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# THE INFLUENCE OF NEUTRAL SALTS, BASES, AND ACIDS ON THE PRECIPITABILITY OF GELATIN BY ALCOHOL.

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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## I.

It has been shown in the previous publications<sup>1</sup> that the effect of neutral salts on gelatin is repressed in the presence of the salt and that we can study such effects with any profit only when the excess of salt is washed away after it has had a chance to act on the protein. Such gelatin reveals qualities which are masked in the presence of the salt and which allow us to gain a clear insight into the effect of the salt. The first striking result was that contrary to the current belief the cation of the salt was found to be the active ion, while the anion did apparently not act at all. All salts with univalent cation and bivalent anion (type  $\text{Na}_2\text{SO}_4$  or  $\text{K}_2$  oxalate) had exactly the same effect as all the salts of the type  $\text{NaCl}$  or  $\text{NH}_4\text{Cl}$  (univalent cation and univalent anion) when the cation concentration was the same in solutions of both salts. While the salts with univalent cation caused an additional swelling and an increase in viscosity of the gelatin after the salt was washed away, the salts with bivalent cation caused little or no such additional swelling and no or only a slight increase in viscosity.

This difference in the effect of univalent and bivalent cations, as well as the repressing effect of the presence of the salt, was explained on the assumption that the neutral salts form with gelatin metal gelatinates which dissociate electrolytically into a negative gelatin ion and a positive cation (that of the salt used), while the anion either does not combine at all with the gelatin

<sup>1</sup> Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343; 1918, xxxiii, 531; xxxiv, 77, 395.

or forms a non- or less dissociable compound. Univalent cations, like Na, form gelatinates which are highly dissociable electrolytically, while the bivalent metals form non- or less dissociable compounds. The presence of the salt represses the electrolytic dissociation of the metal proteinate formed and this repressing effect makes it necessary to remove the excess of salt if we wish to study the effect of the salt on the protein, since many of these effects are due to the ionizing action of the salt on the protein as a consequence of the formation of metal proteinates.

It was pointed out that these results lead to a considerable simplification of protein chemistry. According to our results, gelatin behaves like a weak acid, which, if in contact with a neutral salt, will exchange some of its H ions for the cations of the salt. The anions of the salt do not enter into combination with the gelatin or if they do their effect is not noticeable. When, however, we treat gelatin with a comparatively low concentration of a strong acid, *e.g.* M/100 HCl, gelatin chloride or hydrochloride is formed which should be able to exchange its anion when treated with a neutral salt, while the cation of the latter should not enter into any reaction with the gelatin, or if it does its effect should not be noticeable. This was found to be the case.  $\text{NaNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$  act alike upon gelatin chloride when their  $\text{NO}_3$  concentration is the same, and if the Ca and Na enter into combination with the gelatin chloride their influence is not noticeable. This contradicts the current statement, especially advocated by Pauli,<sup>2</sup> that the action of a neutral salt upon a protein is the algebraic sum of the opposite effects of the anion and cation of the salt, used.

In this paper we wish to test our views further by investigating the effects of a treatment of gelatin by neutral salts upon the precipitability of the gelatin by alcohol. We have occasionally mentioned such investigations but have not yet published any systematic results.

The procedure was as follows. 1 gm. of Cooper's gelatin (grain size between sieve 50 and 60) was put into each of a series of beakers containing 100 cc. of various concentrations of a neutral salt, usually from M/4 or M/2 down to M/2048.<sup>3</sup> One beaker with

<sup>2</sup> Pauli, W., *Beitr. chem. Physiol. u. Path.*, 1903, iii, 225.

<sup>3</sup> If the concentration of the salt is too high it may dissolve some of the gelatin.

distilled water served as a control. The gelatin powder remained in the beaker for 1 hour and was repeatedly stirred during this time. After 60 minutes the gelatin was poured into a cylindrical funnel and the salt solution was allowed to drain off. Then each cylinder was perfused six times with 25 cc. of  $H_2O$  to wash off all the salt solution contained in the capillary spaces between the granules.<sup>4</sup> In the washing the additional swelling appeared which has been discussed in the previous papers. The mass of gelatin was then liquefied by heating for about 10 minutes in a water bath of  $50^\circ C.$ , and the amount of 95 per cent alcohol was ascertained which was required to precipitate 5 cc. of a 1 per cent solution of such gelatin at about  $20^\circ C.$  The method here described has to be followed minutely if the same results as are described in this paper are to be obtained. Differences in the temperature and time of exposure of the gelatin to the salt solution, as well as differences in the amount of washing with distilled water, influence the results.

It is of interest to compare the quantity of alcohol required for precipitation of washed and non-washed gelatin (Table I). The sign  $\infty$  means that the gelatin can no longer be precipitated or that no end-point can be reached with the addition of 20 cc. or more of alcohol.

The reader will notice at a glance the striking difference between the alcohol precipitability of gelatin in the presence of the salt and after the salt is washed away. In the latter case we have a striking *critical point*, which is identical for NaCl and LiCl, namely at  $M/128$ , where the gelatin suddenly becomes non-precipitable, while at the next lower concentration ( $M/256$ ) comparatively little alcohol was required for precipitation.

No such critical point exists when we test the alcohol precipitability of the 1 per cent gelatin solution in the presence of the salt with which it had been treated. The quantities of alcohol required rise slowly with the concentration of the salt, but the differences are too small to select definite values as standards of comparison for the effect of different salts on the gelatin.

<sup>4</sup> On account of the hydrolytic dissociation of gelatin salts an excessive washing will finally lead to the formation of pure gelatin again. The washing, therefore, must be restricted to a removal of the free solution of the original electrolyte existing in the capillary spaces between the granules.

Experiments with acids and alkalis have led Pauli and his pupils to the conclusion that when the gelatin is sufficiently ionized it becomes non-precipitable by alcohol.<sup>5</sup> If we apply this to our experiments with neutral salts we may draw the conclusion that if gelatin is put for 1 hour into a solution of NaCl or LiCl of  $m/128$  or above, it is ionized to such an extent that it ceases to be precipitable by alcohol, provided the excess of salt is washed away, but that if the salt is not washed away the ionization of the sodium or lithium gelatinates is repressed on account of the common ion; hence the gelatin remains precipitable in the presence of the salt.

Table I indicates still another fact worth mentioning; namely,

TABLE I.

		Cc. 95 per cent alcohol required for precipitation of 5 cc. 1 per cent solution of gelatin (at 20°C.) previously treated for 1 hour with												
		M/1	M/2	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	H <sub>2</sub> O
In presence of salt.	NaCl	10.0	8.6	7.4	6.2	5.3	4.1	3.9	4.1	3.4	3.3	3.3	3.4	3.3
	LiCl	11.05	10.45	8.7	7.55	6.65	6.9	5.8	4.55	5.15	3.55	3.25		3.6
In absence of salt (washed six times with H <sub>2</sub> O).	NaCl	∞	∞	∞	∞	∞	∞	∞	∞	6.6	5.8	5.45		5.4
	LiCl	∞	∞	∞	∞	∞	∞	∞	∞	7.6	6.5	6.8	5.6	5.35

that if we perfuse our powdered gelatin six times with 25 cc. of distilled water after it has been kept for 1 hour in 100 cc. of H<sub>2</sub>O, it requires more alcohol for precipitation, namely about 5.5 cc., than when we soak it merely for 1 hour in distilled water; in the latter case it can be already precipitated by about 3.5 cc. of alcohol. The difference is due to the traces of salt which the gelatin contains in the form of impurities and which are removed by sufficient perfusion with distilled water after it has been kept for 1 hour in distilled water. These impurities repress the ionization of the gelatin.

<sup>5</sup> Pauli, *Fortschr. naturwiss. Forschung*, 1912, iv, 223.

The reader will notice that the washed gelatin serving as control has about the same alcohol number in all the experiments reported in this paper. This alcohol number of washed gelatin indicates that normally a certain amount of ionization of gelatin into a negative gelatin ion and a positive hydrogen ion exists since we shall see later that if we add acid to make the gelatin isoelectric a 1 per cent solution precipitates spontaneously on standing at a low temperature and little or no alcohol is required to bring the precipitation to the standard used in these experiments (see Table IV).

Fenn<sup>6</sup> has recently published a series of careful investigations on the influence of electrolytes upon the precipitation of gelatin by alcohol, but all his experiments as well as those of his predecessors on the same effect were made on gelatin in the presence of an excess of electrolytes used. As long as the experiments are made with very dilute acids or alkalies no serious error results, but in the case of higher concentrations of electrolytes (and in the case of salts higher concentrations are required) this method must give ambiguous results since the ionization of the gelatin will be repressed by the presence of the electrolyte. In our experiments this excess of electrolytes was washed away after they had had a chance to act on the gelatin and our experiments have led to entirely different results (Table II).

The reader will see from Table II that under our method of procedure all salts with univalent cation and univalent anion (type NaCl) render the gelatin solution non-precipitable in concentration of  $m/128$  or slightly above (after the salt is washed away). For the majority of cases the critical concentration was  $m/128$ .

All salts with univalent cation and bivalent anion (type  $Na_2SO_4$ ) make the gelatin solution non-precipitable with alcohol at exactly half the molecular concentration of salts of the type NaCl; namely,  $m/256$  or slightly above. For all with the exception of one salt the value was  $m/256$ . This proves that the effect of the salt is determined exclusively by the cation and that the anion does apparently not influence the effect.

When gelatin is treated with neutral salts of the alkali earth

<sup>6</sup> Fenn, W. O., *J. Biol. Chem.*, 1918, xxxiii, 279, 439; xxxiv, 141, 415.

TABLE II.

Type of salt.	Nature of salt.	Cc. 95 per cent alcohol required for precipitation of 5 cc. 1 per cent solution of gelatin (at about 20°C.) previously treated for 1 hour with 100 cc. of one of the following solutions and then freed from the excess of salt by perflusing six times with 25 cc. H <sub>2</sub> O.												
		M/1	M/2	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	H <sub>2</sub> O
I. Uni- valent cation, uni- valent anion.	NaCl....	8	8	8	8	8	8	8	8	6.6	5.8	5.45		5.4
	KCNS...									6.8	6.0	5.6		5.5
	LiBr....	8	8	8	8	8	8	8	8	7.3	6.2	6.0		5.7
	NH <sub>4</sub> Cl....	8	8	8	8	8	8	8	8	9.6	6.0	5.5	5.5	5.35
	LiCl....	8	8	8	8	8	8	8	8	7.6	6.5	6.8	5.6	5.35
	KCl....			8	8	8	8	8	8	8.6	6.2	4.8	5.1	5.0
	LiNO <sub>3</sub> ...			8	8	8	8	8	8	9.8	6.2	5.8	5.4	5.45
	Na acetate....	8	8	8	8	8	8	8	12.4	7.15	5.8	5.7	5.4	5.6
II. Uni- valent cation, bi- valent anion.	Na <sub>2</sub> SO <sub>4</sub> ...		8	8	8	8	8	8	8	8	7.5	5.5		5.5
	Na <sub>2</sub> oxalate....				8	8	8	8	8	8	8	6.8	6.3	5.85
	Na <sub>2</sub> tartrate...	8	8	8	8	8	8	8	8	8.0	6.6		5.7	
	Na <sub>2</sub> succinate.	8	8	8	8	8	8	8	8	8.1	6.15	5.8	5.5	
	K <sub>2</sub> SO <sub>4</sub> ....			8	8	8	8	8	8	8	6.85	6.0	5.7	5.45
	Li <sub>2</sub> SO <sub>4</sub> ...			8	8	8	8	8	8	8.0	6.6	5.3		4.8
III. Bi- valent cation, uni- valent anion.	MgCl <sub>2</sub> ...	6.2	6.3	7.05	7.25	7.6	7.4	7.4	6.2	6.7	5.3	5.8	5.6	5.4
	CaCl <sub>2</sub> ....			5.1	4.9	5.0	5.3	5.0	5.1	5.3	5.3	5.4	5.4	5.4
	SrCl <sub>2</sub> ....		5.3	4.95	5.2	5.6	6.0	5.8	5.5	5.5	5.5	5.5	5.4	5.4
	BaCl <sub>2</sub> ....			4.45	4.8	5.6	4.1	4.7	4.6	4.3	5.0	5.2	6.0	5.6
IV. Bi- valent cation, bi- valent anion.	MgSO <sub>4</sub> ...		6.3	6.5	6.3	6.4	6.6	6.4	6.4	5.5	5.5	5.1	5.1	5.1

metals the gelatin remains precipitable with alcohol after the salt is washed away no matter in what concentration the salt had been used.

A study of the alcohol numbers of the gelatin first treated with the salts of alkali earth metals and then freed from the excess of salt by washing with distilled water shows that only after the treatment with  $MgCl_2$  and  $MgSO_4$  does the alcohol number rise slightly beyond that of gelatin washed with water but not previously treated with salt (under  $H_2O$  in Table II). Hence Mg is the only metal of this group which leads to a slight increase in ionization. It is also worth noticing that  $MgCl_2$  and  $MgSO_4$  act quantitatively alike, thus supporting our statement that the influence of the anion of the neutral salt does not make itself felt in these experiments.

These experiments on alcohol precipitation thus confirm the conclusion, drawn from our experiments on swelling and viscosity, that only the cation of the neutral salts influences the gelatin and that there is a typical difference in the action of univalent and bivalent cations.

The results can be best explained on the assumption that the neutral salts form with gelatin (and perhaps with all proteins which are stronger as acid than as base) metal proteinates, which are highly ionizable when the metal is univalent (Na, Li, K,  $NH_4$ ) and much less ionizable when the salt used is bivalent (Mg, Ca, Sr, Ba).

## II.

If this assumption is correct it follows that treatment of gelatin with solutions of NaOH or KOH should act exactly like treatment with solutions of neutral salts, leading to the production of highly ionizable Na or K gelatinates no longer precipitable with alcohol, and that this ionization should become manifest after the excess of the alkali has been washed away by the repeated perfusion of the powdered gelatin with distilled water.

It would also follow that treatment of the gelatin with  $Ca(OH)_2$  or  $Ba(OH)_2$  should act like the treatment of gelatin with  $CaCl_2$  and  $BaCl_2$ ; *i.e.*, after the base is washed away, the gelatin should remain precipitable no matter what the concentration of the



$\text{Ca}(\text{OH})_2$  or  $\text{Ba}(\text{OH})_2$ .<sup>7</sup> These demands of the theory are fulfilled (Table III). 1 gm. of the finely powdered gelatin was put for half an hour at about 22°C. into 100 cc. of various concentrations of NaOH, KOH,  $\text{Ca}(\text{OH})_2$ , and  $\text{Ba}(\text{OH})_2$ . The gelatin was then put into a funnel, the alkali allowed to drain off, and the gelatin was then perfused four times with 25 cc. of distilled water. After this the gelatin was liquefied and the precipitability of 5 cc. of 1 per cent solution by 95 per cent alcohol was determined at about 20°C. Gelatin which had been previously treated with m/256 or still more dilute KOH or NaOH was precipitable with alcohol, while gelatin treated with m/128 or higher concentrations of these two bases was no longer precipitable with alcohol, after

TABLE III.

Nature of base.	Cc. 95 per cent alcohol required for precipitation of 5 cc. 1 per cent solution of gelatin, at 20°C., previously treated for half an hour with one of the following solutions and then perfused four times with 25 cc. H <sub>2</sub> O.								
	m/16	m/32	m/64	m/128	m/256	m/512	m/1024	m/2048	H <sub>2</sub> O
KOH.....	∞	∞	∞	∞	15.9	7.55	6.4	5.25	5.35
NaOH.....	∞	∞	∞	∞	8.6	6.8	6.2	5.5	5.7
$\text{Ca}(\text{OH})_2$ .....			4.4	5.5	5.3	5.3	5.3	5.9	5.8
$\text{Ba}(\text{OH})_2$ .....		4.3	4.0	4.0	4.3	4.3	5.0	5.4	5.3

the excess of base was washed away. The critical concentration is therefore under the conditions of our experiments the same for NaOH and KOH as for NaCl and KCl.

On the other hand, gelatin treated with weak or strong concentrations of  $\text{Ca}(\text{OH})_2$  or  $\text{Ba}(\text{OH})_2$  did not lose its alcohol precipitability but had the same low alcohol number as gelatin treated with salts of Ca or Ba. No more striking proof of our theory could be expected.

The fact that the alkalis give the same results as the neutral salts confirms our view that the salts combine with the gelatin, forming metal gelatinates, since the combination of alkalis with gelatin can be proved by titration experiments.

<sup>7</sup>The concentrations in which bases can be used are limited by the fact that higher concentrations liquefy the gelatin.

III.

Experiments with acids do not contradict our theory but the agreement is not so perfect as in the case of alkalies. According to our theory all strong monobasic acids should render the gelatin non-precipitable at the same concentration (after the acid is washed away). This seems to be the case, the critical concentration being in the neighborhood of  $M/256$  for HCl,  $HNO_3$ , and trichloroacetic acid (Table IV).

The dibasic acids, like  $H_2SO_4$ , should, however, act like diacidic bases, *e.g.*  $Ca(OH)_2$ , and should not annihilate the alcohol

TABLE IV.

Nature of acid.	Cc. 95 per cent alcohol required for precipitation of 5 cc. 1 per cent solution of gelatin, at 20°C., previously treated for half an hour with one of the following solutions and then perfused four times with 25 cc. $H_2O$ .										
	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	$H_2O$
$HNO_3$ .....		∞	∞	∞	∞	∞	∞	0.7	3.7	4.7	5.1
HCl.....			∞	∞	∞	∞	∞	1.1	4.5		5.3
Trichloroacetic acid..	∞	∞	∞	∞	∞	∞	∞	0.95	3.7	4.95	5.4
$H_2SO_4$ .....				∞	17.0	12.2	10.2	7.1	1.1	4.0	5.5
Tartaric acid.....	∞	∞	∞	∞	∞	∞	∞	6.15	0.9	4.15	5.65
Oxalic acid.....	∞	∞	∞	∞	∞	∞	∞	8.75	1.0	3.4	5.05
Malic acid.....	∞	∞	∞	∞	∞	∞	6.8	1.4	1.4	4.3	5.3

precipitability of gelatin or at least not at low concentrations of the acid. This is true only for  $H_2SO_4$ , and here only partly, but not for the organic dibasic acids, like tartaric, oxalic, and malic. These organic dibasic acids behaved like the monobasic acids (Table IV). The reason for this deviation is not clear. Should it be that the organic acids, like oxalic or tartaric acid, and to a lesser extent also  $H_2SO_4$ , form acid salts with gelatin?

The experiments on acids give us an opportunity to compare our results with those of colloid chemists, especially Pauli. Pauli investigated the effects of acid on proteins but in the presence of the acid. As long as low concentrations of acids are used the error is not so great, but with higher concentrations erroneous

results are unavoidable. Thus Pauli<sup>8</sup> states that if acids are arranged according to their power of influencing in moderate concentrations viscosity and precipitability with alcohol they must be grouped in the following order: trichloroacetic, dichloroacetic, sulfuric, nitric, hydrochloric, monochloroacetic, and acetic acid. A comparison of this statement with the results on gelatin after the excess of acid is washed away (Table IV) shows that Pauli's statement is no longer tenable.

It might be well to point out that our experiments with acids support in another respect the ionization hypothesis very nicely. In these experiments the gelatin was first treated for half an hour at about 20° with 100 cc. of acid and the latter was then washed away by four perfusions with 25 cc. of H<sub>2</sub>O each, as in the experiments with alkalis. The reader will notice that before the gelatin becomes non-precipitable by alcohol it goes through a stage where it can be precipitated with less alcohol than is required for the gelatin not treated with acid. This is due to the fact that gelatin is a stronger acid than base and hence dissociates slightly into H<sup>+</sup> and gelatin. Since gelatin is only a weak acid it requires the addition of only a trace of a second acid to repress this ionization, and for gelatin treated with HCl and then washed four times with 25 cc. of H<sub>2</sub>O this point—the isoelectric point—is reached when the acid used was M/512 (Table IV). It was the same for nitric and trichloroacetic acid. For dibasic acids the isoelectric point was at twice the dilution of that in the case of monobasic acids; namely, M/1024 (Table IV). If more acid is added, a salt formation between acid and gelatin is produced, the acid combining with a NH<sub>2</sub> group of the gelatin, the gelatin salt undergoing stronger ionization. This action of the acid had been noticed and correctly interpreted by Pauli.<sup>5</sup> This observation supports the assumption that the non-precipitability of gelatin treated with electrolytes, acids, alkalis, and neutral salts, is due to ionization of the gelatin.

The fact that the isoelectric point is reached at the same hydrogen ion concentration regardless of the nature of the anion of the acid shows that only the former ion influences the result. Exactly the same is true as far as the alcohol precipitability of

<sup>8</sup> Pauli, *Arch. ges. Physiol.*, 1910, cxxxvi, 483.

gelatin by different monobasic acids is concerned, as our Table IV shows.

The writer wishes to call attention to the fact that a 1 per cent gelatin solution near the isoelectric point becomes on standing at not too high a temperature turbid or completely opaque even if no alcohol is added. This only happens when the gelatin has been treated with acid but not with base or with a neutral salt.<sup>9</sup> Such naturally turbid isoelectric solutions of gelatin are also characterized by a minimum in viscosity, and a minimum in swelling, all of which tends to support the conclusion that these physical qualities are a function of the degree of ionization of the gelatin.

## IV.

Weak acids and bases, *e.g.* acetic acid and  $\text{NH}_4\text{OH}$ , will not cause the gelatin to become non-precipitable. 1 gm. of gelatin

TABLE V.

		Cc. 95 per cent alcohol required for precipitation of 5 cc. 1 per cent solution of gelatin, at 20°C., previously treated for 1 hour with one of the following solutions and then perfused four times with 25 cc. $\text{H}_2\text{O}$ .										
		M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	M/1024	M/2048	$\text{H}_2\text{O}$
Acetic acid.....		6.1	5.5	4.9	4.3	1.8	1.0	0	1.4	3.8	4.7	5.1
$\text{NH}_4\text{OH}$ .....		13.5	8.1	6.3	5.9	5.7	5.8	5.5	5.6	5.5		5.4

was put for 1 hour into beakers containing 100 cc. of different concentrations of these two substances and was afterwards freed from the excess of the solution by four perfusions with 25 cc. of distilled water, as described. It was found that the gelatin so treated remained precipitable by alcohol in all the concentrations used (Table V).

It is noticeable that the values for acetic acid go through a minimum at M/256 while those for  $\text{NH}_4\text{OH}$  do not show such a minimum, as was to be expected.<sup>9</sup>

<sup>9</sup> It is impossible to bring gelatin nearer the isoelectric point with the aid of salts or bases. For amphoteric electrolytes which are stronger acids than bases the isoelectric point is reached when the dissociation of the H ions is repressed to the level of that of the OH ions, and such a result can only be brought about by the addition of acid to the gelatin solution, but not by the addition of a neutral salt. It is, therefore, not correct to state that a salt like  $\text{CaCl}_2$  or  $\text{NaCl}$  can render a protein like gelatin "isoelectric."

The slight effect of acetic acid is not a specific effect of the acetate anion but merely due to the fact that acetic acid is a weak acid, since the weak base  $\text{NH}_4\text{OH}$  shares this peculiarity of the acetic acid. This effect of weak acid and base is a confirmation of our theory, since we assume that electrolytes react in a purely chemical way with gelatin (and proteins in general). Gelatin being a weak acid and a still weaker base, only little gelatin acetate and ammonium gelatinate can be formed with acetic acid and  $\text{NH}_4\text{OH}$  respectively. The mass of the new gelatin salt being small at the best, not as many gelatin ions can be formed as are required to render the gelatin non-precipitable by alcohol.

These negative effects of the treatment of gelatin with weak acid and weak base (after the excess of acid or base is washed away with distilled water) are paralleled by equally negative effects on additional swelling and on viscosity.

#### SUMMARY.

1. The experiments reported in this paper show that if finely powdered gelatin has been treated for 1 hour with the solution of a neutral salt of the type  $\text{NaCl}$  (univalent cation, univalent anion), and if the excess of salt solution is afterwards washed away with  $\text{H}_2\text{O}$ , a 1 per cent solution of such gelatin in distilled water becomes non-precipitable by alcohol when the concentration of the salt solution used exceeds a certain limit, which is about  $m/128$ .

2. When the neutral salt used is of the type  $\text{Na}_2\text{SO}_4$  (univalent cation, bivalent anion) the critical molecular concentration which renders the 1 per cent gelatin solution under the conditions of our experiments non-precipitable by alcohol is exactly one-half of that for salts of type  $\text{NaCl}$ ; namely, about  $m/256$ .

3. When the powdered gelatin is treated with solutions of neutral salts of the type  $\text{CaCl}_2$  or  $\text{MgSO}_4$  (*i.e.* bivalent cation, univalent or bivalent anion) and the excess of salt is washed away the 1 per cent gelatin solution in distilled water remains precipitable with alcohol for all concentrations of the salt used.

4. These experiments support the conclusion reached in the former papers of the writer that only the cation of the salt influences the gelatin.

5. The experiments also support the suggestion made in the writer's former papers that the neutral salts act upon proteins,

which, like gelatin, are stronger acids than bases, by forming metal proteinate which dissociate electrolytically into a positive metal and a negative gelatin ion. The metal gelatinates with univalent cation (Li, Na, K,  $\text{NH}_4$ ) are highly ionizable, while those with bivalent cation (Mg, Ca, Sr, Ba) are less or not at all ionizable. The anion of the salt used behaves as if it did not combine with the gelatin at all or as if it entered into a non- or less dissociable bondage.

6. These conclusions are supported by experiments with alkalis showing that if we treat powdered gelatin for half an hour with different concentrations of NaOH or KOH, and then wash the excess of alkali away, a 1 per cent gelatin solution in distilled water made from powdered gelatin thus treated is no longer precipitable with 95 per cent alcohol when the concentration of the alkali used was  $\bar{\approx}$   $\text{m}/128$ . When, however,  $\text{Ba}(\text{OH})_2$  or  $\text{Ca}(\text{OH})_2$  is used the gelatin solution in distilled water remains precipitable by alcohol no matter what concentration of the alkali was used. In this latter case, Ca and Ba gelatinate are formed which according to our assumption are not or little dissociable electrolytically; in the former case, Na and K gelatinate are formed which are highly ionizable. The fact that the alkalis give the same results as the neutral salts confirms our view that the salts combine with the gelatin, forming metal gelatinates, since the combination of alkalis with gelatin can be proved by titration experiments.

7. If we treat powdered gelatin for half an hour with different concentrations of strong monobasic acids,  $\text{HNO}_3$ , HCl, trichloroacetic acid, and then wash the excess of acid away, a 1 per cent solution of such gelatin in distilled water becomes (under the conditions of our experiment) non-precipitable with alcohol when the concentration of the acid used was  $\bar{\approx}$   $\text{m}/256$ . When we use dibasic acids, the gelatin should remain precipitable by alcohol if the excess of acid is washed away. This is approximately true for  $\text{H}_2\text{SO}_4$  but is not true for the organic dibasic acids, such as tartaric, malic, and oxalic. A possible explanation of this discrepancy between theory and fact is offered.

8. Weak acid (acetic) and weak base ( $\text{NH}_4\text{OH}$ ) act alike inasmuch as neither renders the gelatin non-precipitable (after the acid or base is washed away) which was to be expected if the effect of electrolytes on gelatin is due to a chemical reaction between the two bodies.