to $2y + z - 2\sqrt{y(y+z)}$, where z is the concentration of the chlorine ion due to the ionisation of the gelatin chloride, and y the sum of the chlorine ions due to the hydrochloric acid and the sodium chloride. Loeb considers that as y increases while z remains constant, in the limit when y is very large compared to z, the expression $(2y + z)/2\sqrt{y(y+z)}$ must equal 1, hence $2y + z = 2\sqrt{y(y+z)}$ and the internal osmotic pressure falls to zero. Now when y is large and z is small, the expression $(2y + z)/2\sqrt{y(y+z)}$ does not differ greatly from the expression $(y+z)/\sqrt{y(y+z)}$. Now $y + z$ is the concentration of the chlorine ions in either a gelatin solution or jelly in equilibrium with an external solution in which the concentration of chlorine ions is $y(y + z)$, and Loeb has shown that in the case of a gelatin sol separated from its external fluid by a membrane, a direct titration of the chlorine ions in both the inner and the outer solutions [Loeb, 1922, pp. 183–4] shows that at 25° the value of $(y+z)/\sqrt{y(y+z)}$ becomes 1·06, *i.e.* approximates to 1, as the salt concentration reaches 0·062 M (M/16). Moreover, he shows that if osmotic pressure and the value of the expression $(y + z)/\sqrt{y(y+z)}$ be plotted against the logarithm of the salt content, the two curves are parallel (p. 183), both being S curves which ultimately run parallel to the axis of salt concen-

tration. If the effect of the metallic salts on the volume of the jelly is limited, therefore, to an effect on the membrane equilibrium, and if the contribution of the colloidal ions to the internal osmotic pressure is taken as negligible, then it might be anticipated that increasing the concentration of salt up to $0.06\ M$ would reduce the jelly to the same volume as in distilled water, after which further increase in salt concentration would have little or no effect. Such is, however, by no means the case. A reference to Figs. 1 and 7 shows that in the acid zone increasing concentration of sodium chloride has an increasing repression on gel volume up to a concentration of $2\ M$ and possibly beyond. At $0.5\ M$ sodium chloride the swelling in acid solutions is very close to that in distilled water, but at 1.0 and $2.0\ M$ the water absorption is far below that in distilled water. The experimental curves showing swelling with varying salt content in acid solutions (Fig. 7) are not of similar form to Loeb's theoretical curve. On the contrary, their general form suggests that they represent a compound function, the sum of two simple functions of M, the salt concentration. When $\log M < -0.5$ the swelling curve resembles in form the curve of Loeb's equation, but when $\log M > -0.5$ the swelling is a negative linear function of $\log M$.

It follows, then, that sodium chloride in acid solutions represses swelling by some additional effect as well as by its influence on the membrane equilibrium. This second effect only becomes patent in comparatively strong solutions ($0.5\ M$ or greater).

The swelling in the acid zone is strongly influenced by temperature, the volume of the jelly in salt-free systems being apparently an exponential function of t until on the verge of solution (Fig. 2). An acid gel at p_{H} 2 passes completely into solution at about 20°. This is not due to the appearance of gelatin B in the system, since there is no sudden turning of the volume-temperature curve at about 15–18° as there is in the case of the isoelectric and neutral systems. The influence of temperature on the water absorption of acid gelatin is mainly due to its influence on the ionisation of the weak base gelatin and on the hydrolysis of its salts, with the consequent influence on the membrane equilibrium. A comparison of the acid-binding power of gelatin, as given in the figures of Jordan Lloyd and Mayes [1922] at 20° and of Atkin and Douglas [1924] at 25°, shows that between p_{H} 5 and 2.5 more acid is fixed at the higher temperature. The temperature effect is greater between p_{H} 5 and 2.5, at which point saturation of the amino-groups of the gelatin is complete, than between p_{H} 2.5 and 1. The rotatory power of gelatin salts differs from that of free gelatin [Jordan Lloyd, 1922], but the evidence does not suggest that salt formation is accompanied by a transformation of form A of gelatin into form B. The electrically charged gelatin A as well as gelatin B can form a colloidal solution.

The alkaline swelling zone (p_H 8 to 11·5).

It is not as easy to make accurate and reproducible experiments on the properties of gelatin in alkaline solutions as it is in acid solutions. All experiments and estimations of p_H, etc. must be carried out in closed vessels to prevent the absorption of carbon dioxide from the atmosphere. There is, in addition, the difficulty that, even at low temperatures, the hydroxyl ion catalyses the hydrolysis of gelatin very much more than the hydrogen ion. Perhaps for this reason the zone of alkaline swelling has not been studied so much as that of acid swelling. On referring to Fig. 1 it can be seen that the curve of alkaline swelling in pure sodium hydroxide solution is not symmetrical with the acid-swelling curve. As the p_H value moves from 5·0 to 7·0 the volume of the gel rises to a steady level; then, with further increase from 7·9 to 9·8, to a true maximum. With further increase in alkalinity the gel volume decreases up to p_H 11·5, at which value at 18° solution of the gel sets in. Loeb [1922, p. 81] shows a curve of similar form at 25°. The p_H-swelling curve may be compared with the curve of combination with base at varying p_H. Atkin and Douglas [1924], working at 25°, show that the combination of base with protein takes place in two stages, the first complete at p_H 7–8 and the second at p_H 11–12. Although on the acid side p_H 2·6 marks both maximum combination with acid and maximum swelling, on the alkaline side maximum swelling occurs at p_H 9·8, a point which does not correspond with either of the two maxima of combination indicated by Atkin and Douglas. Neither of these maxima, however, is sharply defined, and Reiner [1926] has suggested that the influence of the gelatin anion on the activities of the other ions causes errors in the calculated results. It seems at least highly probable that alkaline swelling with a sharp maximum at 9·8 is due to the formation of salts between the carboxylic groups of the gelatin and the sodium hydroxide, and that the series of sodium gelatinates ionises, with the formation of colloidal anions which lead to an unequal distribution of the diffusible ions between jelly and external fluid, with an excess of diffusible ions in the jelly, leading to an internal osmotic pressure and the absorption of water.

The maximum of alkaline swelling at p_H 9·8 is in all ways the counterpart of the acid peak of swelling at 2·6. The sharpness of the peak, the relative positions of both ascending and descending branches, and the repression of the alkaline swelling by the addition of sodium chloride (Figs. 1 and 7), all suggest that the two systems are similar. The behaviour of sodium gelatinate in the presence of sodium chloride is, however, apparently simpler than that of gelatin hydrochloride. From the curves shown in Figs. 1 and 7 it can be seen that sodium chloride solutions have no coagulating action on alkaline gelatin and apparently the effect of adding this salt in the alkaline zone is limited to its effect on the membrane equilibrium. The repressive action of the salt on swelling seems to reach its full effect in 1·0 M solutions. The curve of swelling-log M at p_H 10 (Fig. 7) turns and runs parallel to the axis of

$\log M$ in accordance with the theoretical expectations implied in Loeb's equation. The volume of the jelly, however, is never brought back to the volume in distilled water, a fact which suggests that the osmotic pressure of the gelatin ions is not negligible.

The effect of temperature on swelling in the alkaline zone is similar to that in the acid zone, and therefore mainly due to the influence of temperature on the ionisation and hydrolysis constants of the gelatin. Fig. 2 shows the effect of temperature on swelling at p_H 10·5. Swelling increases very rapidly with temperature and is, as with acid jellies, an exponential function of t until
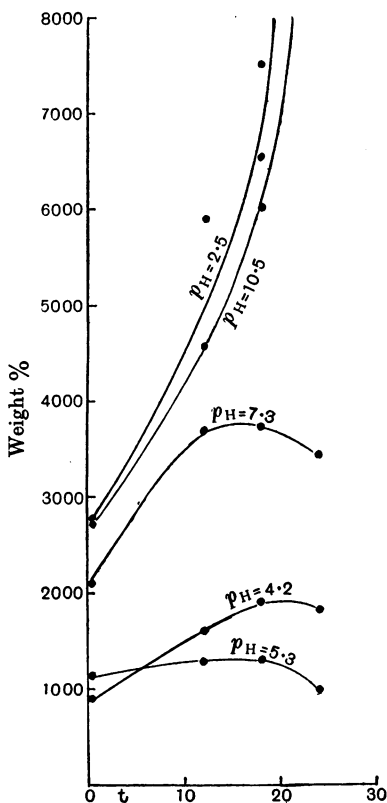

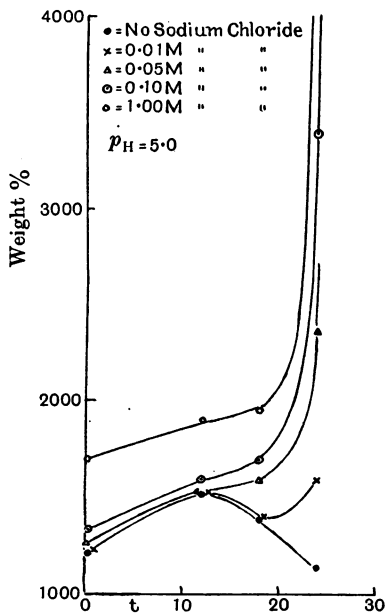Fig. 2. t varying, M constant at zero, p_H constant at 2·5, 4·2, 5·3, 7·3 and 10·5.

Fig. 3. t varying, p_H constant at 5·0, M constant at five values varying from zero to 1·0 M.

the verge of solution. There is no sign of an abrupt change in the form of the curve between 15° and 18° and the jelly passes into solution at about 20°. The rotatory power of the salts of gelatin with bases differs from that of the free gelatin [Jordan Lloyd, 1922], but the evidence does not suggest that the formation of sodium gelatinates is accompanied by a transformation of the A form of gelatin into the B form. The negatively charged particles of gelatin A form a colloidal solution.

The isoelectric zone (p_H 4·6 to 6·0).

The position of minimum swelling in a system free from sodium chloride lies at p_H 4·9 to 5·0, which may be taken as the isoelectric point of the pure gelatin. The presence of sodium chloride in isoelectric or nearly isoelectric solutions causes absorption of water by gelatin A, the amount absorbed at constant temperature being (except in very dilute solutions) proportional to the logarithm of the concentration (Fig. 5, curves at 0°, 12° and 18°). The effect of sodium chloride on water absorption at 24°, when gelatin B may also be assumed to be present, is not directly proportional to log M, but to $e^{\log M}$ (Fig. 5). The p_H of the solution has a marked effect on the influence of the chloride, swelling induced by the action of the salt being greatest at the iso-electric point where the positive and negative gelatin ions are either absent or at a minimum, and increasingly less with increasing concentration of either hydrogen or hydroxyl ions, *i.e.* with increasing concentration of the gelatin ions (Fig. 1). The p_H range over which the swelling effects of sodium chloride are shown is very narrow in dilute solutions, but becomes increasingly wider with increasing salt concentration—at 0·01 M sodium chloride it extends from p_H 4·4 to 5·6 and at 2 M from p_H 3·5 to 6·0 (Fig. 1). The salt influence is evidently limited to the electrically neutral gelatin, and the swelling of gelatin under these conditions is due to an entirely different mechanism from swelling under the influence of the hydrogen or hydroxyl ion.

The effect of temperature on swelling in the isoelectric zone differs strongly from that in the acid and alkaline zones. Below 15° there is a gradual increase of volume with temperature in the salt-free system and at all concentrations of sodium chloride up to 2 M. The influence of the temperature is therefore directly due to action on particles of gelatin A, present in the jelly in the gel form, probably leading to a weakening of the cohesive forces of the rigid structure of the jelly above 15°. The effect of temperature in the absence of salt or at low dilutions (0·01 M) differs from its effect at higher concentrations in an important and striking manner; in the two former cases there is, with further rise of temperature, a decrease in swelling, in the latter a rapid increase (Figs. 2 and 3). This leads to the impression that gelatin B is, in the absence of salt, less hydrated than gelatin A, but is more readily dispersed by sodium chloride. This has already been observed in the smooth but steep rise of the curve showing the influence of salt concentration on swelling at 24° (Fig. 5).

Sodium chloride increases the hydration of electrically neutral gelatin A, but does not disperse it to form a sol. It has a very marked dispersing action on gelatin B.

The zone of neutrality (p_H 6 to 8).

Most proteins are isoelectric in solutions which are slightly acid, hence solutions which are neutral, assuming for the moment that neutrality may be defined with sufficient accuracy as any p_H value between 6 and 8, must contain small quantities of base, both free and combined with the protein.

In a system of gelatin jelly and sodium hydroxide free from diffusible salts, an increase of p_H from 5 towards the more alkaline regions leads at first to a fixation of the base by the protein and to a swelling which increases with increasing p_H. At 18° the swelling reaches a steady level between p_H 7·4 and 7·9. At 25°, and at about the same reaction, the combination of protein and base also reaches a steady level. The swelling can therefore be considered as due to the formation of partially ionisable salts. Evidence of the extent of the ionisation is, however, somewhat conflicting. Reiner has pointed out [1926] that immediately on the alkaline side of the isoelectric point there is a zone where there is no migration of protein in an electric field [see also Svedberg, 1924; Cohn, 1925]; hence, therefore, a zone in which there is no ionisation of the protein salt. Experiments here recorded on the influence of salts in the neutral zone (Fig. 1 a) suggest that part of the sodium gelatinates is present in the ionised form, since the first effect of adding sodium chloride in the neutral zone is exactly the same as in the acid and alkaline zones, i.e. there is a repression of swelling which can be accounted for in the usual manner as due to a readjustment of a membrane equilibrium. In 0·01 M solutions of sodium chloride this is the only effect produced by the salt. At 0·05 and 0·10 M, although there is increased repression with the increased salt concentration, the curve of swelling has taken on a different form, strongly suggesting that, although the salt is repressing swelling by one chemical mechanism, it is simultaneously promoting it at about p_H 7 by another. At concentrations from 0·10 to 2·0 M the effect of the salt is exactly the same as in the isoelectric zone, i.e. at 0°, 12° and 18° the swelling of the jelly increases in proportion to the logarithm of the concentration of the salt (Figs. 1, 6 and 7).

The volumes attained at 18° under the influence of salt are rather greater at p_H 7 than at p_H 5, and, although precise figures are not available, it has been ascertained that the melting-point of the jelly is lower at p_H 7. It is an interesting point that, although jellies can swell to about 7000 % of their dry weight under the action of acid or alkali without going into solution, the presence of sodium chloride leads to the conversion of gel into sol if the swelling approaches 3000 %. In the former case only the gelatin ions pass into the sol state, in the latter the electrically neutral gelatin of the gel framework is dispersed.

The influence of temperature on swelling in the neutral zone (Fig. 2, curve at p_H 7·3) shows an interesting compromise between its influence at p_H 5·3 and at p_H 10·5. In the absence of salt and at the lower temperatures, swelling increases nearly as steeply with temperature as in the alkaline zone, at the higher temperature ($t = 18°$) further rise of temperature causes a repression of swelling as in the isoelectric zone. The influence of temperature in the salt-containing systems (Fig. 4) shows again the mixture of two effects. At the lower salt concentrations swelling increases with temperature up to about 20° at 0·01 M concentration and to 22° at 0·05, after which point further rise of temperature leads to loss of volume. With increasing salt concentration the

optimum temperature of swelling rises, but after 0·1 M has been reached the curves take on a form resembling those obtained at p_H 5 (Fig. 3).
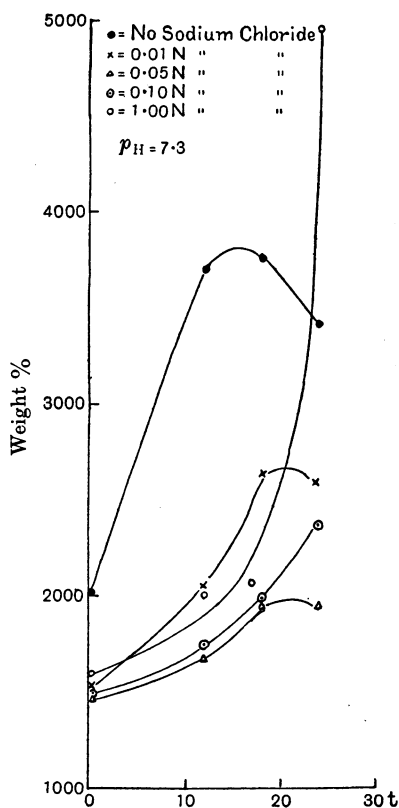

Fig. 4. t varying, p_H constant at 7·3, M constant at five values varying from zero to 1·0 M_i

DISCUSSION.

The water content of a gelatin jelly is determined by the balance of a number of factors. The jelly consists of a solid framework of gelatin in the gel form and an interstitial fluid containing one of the several possible sol forms. Unless both sol and gel forms are present the jelly is unstable [Jordan Lloyd, 1920]. Only gelatin A can assume the gel form, but it seems likely that not only the free ampholyte A but also the unionised salts A.HCl and NaA and possibly the hermaphrodite ions $^+A^-$ postulated by Pauli can all take a place in the framework of the jelly. The characteristic of the gel forms of gelatin seems to be the absence of an electric potential. The sol forms of gelatin may be either electrically charged or electrically neutral; in the former case the sol may be formed by free gelatin ions, A^+ and A^-, in the latter by a dispersion of gelatin A through the action of salts, or by gelatin B. The water

content of the jelly is fixed by a balance between osmotic forces in the sol and the elastic force in the gel framework, the latter also containing water present as a solid solution. Reactions which increase the osmotic forces in the interstitial fluid or those which weaken the elastic forces of the framework lead to swelling; conversely, those which decrease the internal osmotic pressure or increase the elasticity lead to shrinking.
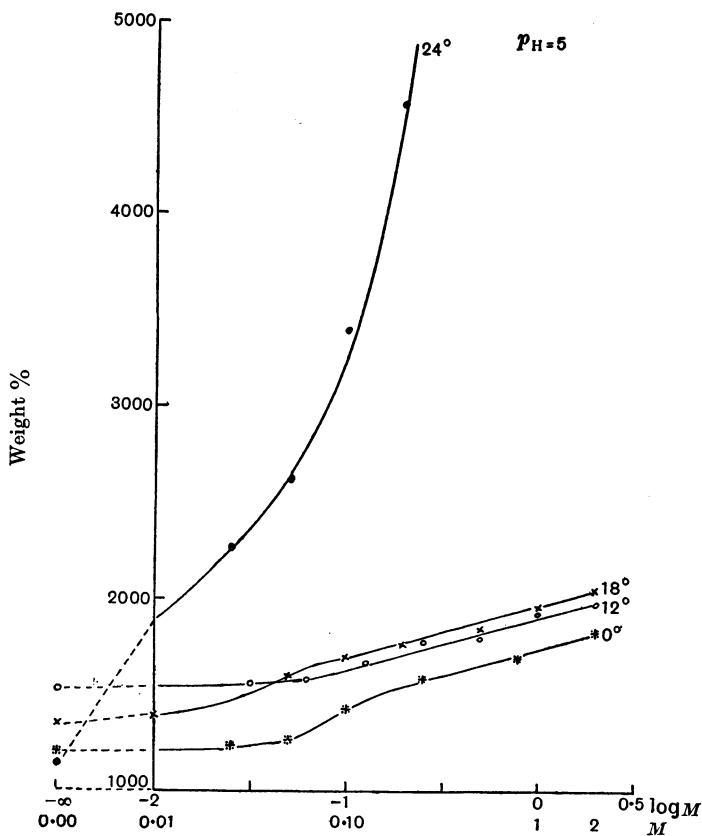

Fig. 5. M varying, p_H constant at 5·0, t constant at 0°, 12°, 18°, 24°.

The formation of ionisable salts between gelatin and hydrochloric acid or between gelatin and sodium hydroxide leads to the appearance in the jelly of colloidal ions with a consequent unequal distribution of diffusible ions across the membrane. The internal osmotic pressure which controls the volume of the jelly is due mainly to the excess in the jelly of the diffusible ions of the gelatin salt, though it can be seen from the results obtained in the alkaline zone that the colloidal ions must contribute some part to the whole. The first effect of the addition of sodium chloride to a system where gelatin hydrochlorides or sodium gelatinates are present in the jelly, is a suppression of swelling which can be accounted for by the readjustment of the membrane

equilibrium. This appears to be the only effect of the salts in the alkaline zone. In the acid zone the sodium chloride, after reaching a concentration of 0·5 M, dehydrates and coagulates the gelatin. In saturated sodium chloride solutions the precipitation in the acid zone is complete [Moeller, 1921]. It is tentatively suggested that the excess of sodium chloride dehydrates A^+, the positive gelatin ion, through the greater attraction of its own ion for the dipoles of water. Dokan [1924] has shown that with colloidal carbohydrates strong salt solutions repress swelling apparently by withdrawing the water
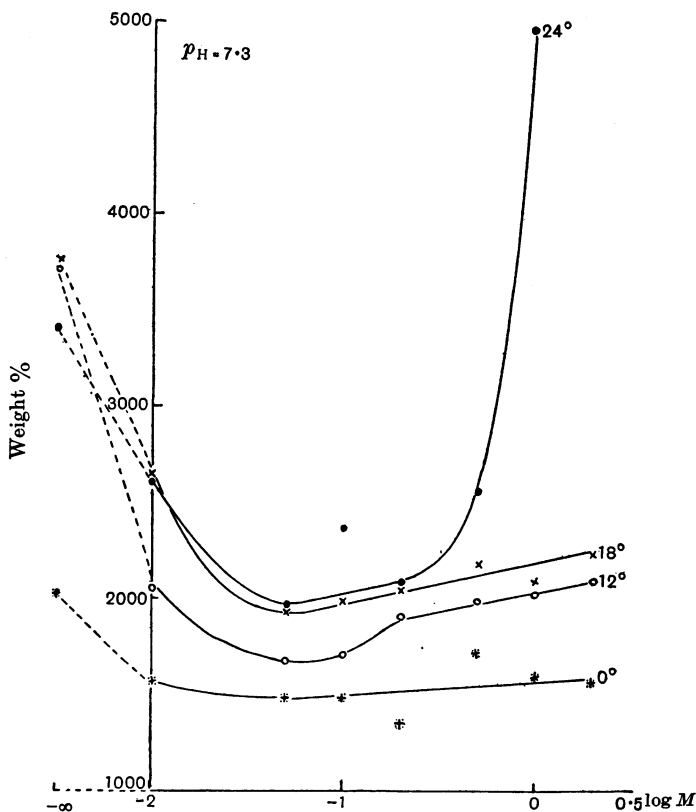

Fig. 6. M varying, p_H constant at 7·3, t constant at 0°, 12°, 18°, 24°.

from the colloid for the hydration of their own ions. The gelatin cation, which other evidence suggests is heavily hydrated, appears to part fairly easily with its water and is therefore flocculated in concentrated salt solutions. It is especially to be noticed that at a concentration of salt in which neutral or isoelectric gelatin may be assumed to be adsorbing both the positive and negative salt ions with their associated water, thus becoming hydrated, the gelatin cation is giving up its water to these same ions free in the solution. Presumably the gelatin cation does not adsorb the ions of the salt so freely as the uncharged gelatin.

The formation of gelatin salts in a jelly causes swelling by increasing the osmotic forces in the interstitial fluid and simultaneously weakens the framework and the elastic recoil. The effect of change of temperature under these conditions is that swelling increases with rise of temperature. This effect seems mainly due to the influence of the temperature on the ionisation and hydrolysis constants of the gelatin. With rising temperature the hydrolysis of the gelatin salts (sol forms) and regeneration of uncombined gelatin (gel form) will be diminished. Hence, rising temperature will weaken the elastic forces of the framework and at the same time it will increase osmotic pressures through increasing the kinetic energy of both colloidal and diffusible ions in the jelly.
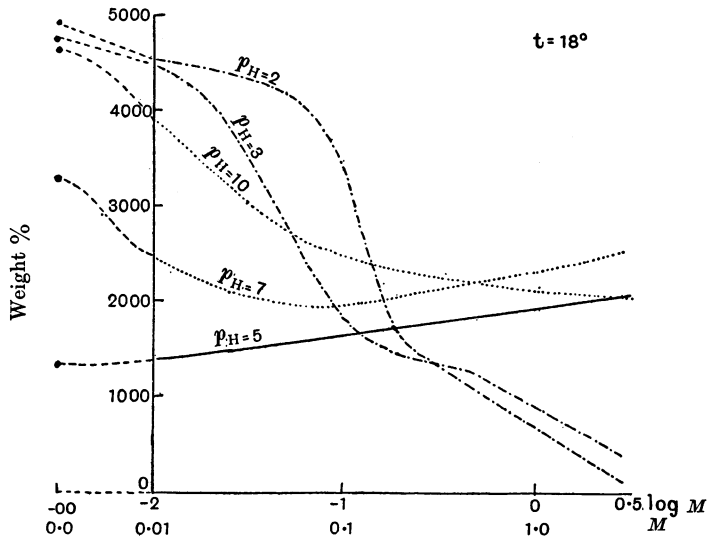

Fig. 7. M varying, t constant at 18°, p_H constant at 2, 3, 5, 7 and 10.

At the isoelectric point the jelly, in equilibrium with water, can be made to swell by a different mechanism from the formation of gelatin salts. At p_H 5 a gelatin jelly free from mineral salts is unstable and the framework collapses under its own forces [Jordan Lloyd, 1920]. With the addition of sodium chloride the conditions for stabilising the jelly appear. The adsorption of both positive and negative ions of the salt by the gelatin, with the resulting hydration of the latter, weakens the elastic forces of the framework. Some of the gelatin will go into the form of an uncharged sol exerting a slight osmotic pressure. Eventually the jelly will be completely dispersed by the salt solution.

Dokan [1924], who has investigated a similar effect of salts on the colloidal carbohydrates, ascribes the action of the salt to the adsorption on to the surface of the electrically neutral colloidal particles of both the positive and negative ions of the salts, each ion carrying into the system its surrounding

shell of water. On this theory the swelling curve should resemble the adsorption curve and be a logarithmic function of the salt concentration, a relation which has been shown to be true for systems consisting mainly of gelatin A.

Temperature has an interesting effect here. A rise of temperature from 0° to 15°–18° slightly weakens the elastic forces of the jelly and the volume increases slightly. At 15°–18° gelatin B, the sol form, begins to appear in the system. Sodium chloride has a strongly dispersing action on gelatin B and salt solutions rapidly dissolve jellies with rise of temperature between 18° and 24°. When no sodium chloride is present, however, rise of temperature over the region 18° to 24° leads to loss of volume by the jelly. Gelatin B, the sol form of uncombined gelatin, is ordinarily more soluble in water than gelatin A, but the latter appears to have a greater capacity of dissolving water in itself to form a solid solution. Further evidence of the behaviour of gelatin in salt-free systems at higher temperatures is needed here.

From the biological point of view the most interesting region studied is the neutral zone, since within this lie the conditions of p_H and salt content generally found in the living cell. The theory of the formation of ionisable sodium gelatinates with a consequent internal osmotic pressure and of the influence of sodium chloride on these, explains the volume relations up to a salt concentration of 0·01 M. As the concentration of sodium chloride reaches the order of value found in the living cell (approximately 0·1 M) the system is found to have undergone a considerable change. The volume now attained by the jelly, both with change of salt concentration and with temperature, is controlled by the same mechanism as at the isoelectric point, or, in other words, as the salt concentration passes a critical value the gelatin becomes electrically neutral and the jelly volume is now due to a balance of osmotic forces derived from an uncharged sol and the elastic forces of a framework weakened by the adsorption of the hydrated ions of the sodium chloride. The effect of sodium chloride at p_H 7 is greater than at p_H 5. Pauli [1926] considers that at the isoelectric point solutions of proteins may contain the protein in four different forms: the charged ions A^+ and A^-, the uncharged pseudo-ampholyte A and the hermaphrodite ion of the internal salt form $+A^-$. The first additions of acid are said to increase the concentrations of $+A^-$. It must remain for the present an interesting speculation whether the first additions of alkali have a similar but possibly even greater effect. Other salts are now under investigation in order to provide further evidence for or against the hypotheses advanced in the paper.

Summary.

1. Sodium chloride, in the presence of hydrochloric acid and up to a concentration of 0·01 M, suppresses swelling of gelatin due to the acid; at greater concentrations it causes a coagulation of the acid gelatin. The maximum of acid swelling is at p_H 2·6.

2. Sodium chloride in the presence of sodium hydroxide suppresses the swelling of gelatin due to the alkali. Even at high concentrations (2 M) the salt has no further action. Maximum alkaline swelling is at p_H 9·8.

3. The swelling of gelatin due to acid or alkali increases as an exponential function of the temperature.

4. The point of minimum swelling of ash-free gelatin (isoelectric point) is at p_H 5·0. At this point sodium chloride promotes water absorption, swelling being proportional to the logarithm of the concentration of the salt.

5. At p_H 5, in the absence of salts, swelling increases slowly with rising temperature up to 15° or 18°, after which, with further rise, there is a decrease of swelling.

6. At p_H 5 the presence of sodium chloride increases swelling slowly with temperature up to about 18°, after which there is very rapid increase ending at about 20° in complete solution of the gelatin. The greater the concentration of sodium chloride present, the lower the temperature at which solution occurs.

7. At p_H 7, in the absence of salts, gelatin shows some swelling due to the alkali present. The addition of sodium chloride up to 0·01 M causes a suppression of swelling; at greater concentrations the salt, as at p_H 5·0, induces a swelling which is in logarithmic ratio to its concentration.

8. At p_H 7, in salt solutions stronger than 0·01 M, the effect of temperature on swelling resembles its effect at p_H 5. When the concentration of sodium chloride is less than 0·01 M, or in the absence of salts, the effect of temperature on swelling at p_H 7 is intermediate between its effects in the alkaline and in the isoelectric zones.

9. When gelatin is in solution as electrically charged particles the effect of adding sodium chloride is mainly electrostatic; when present in electrically neutral forms, the salt acts by adsorption and hydration.

10. The theoretical aspects of swelling are discussed.

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