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## **Ion Series and the Physical Properties of Proteins. II**

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## ION SERIES AND THE PHYSICAL PROPERTIES OF PROTEINS. II.

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### *I. Combining Ratios of Acids and Bases with Gelatin and the Swelling of Gelatin.*

In this paper we will continue the demonstration of the relation between the combining ratios of acids and bases with proteins and the effect of ions on the physical properties of proteins. This demonstration completes the proof that the purely chemical forces of primary valency determine the reactions of proteins with other compounds.

It is generally stated in colloidal literature that gelatin swells more in chlorides, bromides, or nitrates than in water and that it swells less in citrates, acetates, tartrates, phosphates, and sulfates than in water. The author of this statement is Hofmeister<sup>1</sup> who was a pioneer in this work and who cannot be blamed for not having considered certain sources of error in his methods. In Hofmeister's experiments gelatin blocks were put into salt solutions of so high a concentration that—as we now know—no specific ion effects could be expected and the slight differences in swelling actually observed by him were probably merely accidental. He even mentions that sugar solutions have a “dehydrating” effect, and this fact alone should have warned chemists against using his experiments for conclusions concerning the specific effects of ions on the physical properties of colloids. As far as the writer can determine from the literature the discrimination between “hydrating” and “dehydrating” ions originated from these experiments.

<sup>1</sup> Hofmeister, F., *Arch. exp. Path. u. Pharmacol.*, 1891, xxviii, 210.

It is often asserted that Hofmeister's ion series for swelling has been confirmed by other authors. Thus Zsigmondy<sup>2</sup> makes the following statements in support of this impression.

"Wo. Ostwald who compared the efficiency of different acids found that swelling diminishes in the acids in the following order,

HCl > HNO<sub>3</sub> > acetic acid > sulfuric acid > boric acid.

Fischer has shown that the acid and alkali swelling of gelatin as well as that of fibrin is diminished by the addition of salt, and that chlorides, bromides, and nitrates have a less dehydrating action than acetates, sulfates, or citrates. We have here a similar series as in the case of the precipitation of proteins by alkali salts, although the order does not agree entirely."

The writer is inclined to interpret Ostwald's and Fischer's experiments differently from Zsigmondy, since both authors ignored the hydrogen ion concentration of their solutions. We believe to have shown that it is necessary to base conclusions concerning the relative efficiency of ions on experiments with equal hydrogen ion concentration. By ignoring this postulate Ostwald has only succeeded in proving that acetic and boric are weaker acids than nitric but not that gelatin swells less in acetates or borates than in nitrates; and Fischer has only succeeded in proving that citrates and acetates are buffer salts which when added to a solution of a strong acid diminish its hydrogen ion concentration, but not that acetates and citrates diminish the swelling of gelatin. These authors attributed the effects caused by a variation in the hydrogen ion concentration of their solutions erroneously to an influence of the anion. The Hofmeister series of ion effects on swelling has, in reality, never been confirmed.

If we wish to study the specific effects of ions on the swelling of gelatin we must proceed from isoelectric gelatin, bring it to different pH values by different acids or alkalies, and then compare the swelling at the same pH for these different acids or alkalies. If this is done it is found that when gelatin is in combination with the anion of a weak dibasic or tribasic acid, *e.g.* tartaric, citric, phosphoric, its degree of swelling is practically the same as when it is in combination with Cl or NO<sub>3</sub>; since in all these cases the anion of the gelatin salts is monovalent. Only in the case of gelatin sulfate is the swelling considerably less,

<sup>2</sup> Zsigmondy, R., *Kolloidchemie*, Leipsic, 2nd edition, 1918, 373

because the anion is divalent,  $\text{H}_2\text{SO}_4$  combining with gelatin in equivalent and not in molecular proportions as do the weak dibasic or tribasic acids; *e.g.*, tartaric or phosphoric.

A few words are necessary concerning the method of these experiments. We can measure the amount of swelling by determining the increase in weight of a given mass of gelatin or by determining its increase in volume. We have adopted the following simple and quick volumetric method (although we intend to supplement these experiments later with gravimetric experiments).

Dry powdered gelatin, of  $\text{pH} = 7.0$ , was sifted and the grains no longer going through Sieve 50 but going through Sieve 40 or 30 were selected for the experiment. We had therefore fairly uniform grains of not too small a diameter. Doses of 1 gm. each of such powder were weighed out, each dose was put for an hour into 100 cc. of  $\text{M}/128$  acetic acid at  $10^\circ\text{C}$ . to bring the gelatin to the isoelectric point. The powdered mass was then put on a filter and washed five times with 25 cc. of distilled water at  $5^\circ\text{C}$ . In the acetic acid solution and during the washing on the filter the powdered gelatin is stirred constantly.

Each dose of originally 1 gm. of dry powder which had meanwhile absorbed a certain quantity of liquid (which was about the same for each gram of the isoelectric powder) was then put for 1 hour at about  $20^\circ$  into 100 cc. of different concentrations of the acid or base whose influence on swelling was to be tested, and the suspension was constantly agitated. It was found that in an hour the granules of gelatin had reached the maximal swelling in each solution. To measure the relative amount of swelling in different acids or alkalies and at different  $\text{pH}$  the suspension was poured into graduate cylinders of 100 cc. each (and all of the same diameter) in which the granules fell very rapidly to the bottom. The cylinders were kept in a water bath at  $20^\circ\text{C}$ . for about 10 to 15 minutes and the volume occupied by the gelatin granules was then read. This volume included a certain amount of solution between the granules and therefore the real volume of the gelatin was smaller than that read. While therefore the method cannot be used to measure the absolute amount of swelling it allowed us to determine the relative influence of different acids or bases on the swelling for the same  $\text{pH}$ .

The determination of the pH of the gelatin in these experiments requires a short discussion since the pH inside the gelatin is quite different from that in the supernatant liquid, owing to the Donnan equilibrium. Donnan has shown that when a solution of a colloidal salt is separated from the solution of a crystalloidal salt with a common ion by a membrane which is permeable for the crystalloidal but not for the colloidal ions the concentration of the crystalloidal salt is, at the point of equilibrium, always lower on the side of the colloidal solution than on the side of the crystalloidal solution.<sup>3</sup> This was invariably the case in our experiments on osmotic pressure reported in the preceding paper. When, for example, a gelatin chloride solution of pH 3.5 was put inside a collodion bag and the latter was dipped into a solution of HCl (without gelatin) also of a pH 3.5, the pH on the two sides of the membrane did not remain the same since some of the free acid was forced from the colloidal solution into the pure acid solution outside the collodion bag, so that the pH of the outside solution fell while that of the inside rose.

As Procter<sup>4</sup> has pointed out this Donnan equilibrium must play a rôle also in the case of the swelling of gelatin since in this case the surface of the gelatin granule takes the place of the membrane permeable for the crystalloidal electrolyte but not for the colloid.

In our experiments 1 gm. of originally isoelectric gelatin was put for 1 hour at 20°C. into 100 cc. of acid, *e.g.* HCl, of different concentration varying from M/16 to M/8,192. After an hour equilibrium was reached and the pH of the supernatant fluid was determined. The gelatin was put on a filter (after the volume of the gelatin in the graduate cylinder had been measured) and all the acid was allowed to drain off. A trace of outside acid probably remained on the surface of each granule though presumably some of the free acid inside the granule diffused to the surface under the influence of pressure. This error was partly but not completely compensated by adding enough distilled water of pH of about 5.6 to the gelatin after it had

<sup>3</sup> Donnan, F. G., *Z. Electrochem.*, 1911, xvii, 572. Donnan, F. G., and Harris, A. B., *J. Chem. Soc.*, 1911, xcix, 1554. Donnan, F. G., and Garner, W. E., *J. Chem. Soc.*, 1919, cxv, 1313.

<sup>4</sup> Procter, H. R., *J. Chem. Soc.*, 1914, cv, 313. Procter, H. R., and Wilson, J. A., *J. Chem. Soc.*, 1916, cix, 307.

been melted to bring the volume to 100 cc. The pH of the 1 per cent solution of originally isoelectric gelatin was determined colorimetrically. It was found that the pH of the supernatant HCl solution was always considerably smaller than the pH of the gelatin solution (Table I).

The first row in Table I gives the molecular concentration of the 100 cc. of HCl into which the gelatin was originally put. The second row gives the pH of these supernatant HCl solutions after 1 hour, and the third row gives the pH of the gelatin solutions after the supernatant HCl solution had been drained off and after the remaining mass of gelatin had been melted and brought to a volume of 100 cc. by adding enough distilled water of pH 5.6. It will be noticed: first, that the pH of the supernatant HCl solution after 1 hour is higher than the pH of the original HCl solution owing to the fact that some acid combined with the gelatin; and, second, that the pH of the gelatin solution is considerably higher than that of the supernatant solution owing to the Donnan equilibrium, according to which the concentration of free acid outside the gelatin must be greater than inside.

In order to get the correct difference due to the Donnan equilibrium, solutions of gelatin salts were put into collodion bags and these bags were dipped into beakers containing 350 cc. of solution of the same acid or base as that inside the collodion bag and possessing the same pH as the gelatin solution; the outside solution, of course, was free from gelatin. In the case of gelatin-acid salts free acid invariably diffused from the gelatin solution into the pure acid solution, *e.g.* HCl, so that the pH of the latter became smaller and that in the gelatin solution higher. In these experiments the volume of the outside pure HCl solution was 350 cc. and that of the inside 1 per cent gelatin solution only 50 cc. Table II gives the result of one experiment of this kind.

Thus at equilibrium pH the gelatin solution was 3.8 and the outside solution 3.2, or inside 3.3, and outside 4.0. This difference is in the same sense and of nearly but not quite the same order of magnitude as that observed in Table I.

We may therefore conclude that the real pH of the gelatin solution inside the granules of gelatin was slightly less than that measured by our method.

TABLE I.

Original concentration of supernatant HCl solution.	M/16	M/32	M/64	3 M/256	M/128	3 M/512	M/256	M/512	M/1,024	M/2,048	M/4,096	M/8,192
pH of the supernatant	1.25	1.6	1.9	2.1	2.35	2.55	2.8	3.2	3.4	3.6	3.85	4.0
HCl solution after 1 hr.												
pH of 1 per cent gelatin solution after 1 hr.....	2.2	2.3	2.7	3.0	3.25	3.4	3.8	4.2	4.6	4.65	4.7	4.7

TABLE II.

pH at beginning of experiment, inside and outside.....	2.3	2.6	3.0	3.1	3.3	3.5	3.6	3.75	4.1	4.38	4.8
pH of outside HCl solution after 20 hrs.....	2.1	2.5	2.9	3.0	3.15	3.2	3.3	3.4	3.7	4.0	4.8
pH of gelatin solution after 20 hrs.....	2.6	3.2	3.3	3.5	3.7	3.8	4.0	4.1	4.2	4.4	4.8

TABLE III.

Original concentration of supernatant KOH solution.....	M/32	M/64	M/128	M/256	M/512	7 M/4,096	6 M/4,096	5 M/4,096	M/1,024	M/2,048	M/4,096	M/8,192
pH of the supernatant	12.4	12.0	11.6	11.3	8.0	7.2	7.0	6.6	6.6	5.8	5.1	5.2
KOH solution after 1 hr.												
pH of 1 per cent gelatin solution after 1 hr.....	12.0	11.6	11.0	9.8	6.3	5.7	5.5	5.4	5.3	5.1	4.9	4.8

The results of our experiments on swelling are expressed in Figs. 1, 2, and 3. The abscissæ in Fig. 1 are the pH found in the gelatin after equilibrium was established. The ordinates represent the figures for the volume of the granules of 1 gm. of gelatin in different acids. It is obvious that in all cases the volume (or swelling) is a

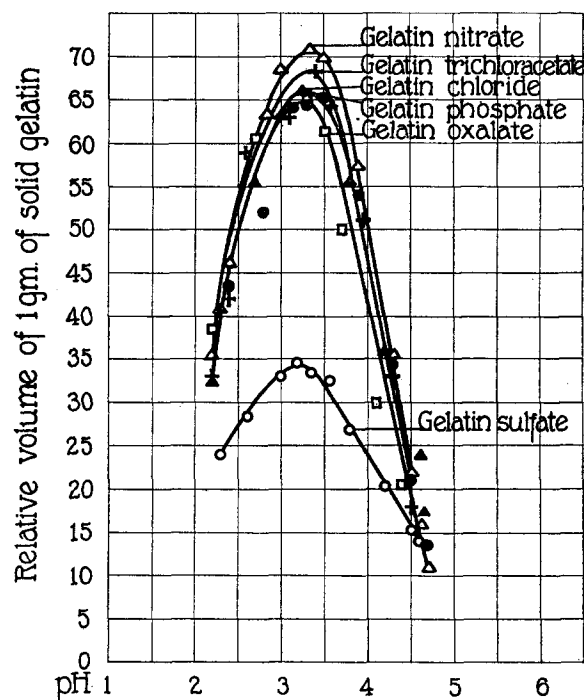


FIG. 1. Influence of HCl, HNO<sub>3</sub>, H<sub>3</sub>PO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, trichloroacetic, and oxalic acids on the swelling of gelatin. Abscissæ are the pH, ordinates the volume of gelatin. The curves for all the acids are practically identical except that for H<sub>2</sub>SO<sub>4</sub> which is about one-half as high as the curves for the other acids.

minimum at the isoelectric point  $\text{pH} = 4.7$ , that it rises with diminishing pH until the maximum is reached at a pH of about 3.2 or 3.3, and that the curve drops steeply with a further diminution of pH (*i.e.* a further increase of hydrogen ion concentration). The fact that the maximum lies here at pH of about 3.2, while in our osmotic pressure curves it was at about 3.3 or 3.4, indicates the degree of error in



the measurement of pH in this case due to the adhesion of some of the original acid on the outside of the granules. This error was partly compensated by the addition of distilled water of pH of about 5.6 in making up the 1 per cent solution of gelatin. On the whole the

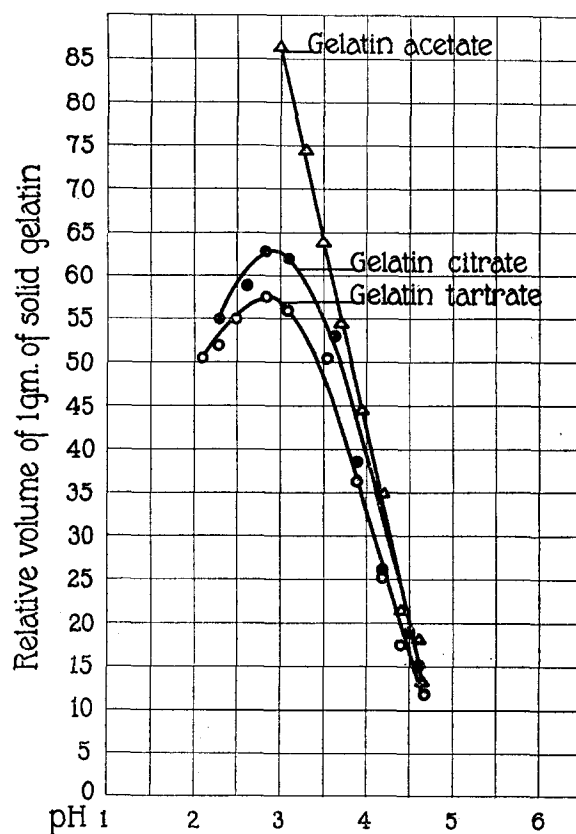


FIG. 2. Influence of citric, tartaric, and acetic acids on swelling of gelatin. The curves for citric and tartaric acids are practically identical with those for HCl and  $\text{HNO}_3$  in Fig. 1. That for acetic acid is a little higher owing probably to some specific and secondary effect of this acid.

probable error was  $+0.1$  or  $+0.2$ ; *i.e.*, the real pH was 0.1 or at the utmost 0.2 greater than in our abscissæ. The most important fact is, however, that the curves for the influence of HCl,  $\text{HNO}_3$ , trichloroacetic, oxalic, phosphoric, citric, and tartaric acids are practically

identical (Figs. 1 and 2), proving that only the effect of the valency and not that of the nature of the anion of the acid used influences the swelling; since we have seen that the anion of weak dibasic or tribasic organic acids combining with the gelatin is always monovalent.

The curve for the swelling of gelatin sulfate, where the anion combining with gelatin is bivalent, is only half as high as the curve for the salts of gelatin with the anion of weak dibasic acids (Figs. 1 and 2).

Acetic acid gives an increasing amount of swelling (Fig. 2), but it must be remembered that  $M/1$  acetic acid had to be used to bring the pH of the gelatin to 3.0, and it is not impossible that in this case a secondary chemical or physical modification of the gelatin may complicate the conditions.

It is of interest to compare these curves with those which should be expected according to the Hofmeister series. In the latter case the curves for phosphate, oxalate, citrate, tartrate, and acetate should coincide with the curve for sulfate instead of coinciding with the curves for Cl and  $\text{NO}_3$ . This difference is due to the fact that the believers in the Hofmeister series did not determine the pH and that they erroneously ascribed the effects due to a variation in the hydrogen ion concentration to a difference in the influence of the anion.

The ratio between the effects of sulfuric acid on swelling and that of the other acids is again not far from 1:2. If we deduct the swelling of the isoelectric gelatin (of about 10 mm.) from the values of our ordinates the swelling of gelatin sulfate at pH of about 3.3 is less than one-half that of the other gelatin-acid salts where the anion in combination with gelatin is monovalent.

When powdered isoelectric gelatin is treated with an alkali, *e.g.* KOH, the supernatant watery solution is less acid or more alkaline than the gelatin granules. The  $\text{CO}_2$  of the air lowers the pH of the solutions a little but this error affects the pH of the supernatant watery solution more than it does the gelatin which has a buffer action. Table III, p. 252, gives the original concentration of the watery solution of KOH, into 100 cc. of which 1 gm. of powdered isoelectric gelatin was put (first row, Table III). After 1 hour the pH of the supernatant watery solution was determined (second row, Table III);

the supernatant solution was drained off from the gelatin, the latter melted, and the volume brought to 100 cc. by adding distilled water of pH of about 5.6, and the pH of the gelatin solution was determined (third row, Table III).

It is obvious that the pH of the supernatant solution is higher than that of the gelatin solution, as we should expect from the Donnan equilibrium.

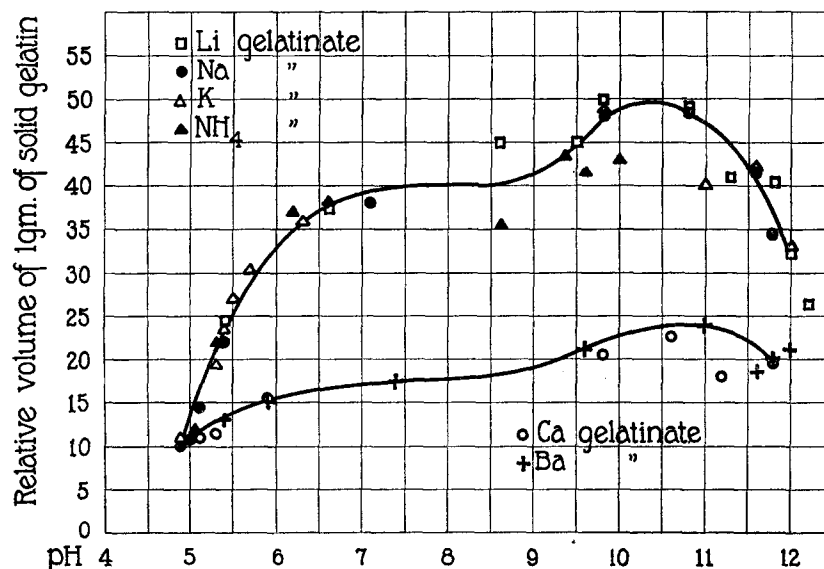


FIG. 3. Curves for the effect of different bases on swelling. Those for LiOH, NaOH, KOH, and  $\text{NH}_4\text{OH}$  are practically identical and about twice as high as those for  $\text{Ca}(\text{OH})_2$  and  $\text{Ba}(\text{OH})_2$ .

Fig. 3 gives the curves for the action of alkalies on swelling. The curves for Li, Na, K, and  $\text{NH}_4$  gelatinate of the same pH are practically identical, except that the values for  $\text{NH}_4\text{OH}$  are irregular for pH above 8.5 possibly on account of the fact that the concentration of  $\text{NH}_4\text{OH}$  required to bring gelatin to such pH is rather high. The main fact is that the ratio of the maximal swelling of gelatin salts with bivalent cation like Ca or Ba is half or possibly a little less than half of that of gelatin salts with monovalent cation, like Na, K, or  $\text{NH}_4$ .

Near  $\text{pH} = 7.0$  the curves run parallel to the axis of abscissæ for the reason that a considerable variation in  $\text{pH}$  signifies only a negligible change in the concentration of gelatin salt formed. The experiments were not carried beyond a  $\text{pH}$  of 12.0 on account of the lack of reliable indicators for that region, and on account of the fact that alkali causes chemical changes in the gelatin.

It should be pointed out that the maximal swelling of gelatin in alkalies was less than that in acids. This was not observed in the osmotic pressure curves.

## *II. Relative Solubility of Different Gelatin Salts in Mixtures of Water and Alcohol.*

When powdered gelatin is brought to the isoelectric point, melted, and made into a 1 per cent solution it is at first transparent. After some time, which is the shorter the lower the temperature, the gelatin solution becomes opaque; and in the course of weeks or months it may settle in the form of a precipitate. This, however, does not happen in each case, possibly for the reason that the precipitation will occur only at a very definite  $\text{pH}$ , while, with a slight deviation from this point in either direction, the result will be only an opacity at room temperature. Raising of the temperature will again result in the clearing of the opacity. The opacity seems therefore to be due to the formation of larger aggregates of protein molecules and these will float as long as they are not too large. The setting of the solution to a gel is a different process from this precipitation since no cloudiness or opacity needs to be connected with this latter phenomenon.

When we add to a freshly prepared solution of isoelectric gelatin only a trace of 95 per cent alcohol the cloudiness which would have formed slowly is noticed at once and if we add a little more alcohol we can produce at once a dense precipitate. In order to standardize the degree of cloudiness produced we add so much 95 per cent alcohol to 10 cc. of a 1 per cent solution of isoelectric gelatin in a test-tube of definite diameter until certain letters become illegible when looked at through the test-tube filled with the gelatin-alcohol-water mixture. Since the addition of alcohol to the watery solution raises the temperature and since this has the tendency to diminish the degree of

opacity of the mixture it was necessary to dip the test-tube in ice water during the process of mixing and keep the gelatin solution approximately at 10°C.

When we prepare gelatin chloride by adding small quantities of HCl to isoelectric gelatin we need the more alcohol the lower the pH and very soon a limit is reached when the addition of 25 cc. or more alcohol no longer brings about any precipitate or even cloudiness. Thus 10 cc. of isoelectric gelatin required 2 cc. of 95 per cent alcohol to bring about that high degree of opacity at which the test letters were no longer legible. When the pH of the gelatin was lowered to 4.55

TABLE IV.

Cc. of 95 per cent alcohol required to bring 10 cc. of 1 per cent gelatin-salt solution to standard opacity.										
	pH of gelatin-acid salt.									
	4.6	4.55	4.45	4.4	4.2	3.75	3.3	3.1	2.6	2.1
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Gelatin chloride.....		5.5	∞	∞	∞	∞	∞	∞	∞	∞
“ oxalate.....	5.3	18.1		∞	∞	∞	∞	∞	∞	∞
“ phosphate.....		28.0		∞	∞	∞	∞	∞	∞	∞
“ tartrate.....		11.6		∞	∞	∞	∞	∞	∞	∞
“ succinate.....		3.0	5.4	∞	∞	∞	∞	∞	∞	∞
“ citrate.....		4.2		∞	∞	∞	∞	∞	∞	∞
“ acetate.....		2.7	20.0	∞	∞	∞	∞	∞	∞	∞
“ monochloracetate.....		4.1	∞	∞	∞	∞	∞	∞	∞	∞
“ dichloracetate.....		5.9	∞	∞	∞	∞	∞	∞	∞	∞
“ trichloracetate.....	2.9			∞	∞	∞	∞	∞	∞	∞
“ sulfate.....		6.8		9.0	11.2	12.8	14.6	14.4	13.2	13.3

by the addition of HCl, 5.5 cc. of alcohol were required for the same degree of opacity. When the pH of the 1 per cent gelatin chloride solution was only a trifle lower, namely 4.50, the addition of 25 cc. of alcohol or more did not suffice for bringing about the degree of opacity required for our test; only a lower degree of turbidity resulted. A gelatin chloride solution of pH 4.45 remained perfectly clear (with a bluish tint) regardless of how much alcohol was added. We may say that gelatin chloride becomes soluble in an alcohol-water mixture containing more than 75 per cent alcohol as soon as its pH is  $\geq$  4.45.

It seemed of interest to compare the relative solubility of other gelatin-acid salts with that of gelatin chloride. Table IV gives the

result. The figures indicate the number of cc. of 95 per cent alcohol which when added to 10 cc. of 1 per cent gelatin solution brings about the standard degree of opacity. When the addition of 30 cc. or more alcohol to 10 cc. of the 1 per cent solution of gelatin-acid salt leaves the solution perfectly clear we indicate this by the sign  $\infty$ .

The result (which agrees with the results of a previous publication by the writer<sup>5</sup>) is unequivocal: all those gelatin-acid salts in which the anion in combination with gelatin is monovalent can no longer be precipitated by 95 per cent alcohol when the pH is  $\geq 4.4$ ; while the only gelatin-acid salt in combination with a bivalent anion, namely gelatin sulfate, can be precipitated at any pH down to 2.0 (or even below). The relative solubility of gelatin-acid salts in alcohol shows,

TABLE V.

Cc. of 95 per cent alcohol required to bring 10 cc. of 1 per cent gelatin-salt solution to standard opacity.								
	pH of metal gelatinate.							
	4.9	5.0	5.4	6.4	9.6	10.2	11.4	12.0
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Li gelatinate.....	2.3	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$
Na " .....	2.0		$\infty$	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$
K " .....	1.9	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$
NH <sub>4</sub> " .....	1.9	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$	$\infty$
Ca " .....		2.8	4.4	8.2	8.2	10.5	12.0	7.2
Ba " .....		2.1	4.2	6.6	7.9	8.1	5.9	5.3

therefore, the same influence of the valency (and lack of influence of the nature of the anion) which we have found in connection with the other properties of proteins like swelling, osmotic pressure, and viscosity.

The same agreement exists in regard to metal gelatinates. 10 cc. of a 1 per cent solution of Li, Na, K, and NH<sub>4</sub> gelatinate can no longer be precipitated by the addition of 95 per cent alcohol when the pH is  $\geq 5.0$ . The deviation from the isoelectric point is minute. 10 cc. of 1 per cent Ba and Ca gelatinate, however, can be precipitated with comparatively small quantities of 95 per cent alcohol at any pH (Table V).

<sup>5</sup> Loeb, J., *J. Biol. Chem.*, 1918, xxxiv, 489.

We should expect that when the hydrogen ion concentration of a gelatin chloride solution becomes very high its solubility in an alcohol-water mixture will be diminished again. This is indeed the case, and happens when in 100 cc. of 2 per cent solution of isoelectric gelatin are contained 30 or 40 cc. of  $M/1$  HCl. When to 5 cc. of such a solution are added 25 or 20 cc. of 95 per cent alcohol, the turbidity occurs again. When 100 cc. of the solution contain 50 cc. of  $M/1$  HCl only 14.7 cc. of 95 per cent alcohol are required.

The same result was obtained with Na gelatinate which can also be precipitated again by alcohol when its pH exceeds 12 or 13.

The fact that the gelatin-acid salts (with the exception of gelatin sulfate) become completely soluble in alcohol when the pH reaches the low value of 4.4 is not easy to harmonize with the hypothesis of Pauli that this is due to the ionization of the gelatin, since the relative amount of ionized gelatin is exceedingly small at pH 4.4.

The experiments on the relative solubility of different gelatin salts therefore show the same influence of the valency of the ion in combination with gelatin as was shown in regard to the other physical properties of proteins.

### *III. Conductivity and Ionization of Gelatin Solutions.*

The influence of ions on the conductivity of protein solutions should run parallel to the influence on swelling, viscosity, and osmotic pressure, if it be true that these properties depend on the concentration of the protein ions in the solution. According to this theory, first proposed by Laqueur and Sackur<sup>6</sup> and elaborated by Pauli,<sup>7</sup> the values for the physical properties of proteins are a minimum at the isoelectric point for the reason that the ionization of the protein molecules is a minimum at that point. When we add acid, *e.g.* HCl, protein chloride is formed which is highly ionized and the increase in the viscosity, swelling, and osmotic pressure with the increase of acid is explained by the ionization theory on the assumption of an increase in the concentration of the protein ions in the solution. When, however, too much acid is added, *i.e.* as soon as the pH of the gelatin solution

<sup>6</sup> Laqueur, E., and Sackur, O., *Beitr. chem. Physiol. u. Path.*, 1903, iii, 193.

<sup>7</sup> Pauli, W., *Kolloidchemie der Eiweisskörper*, pt. 1, Dresden and Leipsic, 1920.

falls below 3.3, the swelling, osmotic pressure, and viscosity of the solution diminish again upon the addition of further acid. This would be explained by the ionization theory on the assumption that the concentration of ionized protein in the solution reaches a maximum at a pH of about 3.3, and that a further increase of acid lowers the concentration of ionized gelatin in the solution. The same theory should also explain the fact that the curves for the physical properties of gelatin salts with a bivalent ion are so much lower than the gelatin salts with a monovalent ion by the assumption that the latter are more highly ionized than the former.

We can determine the concentration of ionized gelatin in solution with the aid of conductivity measurements of the solution of a gelatin salt, *e.g.* gelatin chloride, if we deduct the conductivity of the free HCl in the solution from the total conductivity of the gelatin solution, since our gelatin solutions contain no other electrolyte except the free acid, *e.g.* HCl, and the gelatin salt; *e.g.* gelatin chloride. This is proved by the fact that at the isoelectric point our gelatin solutions had practically the conductivity zero (Figs. 4, 6, 8, and 9). Our method of procedure was as follows: doses of 1 gm. of powdered gelatin were brought to the isoelectric point and to each gram of isoelectric gelatin were added different quantities of 0.1 N acid or alkali and some water; the mass was melted by heating to 40° and then so much H<sub>2</sub>O was added that the volume of the solution was 100 cc. After that the pH of the gelatin solution and the conductivities were determined.

Fig. 4 gives the curves for such measurements in the case of gelatin chloride. The abscissæ are the pH, the ordinates the specific conductivities multiplied by 10<sup>4</sup>. The curve to the right is the total specific conductivity  $\times 10^4$  of the gelatin chloride solution of different pH. The curve to the left represents the measurements of the specific conductivities  $\times 10^4$  of pure HCl solutions (without gelatin) for different pH. By deducting the ordinates of this latter curve from the ordinates of the curve for total conductivity we get the curve in the middle representing the specific conductivity  $\times 10^4$  of the pure gelatin chloride solution. Since it had been shown before that the viscosity of the solution does not influence the conductivity in this case (Hardy,



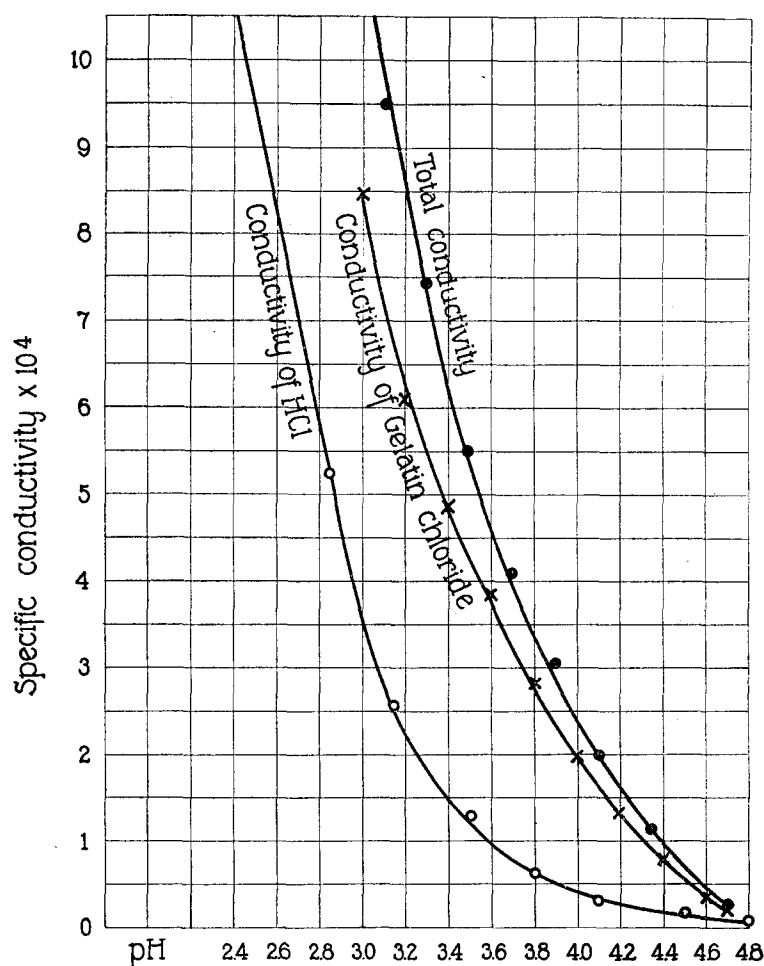


FIG. 4.

FIGS. 4 and 5. Specific conductivity of gelatin chloride solutions of different pH (but all 1 per cent in regard to isoelectric gelatin). Abscissæ are the pH, ordinates specific conductivity  $\times 10^4$ . Total conductivity means specific conductivity  $\times 10^4$  of the gelatin solution measured directly. From this is to be deducted the specific conductivity of HCl of the same pH as the gelatin solution, to obtain the real curve for the specific conductivity of gelatin chloride.

Loeb, Northrop<sup>8</sup>), we may conclude that the middle curve represents the specific conductivity of the gelatin chloride solution and that it

<sup>8</sup> Northrop, J. H., *J. Gen. Physiol.*, 1919-20, ii, 605.

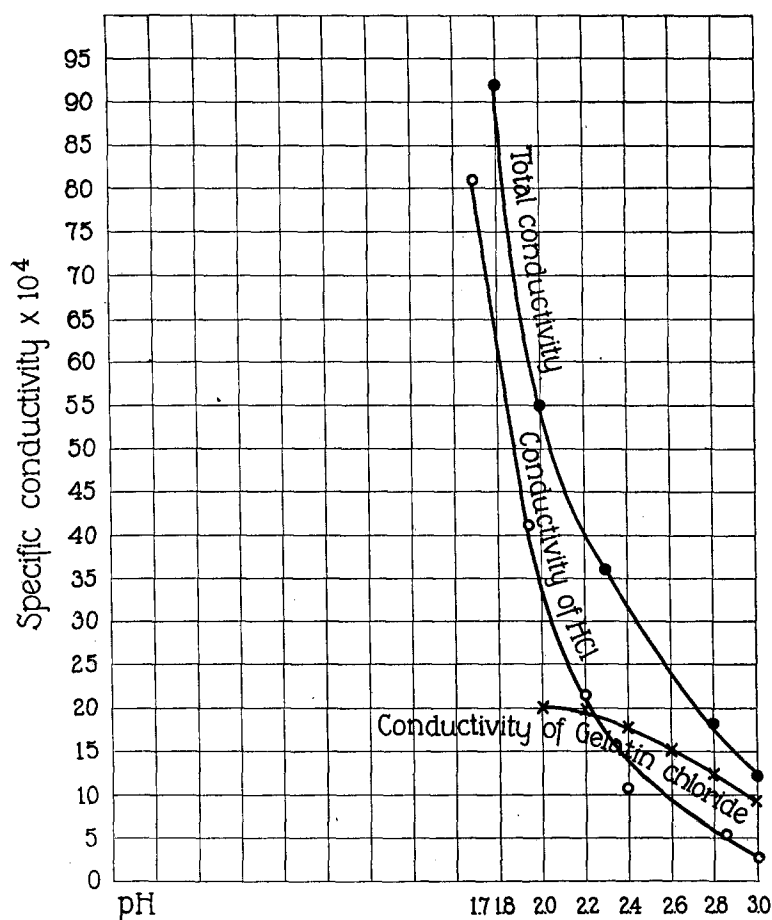


FIG. 5.

can hence be used as a measure of the concentration of the ionized gelatin in the solution. Fig. 4 shows that the curve for the conductivity of gelatin chloride rises continually with increasing hydrogen ion concentration. Fig. 5 is a completion of Fig. 4 for pH down to 2.0. (The ordinates are on a smaller scale in Fig. 5 than in Fig. 4.) It is obvious that at no time does the conductivity curve for gelatin chloride, *i.e.* the curve representing the concentration of ionized protein, show the drop observed in the curves representing the other properties of proteins.

Figs. 6 and 7 show that the same is true for the conductivity curve for gelatin sulfate; Fig. 6 gives the specific conductivities for pH 4.7 to 3.0, and Fig. 7 for pH 3.0 to 2.2 (the ordinates in Fig. 7 are on a

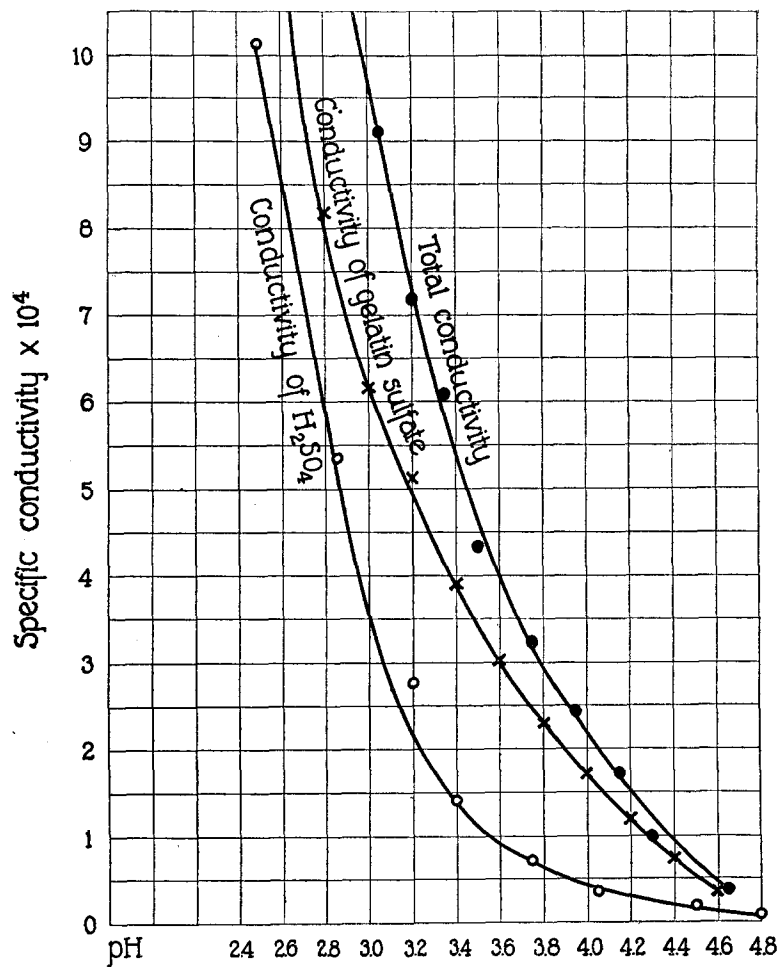


FIG. 6.

FIGS. 6 and 7. Conductivity curves for gelatin sulfate. See legend for Figs. 4 and 5.

smaller scale than in Fig. 6). Experiments on the conductivity of gelatin acetate, trichloracetate, phosphate, and oxalate all give a

similar result. These experiments do not support the hypothesis that the drop in the curves for viscosity, swelling, and osmotic pressure of gelatin-acid salts at or near pH 3.3 is due to a corresponding drop in the degree of ionization of the gelatin salts mentioned.

No drop was discovered in the conductivity curves for metal gelatinates (Na gelatinate, Fig. 8, and Ba gelatinate, Fig. 9).

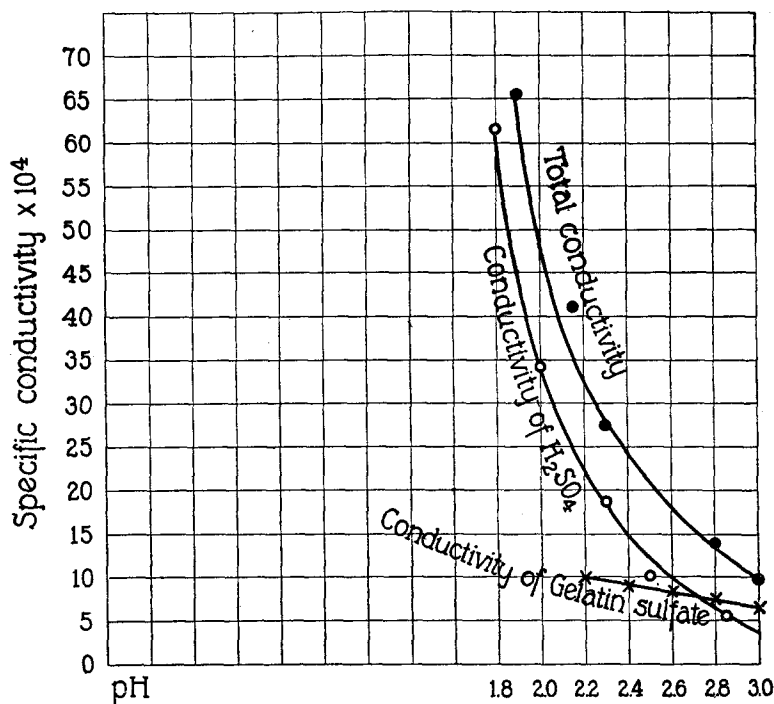


FIG. 7.

The question now arises whether we can explain the difference in the swelling, osmotic pressure, and viscosity of gelatin sulfate on the one hand and gelatin chloride and oxalate, etc., on the other hand on the basis of the ionization theory. If the ionization theory is correct the conductivity of gelatin oxalate, and of gelatin chloride should be twice or almost two and one-half times as great as that of gelatin sulfate. Yet Table VI shows that there is very little difference

between the conductivities of gelatin oxalate and gelatin sulfate; and also a difference of only 20 per cent between gelatin sulfate and gelatin chloride at pH 3.7. As a matter of fact the difference in conduc-

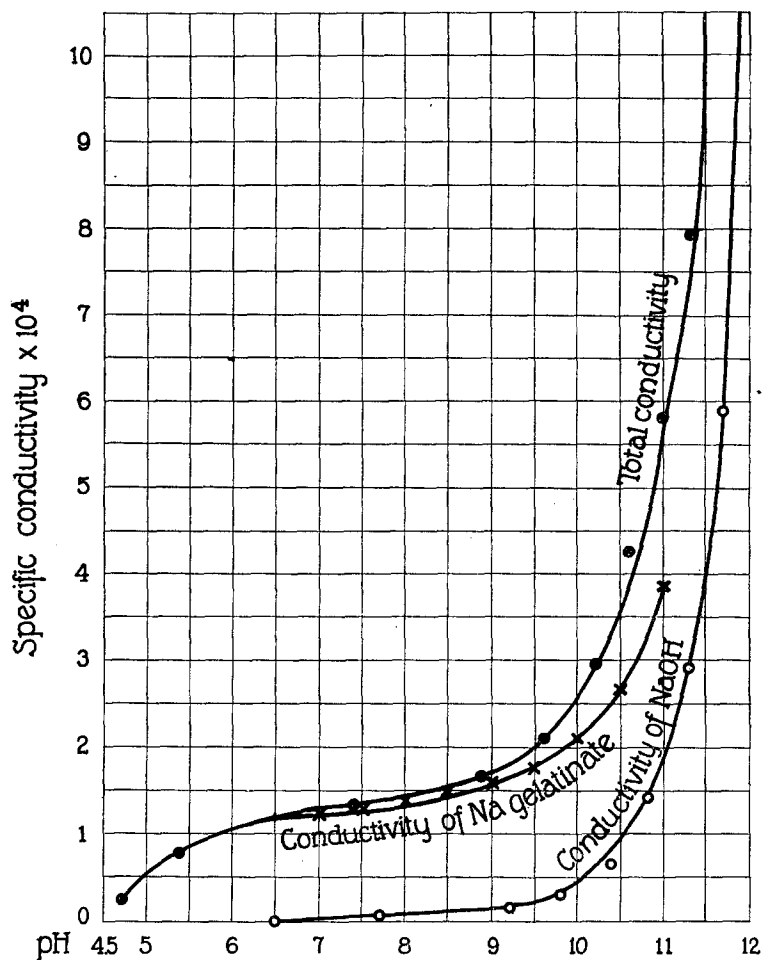


FIG. 8. Conductivity curve for Na gelatinate.

tivity between gelatin oxalate and gelatin chloride which show equal swelling, viscosity, and osmotic pressure is greater than the difference in conductivity between gelatin chloride and gelatin sulfate which are so enormously different in regard to swelling, osmotic pressure, etc.

The three salts, gelatin chloride, sulfate, and oxalate were chosen, since the ionic mobilities of  $\text{Cl}$ ,  $\frac{1}{2} \text{SO}_4$ , and  $\frac{1}{2}$  oxalate are so nearly alike.

It had been pointed out by the writer in a previous paper that the difference in conductivities of Na and Ba gelatinate and of gelatin

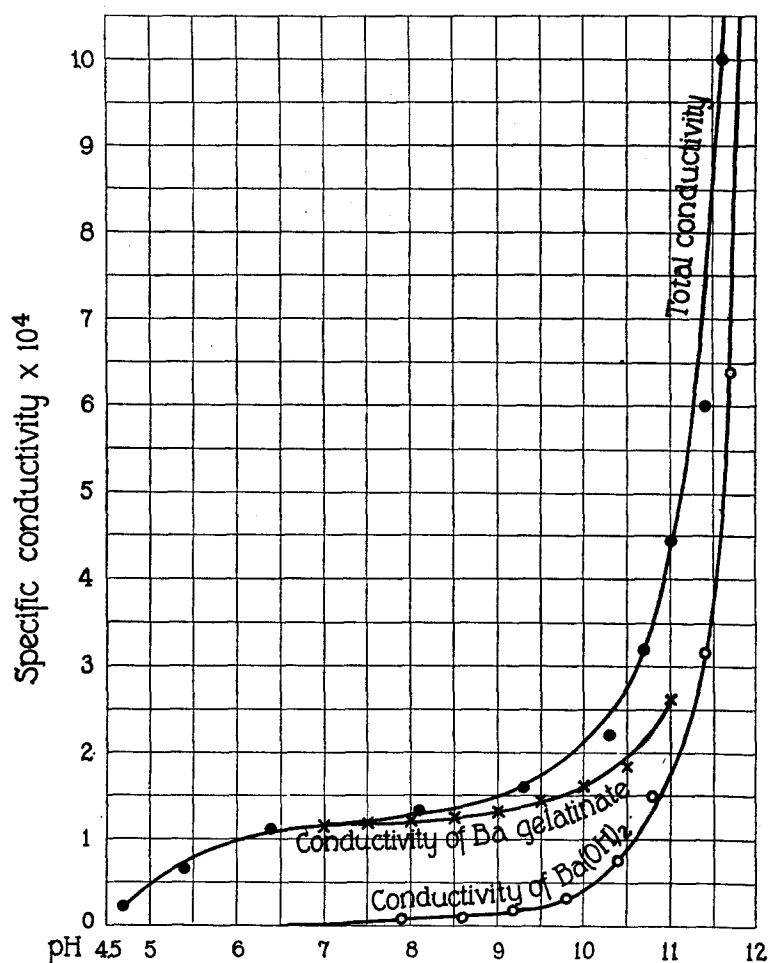


FIG. 9. Conductivity curve for Ba gelatinate.

bromide and gelatin sulfate is too small to account for the difference in the osmotic pressure of solutions of these two types of gelatin salts on the basis of differences in the ionization of the two protein salts.<sup>9</sup>

<sup>9</sup> Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 483, 569.

These data lend no support to the assumption that the difference between the swelling, viscosity, and osmotic pressure of gelatin sulfate on the one hand, and of gelatin chloride and gelatin oxalate on the other is due to differences in the degree of ionization of proteins.

TABLE VI.

*Specific Conductivity of 1 per cent Solutions of Gelatin Chloride, Gelatin Sulfate, and Gelatin Oxalate.*

	pH of gelatin-acid salt.				
	4.4	4.0	3.7	3.4	3.0
Gelatin oxalate.....	0.65	1.45	2.15	3.15	5.25
“ sulfate.....	0.75	1.75	2.60	3.95	6.15
“ chloride.....	0.8	2.0	3.25	4.85	8.5

## SUMMARY AND CONCLUSIONS.

1. Our results show clearly that the Hofmeister series is not the correct expression of the relative effect of ions on the swelling of gelatin, and that it is not true that chlorides, bromides, and nitrates have “hydrating,” and acetates, tartrates, citrates, and phosphates “dehydrating,” effects. If the pH of the gelatin is taken into consideration, it is found that for the same pH the effect on swelling is the same for gelatin chloride, nitrate, trichloracetate, tartrate, succinate, oxalate, citrate, and phosphate, while the swelling is considerably less for gelatin sulfate. This is exactly what we should expect on the basis of the combining ratios of the corresponding acids with gelatin since the weak dibasic and tribasic acids combine with gelatin in molecular proportions while the strong dibasic acid  $H_2SO_4$  combines with gelatin in equivalent proportions. In the case of the weak dibasic acids the anion in combination with gelatin is therefore monovalent and in the case of the strong  $H_2SO_4$  it is bivalent. Hence it is only the valency and not the nature of the ion in combination with gelatin which affects the degree of swelling.

2. This is corroborated in the experiments with alkalis which show that LiOH, NaOH, KOH, and  $NH_4OH$  cause the same degree of swelling at the same pH of the gelatin solution and that this swell-

ing is considerably higher than that caused by  $\text{Ca}(\text{OH})_2$  and  $\text{Ba}(\text{OH})_2$  for the same pH. This agrees with the results of the titration experiments which prove that  $\text{Ca}(\text{OH})_2$  and  $\text{Ba}(\text{OH})_2$  combine with gelatin in equivalent proportions and that hence the cation in combination with the gelatin salt with these two latter bases is bivalent.

3. The fact that proteins combine with acids and alkalies on the basis of the forces of primary valency is therefore not only in full agreement with the influence of ions on the physical properties of proteins but allows us to predict this influence qualitatively and quantitatively.

4. What has been stated in regard to the influence of ions on the swelling of the different gelatin salts is also true in regard to the influence of ions on the relative solubility of gelatin in alcohol-water mixtures.

5. Conductivity measurements of solutions of gelatin salts do not support the theory that the drop in the curves for swelling, osmotic pressure, or viscosity, which occurs at a pH 3.3 or a little less, is due to a drop in the concentration of ionized protein in the solution; nor do they suggest that the difference between the physical properties of gelatin sulfate and gelatin chloride is due to differences in the degree of ionization of these two salts.