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The Influence of Neutral Salts Upon the Viscosity of Gelatin Solutions

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THE INFLUENCE OF NEUTRAL SALTS UPON THE VISCOSITY OF GELATIN SOLUTIONS.

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I.

When dried pig's bladder or powdered gelatin is treated with a neutral salt a certain swelling occurs; after the salt is washed away a much greater additional swelling is noticed when the cation of the neutral salt used was monovalent. When the cation of the salt used was bivalent no such additional swelling occurs.[1] These phenomena had been explained by the writer on the assumption that neutral salts with monovalent cation (Li, Na, K, NH_4) form with gelatin and certain other proteins highly ionizable metal proteinates which dissociate electrolytically into a positive metal ion (that of the salt used) and a negative protein (gelatin) ion. It is the latter which is responsible for the additional swelling after the excess of the salt is washed away, the ionization of the metal gelatinate being repressed in the presence of the excess of salt. The salts with bivalent cation (Mg, Ca, Sr, Ba) were assumed to form non- or less ionizable metal gelatinates and hence no or little additional swelling of gelatin was observed after the excess of neutral salts of bivalent cations was washed away.[2,3]

The new experiments reported in this note were undertaken with the intention of testing the idea that the difference in the influence of the monovalent and bivalent cations upon the additional swelling is due to a difference in the degree of ionization of the metal gelatinates with monovalent and bivalent cations.

[1] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343.
[2] Loeb, *J. Biol. Chem.*, 1918, xxxiii, 531.
[3] Loeb, *J. Biol. Chem.*, 1918, xxxiv, 77.

It has been pointed out by various authors—Laqueur and Sackur,[4] Hardy,[5] Pauli,[6] Bottazzi,[7] and Robertson[8]—that solutions of ionized protein have a much higher viscosity than solutions of the same protein in non-ionized condition. These statements are based on experiments with acids and bases, but not with neutral salts. If they are correct—as they seem to be—then the measurements of viscosity should offer a means of testing our hypothesis that neutral salts with univalent metal (type NaCl and Na_2SO_4) react with gelatin under formation of highly ionizable metal gelatinates (e.g. sodium gelatinate), while neutral salts with bivalent metal (type $CaCl_2$) form metal gelatinates (e.g. calcium gelatinate) which are not or only slightly capable of ionization.

II.

The method of our procedure was as follows. 1 gm. of finely powdered gelatin was put into 100 cc. of a neutral salt solution, whose concentration was generally m/8, and left in this solution for about 1 hour. The powder was stirred repeatedly in the solution. It was then poured into one of the cylindrical funnels described in the previous papers and the salt solution was allowed to filter off as much as possible. To remove the last traces of salt solution the gelatin mass was then perfused four times with 25 cc. of H_2O. Tests with silver nitrate showed that the water filtering off in the fourth perfusion no longer gave a precipitate when the salt used was a chloride. The removal of the salt solution is necessary since its presence represses the ionization of the metal gelatinate formed. It was the neglect of this precaution which led to the erroneous results of some previous experimenters on the action of neutral salts on proteins. Hence our viscosity measurements were all made with gelatin freed from the excess of the salt (with which it had been treated) *and dissolved*

    [4] Laqueur, E., and Sackur, O., *Beitr. chem. Physiol. u. Path.*, 1903, iii, 193.

    [5] Hardy, W. B., *J. Physiol.*, 1905-06, xxxiii, 251.

    [6] Pauli, W., *Fortschr. naturwiss. Forschung*, 1912, iv, 223.

    [7] Bottazzi, F., *Atti. Accad. Lincei*, 1913, xxii, pt. 2, 141, 263.

    [8] Robertson, T. B., The Physical Chemistry of the Proteins, New York, 1918, 320.

in distilled water (and not in the salt solution with which the gelatin had previously been treated).

It is well known that the viscosity of gelatin solution increases with standing even if the solution remains liquid. This has been ascribed to the fact that the gelatin solution has a similar structure to the solid gelatin,[8] but that this structure is only gradually reached by the liquid. We may say that gelatin solution upon standing changes towards a gel structure though it may never reach the actual gel stage. Since we now know through the work of Langmuir that molecules are not spherical, but that we may have to conceive them in the case of proteins as having a chain structure, we may assume that the steady increase of viscosity of gelatin solution upon standing is due to a gradual orientation of gelatin molecules or ions towards the arrangement they possess in a solid gel. On that assumption we understand also the influence of temperature upon the viscosity of gelatin solutions.

Reproducible measurements of viscosity of gelatin solutions can only be obtained when these two chief influences, time and temperature, are kept equal. Our mode of procedure was as follows.

After the mass of gelatin had been washed free from the salt solution with which it had been treated it was liquefied by heating in a water bath at about 50°C. for about 10 minutes. The solution containing 1 gm. of the dry gelatin was then made up to a volume of 100 cc. by adding the necessary amount of *distilled water* and the temperature of the solution lowered to 24°C. The viscosity was determined *immediately* at that temperature, the Ostwald viscometer being in a water bath of 24°C.

In this way we got comparatively constant and reproducible results. The slight variations observed for the same solution were due to the fact that in some cases the gelatin was liquefied immediately after perfusion while in other experiments we waited until the next morning. In the latter case the viscosity was always lower, possibly on account of some hydrolysis or putrefaction.

The time of outflow of distilled water through our viscometer was 53 seconds at 24°C.

A 1 per cent gelatin solution made up of powdered gelatin washed repeatedly with distilled water gave in different experi-

ments the following times of outflow: 84, 83, 78, 84, 86, 80, 83, 76, 83, 82, 83, and 84 seconds, averaging 82 seconds. We may therefore say, a 1 per cent solution of the gelatin used by us (possessing a negligible degree of electrolytic dissociation) has a viscosity corresponding to a time of outflow of 82 seconds through our viscometer.

It seemed advisable to determine the influence of the concentration of the salt solution used upon the result. For this purpose 1 gm. of finely powdered Cooper's non-bleached gelatin was put for 1 hour into each of a series of beakers containing 100 cc. of a NaCl solution varying from M/2 to M/1024 in concentration. After this the gelatin from each solution was put into a separate cylindrical funnel, drained off, and perfused four times with distilled water.[9] The water running off in the fourth perfusion was practically free from chloride when the gelatin had been treated with an M/8 solution of NaCl. The gelatin was therefore free from salt except that held in chemical combination. Three effects of the neutral salts upon the gelatin were measured, namely:

1. The additional swelling; i.e., the increase in swelling which occurred in the cylindrical column of gelatin after the salt was washed away (and the ionization was no longer repressed). The additional swelling was measured in mm. of the height of the column of gelatin in the cylindrical funnels which all had the same diameter.

2. The viscosity of the washed gelatin. The viscosity was expected to increase parallel with the additional swelling, if both additional swelling and increase in viscosity were due to the same factor; namely, increased ionization of the gelatin. The viscosity measurements are given in times (seconds) of outflow through the viscometer.

3. The amount of 95 per cent alcohol required to precipitate 5 cc. of a 1 per cent gelatin solution (washed) and made up in distilled water. According to Pauli, ionized protein loses its precipitability by alcohol. Hence when the ionization of the gelatin exceeds a certain limit it should cease to be precipitable by alcohol. We assumed that this limit was reached when 25 cc.

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[9] Those treated with M/2 and M/4 NaCl were perfused six times with 25 cc. of distilled water.

or more of alcohol no longer gave a precipitate and we indicate this by the symbol ∞.

The reader will notice that in all cases the measurements were made on gelatin in the *absence* of the salt solution with which it had been treated. As has been stated in this and the previous paper, the measurements in the presence of salts are of restricted value, since the ionizing effect of the salt on the protein is concealed in the presence of the salt.

Table I gives the three groups of measurements.

TABLE I.

1 gm. gelatin treated for 1 hour with the following solutions of NaCl and then freed from the supernatant solution by four perfusions with 25 cc. of distilled water.

	$M/2$	$M/4$	$M/8$	$M/16$	$M/32$	$M/64$	$M/128$	$M/256$	$M/512$	$M/1024$	H_2O (control)
Additional swelling in mm. of the height of the cylindrical column of gelatin..	43	40	41	38	40	30	24	20	9	9	5.5
Viscosity of 1 per cent gelatin solution in H_2O in seconds of time of outflow......................	99	99	98	97	95	93	90	89	87	86	85
Cc. of 95 per cent alcohol required to precipitate 5 cc. 1 per cent gelatin solution in H_2O...............	∞	∞	∞	∞	∞	∞	11.3	6.6	5.7	5.7	5.5

These measurements show that beyond a concentration of M/8 a further increase of concentration does not influence the results materially. We therefore used salts with univalent ions in this concentration, while salts with bivalent ions were generally though not always used in M/16 concentration.

III.

We now proceeded to test our hypotheses, namely:

1. That neutral salts with univalent cation (type NaCl and Na_2SO_4) form with common gelatin highly ionizable gelatin compounds (metal gelatinates).

2. That neutral salts of the alkali earth metals (type CaCl₂) form compounds with gelatin (metal gelatinates) which are only slightly dissociable.

If these hypotheses are correct, we should expect, first, that gelatin treated for 1 hour with an M/8 solution of any neutral salt with univalent cation (Li, Na, K, NH₄) should (when freed from the excess of salt by perfusion with H₂O) have a considerably increased viscosity; *i.e.*, a time of outflow considerably higher than 82 seconds. Moreover, the time of outflow should be exactly the same for all the salts used regardless of the nature of the cation as long as this is univalent, and regardless of the nature of the anion, whether Cl, NO₃, acetate, sulfate, tartrate, succinate, etc.; the only provision being that the salt solution originally applied should always have the same concentration of the univalent cation (M/8 in our experiments). All this turned out as our theory demanded. In our experiments 1 gm. of powdered gelatin was allowed to interact for 1 hour with an M/8 solution of salts of the type NaCl and with M/16 solutions of salts of the type Na₂SO₄ (bivalent anion). When the salt in the capillary spaces of the gelatin granules was washed away with distilled water, the times of outflow were found to be about 97 seconds (varying between 95 and 102), regardless of the nature of the anion or cation (as long as the latter was univalent) of the salt used. This was an increase in the viscosity of about 20 per cent.

Second, if our hypothesis is correct, gelatin treated for 1 hour with M/8 or M/16 solutions of neutral salts of the alkaline earth metals should, when the salt solution in the capillary spaces of the powdered mass is washed away with H₂O, give times of outflow not much higher than those found for common gelatin (82 seconds). This also was found to be the case. Gelatin treated previously for 1 hour with M/8 or M/16 MgCl₂, CaCl₂, SrCl₂, BaCl₂, gave outflows between 82 and 87, which is only a slight if any increase over that of common gelatin.

Gelatin treated with M/8 cane sugar, M/8 glycerol, or M/8 ethyl alcohol, gave times of outflow identical with those found for *common gelatin. Table II gives the full results. The slight* variations between members of the same group of salts are due to the fact that some viscosity determinations were made on the same day on which the gelatin was treated with the salt solution and others on the day following.

TABLE II.

Type of salt.	Molecular concentration of salt.	Nature of salt with which gelatin was treated.	Viscosity (expressed in time of outflow) of 1 percent gelatin solution; gelatin treated with salt, then with H_2O to wash away salt.
			sec.
	M/8	NaCl..................	95
	M/8	NaNO₃................	97
	M/8	Na acetate............	95
I.	M/8	NaCNS...............	96
Univalent cation, uni-	M/8	KCl..................	99
valent anion.	M/8	LiNO₃................	96
	M/8	KCNS................	96
	M/8	LiBr.................	99
	M/8	NH₄Br...............	96
	M/8	Na₂SO₄................	95
	M/8	Na₂ oxalate...........	100
	M/16	Na₂ tartrate...........	99
II.	M/16	Na₂ malate............	97
Univalent cation, bi-	M/16	Na₂ succinate.........	100
valent anion.	M/16	Li₂SO₄................	99
	M/16	K₂SO₄................	99
	M/16	(NH₄)₂SO₄.............	98
	M/16	K₂ tartrate............	102
III.	M/8	MgCl₂................	87
Bivalent cation, uni-	M/16	CaCl₂................	82
valent anion.	M/8	SrCl₂.................	87
	M/16	BaCl₂................	82
IV.			
Bivalent cation, bi-	M/8	MgSO₄................	90
valent anion.			
V.		H_2O...................	82
Non-electrolytes.	M/8	Glycerol..............	79
	M/8	Cane sugar............	80
	M/8	Ethyl alcohol..........	81

If it is true that the increase in viscosity is the expression of an increase in ionization of the gelatin, the facts found by us support our hypotheses: (1) that neutral salts with univalent cations form

with gelatin highly ionizable metal gelatinate compounds which dissociate into a positive metal and a negative gelatin ion; and (2) that neutral salts with bivalent cation form metal gelatinates which are considerably less ionizable. As far as the anion is concerned, its influence is neither noticeable in the additional swelling nor in the increase in viscosity. $MgSO_4$ and $CaSO_4$ act not differently from $MgCl_2$ and $CaCl_2$, and NaCl not differently from Na_2SO_4, as long as the concentration of the cation is the same. This means that the anion of the salt used either does not combine with the protein at all or that if it combines it enters into a bondage where its influence is not noticeable.

Finally, the results on viscosity support our statement drawn from the experiments on swelling that the action of neutral salts upon gelatin is of a stoichiometrical character, since the increase in the viscosity of the gelatin caused by a previous treatment with a salt solution is exactly the same for all salts with univalent cations regardless of the nature of the anion or cation as long as the concentration of the cation is the same. This stoichiometrical result is entirely independent of the hypothesis that the increase in viscosity is due to an increase in ionization.

IV.

If we treat gelatin with HCl, gelatin chloride (or hydrochloride) is formed, which according to our previous observations should react only with the anion of neutral salts, since such a compound dissociates preeminently or exclusively into a positive gelatin ion and a negative chlorion. Hence we should expect that a treatment of gelatin chloride with salts of the calcium group would affect the viscosity of gelatin chloride (after the salt solution is washed away) qualitatively and quantitatively like the treatment with salts of the type NaCl, provided the concentration of the Cl ion is the same; while the influence of the cations, Na and Ca, which was so strong in the case of common gelatin, should not be noticeable. All this turned out as expected. Hence neutral salts of the calcium group as well as neutral salts of the sodium group form highly ionizable salts with gelatin chloride—as long as the anion of the salt is univalent. Neutral salts with bivalent anion, however, should form with gelatin chloride new compounds

(gelatin sulfate, gelatin oxalate, gelatin malate, etc.) which are less ionizable than gelatin chloride (or hydrochloride), and this was also borne out by the experiments which we will now describe in more detail.

1 gm. of powdered gelatin was put for half an hour into a beaker containing 100 cc. $M/100$ HCl and the gelatin was repeatedly stirred. The gelatin was then put into the cylindrical funnel and the HCl solution (or what was left of it) allowed to filter off. After this the gelatin in the funnel was perfused three times with 25 cc. of the salt solution ($M/8$ or $M/16$ respectively) and then four times with 25 cc. of H_2O to remove any free salt solution. After this water had run off the gelatin was made up into a 1 per cent solution (in distilled water) and its viscosity was determined immediately as described.

The time of outflow of liquid gelatin chloride produced by treatment with $M/100$ HCl, when perfused three or four times with water to remove the remnant of acid in the capillary spaces between the gelatin granules, was for a 1 per cent solution generally between 120 and 130 seconds, and sometimes higher.

Gelatin chloride produced by a half hour treatment of 1 gm. of powdered gelatin in 100 cc. $M/100$ HCl was put into a funnel, the HCl solution was allowed to filter off, and the gelatin was perfused three times with $M/8$ $MgCl_2$ or $CaCl_2$ or $SrCl_2$, and then perfused four times with H_2O. The time of outflow varied between 132 and 135 seconds (Table III), a value which was identical with the one obtained when salts with a monovalent cation and anion were used for perfusion, such as NaCl, $NaNO_3$, KCl, KCNS, LiBr, NH_4Br, etc., the time of outflow varying in this case between 129 and 136 seconds (Table III). Only Na acetate proved to be an exception as was the case in similar experiments on swelling. This suggests that in the reaction between gelatin chloride and neutral salts only the anion of the neutral salts reacts with the gelatin or that at least the effect of the cation is not noticeable. This is exactly the same conclusion to which our experiments on swelling had led us. On the other hand, the same theory demanded that when gelatin chloride reacts with salts with bivalent anion, like SO_4, oxalate, etc., gelatin sulfate, gelatin oxalate, etc., should be formed with a much lower degree of ionization and hence a much lower degree of viscosity. This was also

found to be true. When gelatin chloride obtained by interaction for half an hour between 100 cc. $M/100$ HCl and 1 gm. gelatin was perfused three times with one of these salts with bivalent anion and then freed from the salt by washing four times with

TABLE III.

Type of salt.	Molecular concentration of salt.	Nature of salt with which gelatin was treated.	Viscosity (expressed in time of outflow) of 1 percent gelatin solution; gelatin first treated with $M/100$ HCl, then with salt, then with H_2O to wash away salt.
			sec.
	$M/8$	NaCl..................	131
	$M/8$	NaNO₃................	136
I.	$M/8$	Na acetate............	107
Univalent cation, uni-	$M/8$	KCl..................	131
valent anion.	$M/8$	LiNO₃................	130
	$M/8$	KCNS................	129
	$M/8$	LiBr.................	132
	$M/8$	NH₄Br...............	132
	$M/8$	Na₂SO₄...............	105
	$M/8$	Na₂ oxalate...........	94
II.	$M/16$	Na₂ tartrate..........	102
Univalent cation, bi-	$M/16$	Na₂ malate............	109
valent anion.	$M/16$	Na₂ succinate.........	106
	$M/16$	(NH₄)₂SO₄............	106
	$M/16$	K₂ tartrate...........	97
III.	$M/8$	MgCl₂................	132
Bivalent cation, uni-	$M/8$	CaCl₂................	133
valent anion.	$M/8$	SrCl₂................	135
IV.			
Bivalent cation, bi-	$M/8$	MgSO₄...............	103
valent anion........			

H_2O, the viscosity was lowered more than 20 per cent, the time of outflow varying between 94 and 109 seconds (Table III). Hence sulfates, oxalates, malates, succinates, act upon gelatin chloride in the same way as do Ca or Mg or Ba upon common gelatin; namely, forming little or less dissociable gelatin salts. This

agrees with the conclusion drawn from the experiments on the effect of neutral salts upon the swelling of gelatin.

The fact that gelatin treated with M/100 HCl has a higher viscosity (and swells more) than gelatin treated with M/8 NaCl may be due to the difference in the mass of gelatin salt formed in the two cases.

<div align="center">V.</div>

A third test for our theory should consist in the proof that the influence of neutral salts upon the viscosity of sodium gelatinate produced by the action of NaOH upon 1 gm. of gelatin runs parallel to the influence of neutral salts upon the viscosity of common gelatin. The test turned out as our theory demanded.

1 gm. of finely powdered gelatin was put for half an hour into a beaker containing 100 cc. M/100 NaOH. The gelatin was then put into a funnel in order to allow the NaOH to filter off, and after this the gelatin was perfused three times with 25 cc. of an M/8 or M/16 salt solution and four times with 25 cc. of H₂O to wash out the salt solution from the capillary spaces. The results were as follows.

When gelatin is treated with M/100 NaOH the viscosity measured in times of outflow is raised from 82 seconds to from 115 to 118 seconds. When it is washed with water a slightly lower value is obtained, due possibly to the washing away of some of the NaOH formed by hydrolytic dissociation of sodium gelatinate with subsequent diminution of sodium gelatinate. The value 115 to 118 seconds is higher than the value obtained by the interaction between M/8 NaCl and gelatin, which was about 98 seconds, indicating that more sodium gelatinate is formed by the interaction of 1 gm. of gelatin with 100 cc. M/100 NaOH than with 100 cc. M/8 NaCl.

The theory which we developed on the basis of the swelling experiments demands that if sodium gelatinate produced by the action of M/100 NaOH be treated with a neutral salt with univalent cation the viscosity should be the same no matter what the nature of the anion or whether the latter is monovalent or bivalent, provided that the concentration of the cation is the same in all cases and provided that the salt in the capillary spaces between the granules of gelatin is washed away before the viscosity is

determined. The experiments confirmed this expectation. For salts of the type NaCl as well as for salts of the type Na_2SO_4, the viscosity measured in time of outflow was the same; namely, in the neighborhood of 118 (Table IV). This again indicates

TABLE IV.

Type of salt.	Molecular concentration of salt.	Nature of salt with which gelatin was treated.	Viscosity (expressed in time of outflow) of 1 percent gelatin solution; gelatin first treated with M/100 NaOH, then with salt, then with H_2O to wash away salt.
			sec.
	M/8	NaCl................	115
	M/8	NaNO₃..............	119
I.	M/8	Na acetate..........	120
Univalent cation, univalent anion.	M/8	KCl.................	120
	M/8	LiNO₃..............	116
	M/8	KCNS..............	116
	M/8	LiBr...............	118
	M/8	NH₄Br.............	110
	M/16	Na₂SO₄............	116
	M/16	Na₂ oxalate........	112
II.	M/16	Na₂ tartrate........	120
Univalent cation, bivalent anion.	M/16	Na₂ malate.........	120
	M/16	Na₂ succinate.......	119
	M/8	(NH₄)₂SO₄.........	110
	M/16	K₂ tartrate.........	117
III.	M/16	MgCl₂.............	87
Bivalent cation, univalent anion.	M/16	CaCl₂.............	96
	M/8	SrCl₂.............	98
IV. Bivalent cation, bivalent anion.	M/8	MgSO₄.............	103

that only the cation of the neutral salt interacts with sodium gelatinate. When $MgCl_2$, $CaCl_2$, and $SrCl_2$ were substituted for the salts with univalent cation the time of outflow was reduced to from 87 to 98 seconds (Table IV), indicating that lowering of the degree of electrolytic dissociation which our theory demanded.

This lowering was due to the formation of non- or less dissociable calcium gelatinate or magnesium gelatinate from sodium gelatinate.

VI.

Attention had been called in this and the writer's former papers to the fact that the additional swelling due to the action of a neutral salt upon gelatin is only noticeable after the excess of salt solution has been washed away, and this was explained on the assumption that the additional swelling was due to the gelatin ions formed under the influence of the salt, but owing to the repression of ionization in the presence of the salt the additional swelling could only show itself after the excess of salt solution was washed away. The same is true for the influence of salts upon the viscosity. When we measure the influence of neutral salts upon the viscosity of gelatin solution in the presence of the salt we get negative if not confusing results. Thus measurements of the viscosity of gelatin in $M/8$ $MgCl_2$, $CaCl_2$, or $SrCl_2$ gave times of outflow between 74 and 85 seconds, while the viscosity measurements of gelatin in the presence of salts of the type NaCl gave values between 72 and 76 seconds, and in the presence of salts of the type Na_2SO_4 the times were 80 to 83 seconds. Almost similar figures were obtained for gelatin treated first with $M/100$ HCl and then with $M/8$ neutral salts. This agrees with our theory that the effect of neutral salts upon the additional swelling and upon the increase of viscosity is due to the ionization of gelatin salts with univalent anion or cation; and that this ionization is repressed by the presence of as high a concentration of a neutral salt as $M/8$ or $M/16$. This confirms our statement that any theory of the action of the neutral salts upon proteins must be based on experiments in which the excess of salts which have acted on the protein has been washed away.

VII.

We have called attention to the fact that if dried pig's bladder or powdered gelatin is treated with 100 cc. $M/8$ NaCl containing quantities of $M/8$ $CaCl_2$ varying from 1 to 32 cc. the additional swelling noticeable after the salt is washed away diminishes with

the amount of $CaCl_2$ added.   This is a case of antagonistic salt action.   It was of interest to find out whether this antagonistic effect of the addition of $CaCl_2$ to NaCl would also become noticeable in the viscosity of the gelatin after the salt was washed away.   This was the case.   1 gm. of powdered gelatin was put for 1 hour into 100 cc. M/8 NaCl containing 0, 1, 2, 4, 8, 12, 16, and 32 cc. M/8 $CaCl_2$.   As controls gelatin treated with H_2O alone and gelatin treated for 1 hour with M/8 $CaCl_2$ were added. After staying 1 hour in these solutions the gelatin was put into the cylindrical funnels where it was washed four times with 25 cc. of distilled water, until the filtrate no longer gave a precipitate with silver nitrate.   The additional swelling (expressed in mm. of the height of the cylindrical column of gelatin), the viscosity expressed in time of outflow, and the alcohol precipitability of 1 per cent gelatin solutions treated in the way mentioned are given in Table V.   The reader's attention is called to the fact that all the measurements were made on gelatin washed free from the salt originally used and dissolved in H_2O.

TABLE V.

1 gm. gelatin put for 1 hour into the following solutions and then put into funnels and perfused four times with distilled water.

	100 cc. M/8 NaCl + cc. M/8 CaCl₂.							M/8 CaCl₂	H₂O
	0	1	2	4	8	16	32		
Additional swelling in mm. of the cylindrical column of gelatin...	36	38	35	25	23	22	17	6.0	6.5
Viscosity of 1 per cent gelatin solution in H_2O in seconds of time of outflow..............	98	96	96	92	91	88·	90	88	84
Cc. of 95 per cent alcohol required to precipitate 5 cc. 1 per cent gelatin solution in H_2O.	∞	∞	∞	∞	About 20	9.5	6.5	5.4	5.5

The additional swelling is markedly lowered by the addition of 4 cc. or more of M/8 $CaCl_2$ to 100 cc. M/8 NaCl, and the addition of 1 cc. or more of $CaCl_2$ lowers the viscosity.   The precipitability with alcohol begins when more than 8 cc. M/8 $CaCl_2$ is added to 100 cc. M/8 NaCl.

Viscosity and additional swelling give parallel results, supporting our view that in this case the antagonism is due to the diminution in the ionization of a metal gelatin through the transformation of the highly ionizable sodium gelatinate into calcium gelatinate which is only slightly ionizable.

There exists a second type of antagonistic salt action due to the transformation of the highly ionizable gelatin chloride into the less ionizable gelatin sulfate. It was of interest to apply the three tests, additional swelling, viscosity measurements, and precipitation with alcohol, to this assumption.

1 gm. of powdered gelatin was put for half an hour into each of a series of beakers containing 100 cc. $M/100$ HCl to transform the gelatin into gelatin chloride or hydrochloride. The gelatin was then put into a series of cylindrical funnels which were perfused three times with 25 cc. of an $M/8$ solution of NaCl containing varying quantities of $M/16$ Na_2SO_4; namely, the following mixtures, 100 cc. $M/8$ NaCl containing 0, 1, 2, 4, 8, 16, and 32 cc. $M/16$ Na_2SO_4. As a control one funnel was perfused with $M/16$ Na_2SO_4. After this each funnel was perfused four times with 25 cc. H_2O to wash away the salt solution in the capillary spaces between the granules.

Table VI gives the results. The additional swelling is the swelling observed at the end of the washing with water, in excess of the swelling which occurred in the gelatin while the latter was in the acid solution. The reader will notice that the addition of Na_2SO_4 to NaCl diminishes the ionizing effect of the NaCl, inasmuch as with the increase in the quantity of Na_2SO_4 added to the NaCl solution the additional swelling, as well as the viscosity, is diminished (Table VI).

It is obvious again that the values for viscosity are extremely regular and that they diminish with the amount of Na_2SO_4 added. The viscosity of a 1 per cent gelatin solution only perfused with H_2O corresponded to a time of outflow of 86 seconds.

These viscosity measurements therefore support our hypothesis that antagonistic salt action can be produced by the transformation of a more ionizable gelatin salt of the type sodium gelatinate into a less ionizable gelatin salt of the type calcium gelatinate; or of the more ionizable salt of the type gelatin chloride into a less ionizable salt of the type gelatin sulfate.

TABLE VI.

1 gm. gelatin put for 30 min. into 100 cc. M/100 HCl to produce gelatin chloride and then put into funnels and perfused three times with 25 cc. of the following salt solutions and four times with 25 cc. H₂O to wash away the salt.

	100 cc. M/8 NaCl + cc. M/16 Na₂SO₄.							M/16 Na₂SO₄
	0	1	2	4	8	16	32	
Additional swelling in mm. of the height of the cylindrical column of gelatin............	36	34	29	25.5	26.5	24	14	−5.5
Viscosity of 1 per cent gelatin solution in H₂O in seconds of time of outflow..........	138	137	135	131	130	125	118	108
Cc. of 95 per cent alcohol required to precipitate 5 cc. 1 per cent gelatin solution in H₂O......................	∞	∞	∞	∞	∞	∞	∞	9

Theoretical Remarks.

In three former papers we have shown that when powdered gelatin or dried pig's bladder is treated with the solution of a neutral salt, the gelatin or bladder undergoes a certain amount of swelling and that after the salt solution is washed away with distilled water a considerable additional swelling of the gelatin occurs. In the same papers we had also shown that the influence of neutral salts upon the additional swelling of gelatin or pig's bladder is of a stoichiometrical character, depending purely upon the concentration and valency of the cation of the salt used. Our present experiments on the influence of neutral salts upon the viscosity of gelatin solution show that the influence of neutral salts upon the viscosity is also of a stoichiometrical character, inasmuch as it also depends exclusively or preeminently upon the concentration and valency of the cation of the neutral salt used. The anion seemed to be without influence. The writer had found in his former experiments that the neutral salts with mono-valent cation cause a considerable additional swelling of the gelatin while the neutral salts with bivalent cation cause no such swelling, and to explain this difference he had assumed that neutral salts with monovalent cation form with gelatin metal

gelatinates which are highly ionizable, while salts with bivalent cations form with gelatin metal gelatinates which are much less or only slightly ionizable. The experiments on viscosity support this conclusion, if the statement generally made is correct that ionization increases the viscosity of a protein solution.

In publications on the peculiarities of colloids the statement is frequently made that the oppositely charged ions of a neutral salt affect a colloid, e.g. a protein, in the opposite sense and that the effect of the salt is always the algebraic sum of these two opposing forces. This statement does not hold for the influence of neutral salt on those qualities of colloids which have thus far formed the subject of our investigation; namely, additional swelling, viscosity, and precipitability by alcohol after the salt has been washed away. Common gelatin (and the same may be true for all proteins which are much stronger as acids than as bases) reacts with neutral salts as if only the cation of the salt were active; salts of the type NaCl and of the type Na_2SO_4 act qualitatively and quantitatively alike as long as the concentration of the cation is the same, and the nature of the anion does not influence the result. It was also possible to show that $MgCl_2$ acts like $MgSO_4$ and $CaCl_2$ like $CaSO_4$ as long as the concentration of the cation is the same. All this would be impossible if the anion acted upon the protein in a sense opposite to that of the cation. The qualities which we have investigated depend apparently on the ionized negative gelatin ion and this combines only with the cation of the neutral salt applied. If the anion of the latter enters at all into combination with the negative gelatin ion its influence does not show itself in those qualities which thus far have formed the subject of our investigations.

When we produce gelatin chloride (or hydrochloride) by treating the gelatin with $M/100$ HCl, and wash away the superfluous acid the gelatin chloride is only influenced by the anion of a neutral salt, and salts of the type $CaCl_2$ act qualitatively and quantitatively like salts of the type NaCl if the concentration of the Cl ion is the same. The cation does not act in the opposite sense from the anion of the salt but does not act at all, or at least its influence is not noticeable, and Ca, which influences common gelatin and sodium gelatinate produced by treatment with NaOH so powerfully, is apparently without influence when acting upon

gelatin chloride. Our experiments have therefore led to a remarkable simplification of our conception of the influence of neutral salts upon proteins inasmuch as they show that for the physical characters thus far studied by us only one of the ions of the neutral salts is to be considered; namely, the one which has the opposite charge from the protein ion with which it reacts.

There are other qualities of proteins which are not confined to the ionized protein. Our investigations have not yet been extended to these qualities and we are not prepared to say whether or not qualities depending upon the non-dissociated protein molecules are influenced in an opposite sense by the oppositely charged ions of neutral salts.

SUMMARY.

1. Our experiments have shown that when we treat gelatin with M/8 or M/16 solutions of neutral salts with univalent cation, the viscosity of the gelatin is increased considerably after the excess of salt is washed away. When we treat gelatin with M/8 or M/16 solutions of neutral salts with bivalent cation no or only a slight increase of viscosity of the gelatin is noticeable after the excess of salt is washed away.

2. Neutral salts with univalent cation (Li, Na, K, NH$_4$) produce the same increase in the viscosity of the gelatin solution as long as the concentration of cation is the same,—namely M/8 in our experiments,—regardless of the nature of the anion and regardless of whether the anion is univalent or bivalent.

3. The statements made concerning the influence of neutral salts upon the viscosity of gelatin hold also for the influence of these salts upon the viscosity of gelatin previously treated with an alkali; e.g., NaOH.

4. The action of neutral salts upon the viscosity of gelatin previously treated with HCl shows that salts with bivalent anion lower the viscosity of such gelatin; while salts with univalent anion have the opposite effect. The influence of the cation was not noticeable in our experiments.

5. These observations do not agree with the statement frequently made that the action of a neutral salt upon a protein is the algebraic sum of the opposite action of the oppositely charged

ions of the neutral salts. The viscosity of a gelatin solution is
influenced chiefly if not exclusively by one of the two ions of the
neutral salt; namely, the one which has the opposite electrical
charge from that of the protein. Since common gelatin is
stronger as acid than as base, its viscosity is influenced only by
the cation of a neutral salt; and the same is true for the influence
of neutral salts upon the viscosity of metal gelatinates produced
by the action of bases upon gelatin. The viscosity of gelatin-
acid salts (produced by the action of acids upon gelatin) which
yield a positive gelatin ion is influenced chiefly if not exclusively
by the anion of a neutral salt.

6. These conclusions are in agreement with the conclusions
drawn from the experiments on the limiting concentration of
neutral salts upon additional swelling reported in the writer's
previous publications.

7. If it is true that the increase in the viscosity of gelatin solu-
tion under the influence of electrolytes is due to an increase in
the ionization of the gelatin, our results support the hypothesis
that neutral salts with univalent cation form highly ionizable
metal proteinates with proteins of the type of gelatin (which is
much stronger as acid than as base), while neutral salts with
bivalent cation form less or non-dissociable metal proteinates
with gelatin.