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# THE STOICHIOMETRICAL CHARACTER OF THE ACTION OF NEUTRAL SALTS UPON THE SWELLING OF GELATIN.

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

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## I.

It is generally believed that the reactions of colloids do not obey the stoichiometric laws of general chemistry. This viewpoint is illustrated clearly in the discussion of the action of neutral salts upon such physical qualities of proteins, as the swelling power of gelatin. The colloid chemists state that acetates, sulfates, tartrates, and citrates influence the swelling in the opposite sense from chlorides, bromides, and nitrates, the latter causing more swelling of the gelatin than distilled water, while the former cause a shrinking of the gelatin. Moreover, they claim that the relative efficiency of the anions of the same group varies in a definite order. The influence of the cations is stated to be negligible. In a previous paper the writer has already reported facts showing clearly that the effect of neutral salts upon the swelling of gelatin is of a stoichiometrical character.<sup>1</sup> Thus it was shown that all the univalent cations of neutral salts, Li, Na, K, and NH<sub>4</sub>, influence the swelling of gelatin at exactly the same concentration no matter what the nature of the anion; and the writer had also proved that only an unsuitable method was responsible for the erroneous statements of the colloid chemists concerning the influence of salts upon the swelling of gelatin.

The source of error into which the colloid chemists have fallen in this case is not difficult to understand. It happens that an adequate chemical reaction between neutral salts and proteins

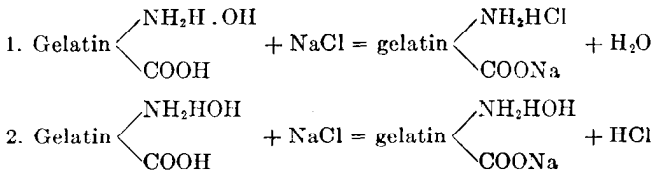
<sup>1</sup> Loeb, J., *J. Biol. Chem.*, 1918, xxxiii, 531.

occurs only when the salt is added in excess, and it also happens that a number of the physical qualities of the new salt-protein compound only become apparent if the excess of the salt which acted upon the protein is afterwards washed away. In most previous experiments the effect of the salt upon the protein was tested in the presence of the salt; so that when little salt was used no or too small an effect on the protein was produced and when too much salt was used the salt present prevented the manifestation of the new qualities of the salt-protein product. Thus in Hofmeister's experiments<sup>2</sup> the swelling of a block of gelatin was measured in the presence of an excess of salt and he measured in reality the resultant of two opposite effects; namely, the mass action of the salt upon the protein and the inhibiting effect of the salt upon the swelling of the new salt-protein compound. It is no wonder that with such methods no stöchiometrical results were obtained.

Our method consisted in perfusing 2 gm. of finely powdered, non-bleached gelatin of equal size of grains (going through sieve size 50 but not through 60) twice with 25 cc. M/8 NaCl. It was assumed that with this excess of salt a certain percentage of the gelatin molecules would react with the NaCl and form a new compound. The gelatin molecules being amphoteric but stronger as acid than as base should form with the salt a compound which we will symbolize as Na-G (sodium gelatinate). When we remove the salt by perfusing the powder four times with 25 cc. of H<sub>2</sub>O the Na-G formed is supposed to remain after the salt solution is washed away. Hence it is the additional swelling of this sodium gelatinate which served for the experiments in our previously published notes.

This compound dissociates electrolytically into  $\overset{-}{G} + \overset{+}{Na}$ , as was indicated by the fact that in the electrical field gelatin treated with NaCl, and then washed with H<sub>2</sub>O, migrates exclusively to the anode (as does the gelatin not treated with salt, which is a stronger acid than base). This leaves the question open whether or not the Cl combines also with the gelatin or whether the reaction is only between Na and gelatin; in other words, Which of the two following possible reactions takes place?

<sup>2</sup> Hofmeister, F., *Arch. exp. Path. u. Pharm.*, 1891, xxviii, 210.



If the second type of reaction takes place, free acid, HCl, should appear in the supernatant salt solution. This was apparently not the case in our experiments. It should, however, be borne in mind that at least part of any free acid formed would combine with the gelatin and this combination between HCl and gelatin might occur not only in molecules of the type

gelatin  $\begin{cases} \text{NH}_2\text{HOH} \\ \text{COONa} \end{cases}$  but also in the molecules not in combination with

Na; namely, gelatin  $\begin{cases} \text{NH}_2\text{HOH} \\ \text{COOH} \end{cases}$  It is very probable that the

reaction between gelatin and NaCl in our experiment is not complete; *i.e.*, does not transform all the gelatin molecules into Na gelatinate. If, therefore, part of the acid is caught by the gelatin, the fact that the reaction of the supernatant salt solution does not become markedly acid does not speak against the assumption that the reaction which occurs is the second reaction, which results in the formation of asodium gelatinate. The decision of the question is not essential for our present purpose. We may state that when we treat gelatin with an excess of NaCl a compound is formed

<sup>+</sup> which dissociates electrolytically into Na and a negatively charged gelatin ion. If this gelatin ion contains also Cl the Cl is held in non-dissociable or less dissociable bondage; so that for all practical purposes we are dealing with a compound which we may call sodium gelatinate and which we will symbolize as Na-G.

Non-treated gelatin of the type gelatin  $\begin{cases} \text{NH}_2\text{H} \cdot \text{OH} \\ \text{COOH} \end{cases}$  swells in distilled water, but the swelling soon reaches its maximum. Sodium gelatinate swells also when perfused with an  $m/8$  NaCl solution and reaches a maximum (which is lower than that of non-treated gelatin in  $\text{H}_2\text{O}$ ). This swelling is the *initial* swelling which has nothing or little to do with the effect of the salt and which will be

disregarded by us. If we remove, however, the excessive salt by perfusing the powder repeatedly with a NaCl solution weaker than  $m/8$  we find that below a definite concentration ( $m/64$ ) of NaCl an *additional* swelling of the gelatin takes place, which becomes the greater the greater the dilution of the washing solution. This *additional* swelling which is the true effect of the salt upon the swelling of the gelatin we assumed to be due to the existence of the negative gelatin ion. That the gelatin ion has a negative charge in this case has nothing to do with the phenomenon of additional swelling, since positively charged gelatin ions (*e.g.*, gelatin treated with acid) also give rise to an additional swelling. The assumption that the ionization of the gelatin is responsible for the *additional* swelling is not necessary for the proof of the stoichiometrical character of the influence of neutral salts on the swelling, though everything speaks in favor of this assumption.

The fact of importance for us is the following: An excess of NaCl is necessary to cause the formation of Na gelatinate and the *additional* swelling only begins to appear if the excess of the salt is removed and the  $m/8$  NaCl solution is replaced by a  $m/64$  NaCl solution. If the  $m/8$  NaCl is replaced by a still weaker NaCl solution the additional swelling becomes still greater. *The stoichiometrical character of the inhibiting influence of neutral salts upon the additional swelling is revealed in the following facts.*

1. All neutral sodium salts with monovalent anion have under the conditions of the experiment the same limiting molecular concentration for the beginning of the additional swelling, namely  $m/64$  (Table I, Group I).

2. All neutral sodium salts with bivalent anion have the same limiting molecular concentration for the beginning of the additional swelling which is  $m/128$ —exactly half as large as that for the salts with monovalent anion. Since a  $m/128$  solution of  $\text{Na}_2\text{SO}_4$  has approximately as many sodium ions in solution as a  $m/64$  NaCl solution, we can say that in all neutral solutions of a sodium salt with monovalent or bivalent anion the limiting concentration for the additional swelling is determined by the concentration of Na ions in the solution which is  $m/64$  regardless of the anion (Table I, Group II).

3. The limiting concentration for the additional swelling remains the same when the salts treated first with  $m/8$  NaCl are after-

wards perfused with neutral solutions of salts of Li, K, and  $\text{NH}_4$  with monovalent or bivalent anion (Table I, Groups I and II).

4. Non-electrolytes, like cane sugar, glycerol, alcohol, have no such limiting effect upon the additional swelling in concentrations of 2 M and below, but behave like distilled water (Table I, Group V).

These four facts are only comprehensible on the assumption that the additional swelling of gelatin depends upon the formation of well defined metal gelatinates in which the metal ions, Li, Na, K, and  $\text{NH}_4$ , can replace each other in the same stoichiometric way as in the case of simple inorganic salts. The fact that the nature of both bivalent and univalent anions and univalent cations of the salt are of little if any importance fits well with the assumption that it is the ionization of the metal gelatinate which determines the additional swelling (Table I).

In order to understand the figures it should be stated that the perfusion of gelatin occurred in cylindrical funnels of the same diameters so that the increase in the height of the cylinder, expressed in the table in millimeters, was the measure of the swelling. The measurements were usually made after 24 hours to allow all the water which was not held by the gelatin or in the minute capillary spaces between the granules to run off.<sup>3, 1</sup>

The perfusion of the 2 gm. of gelatin with 50 cc. M/8 NaCl causes a considerable initial swelling which does not increase when the perfusion with M/8 NaCl is repeated. When, however, for the subsequent perfusion (following that with M/8 NaCl) a weaker solution, namely M/64 or M/128 NaCl, is used, *additional* swelling takes place and this *additional* swelling is the true effect of the previous salt treatment upon the swelling of gelatin. As long as this additional swelling is less than 3 or 4 mm. in the height of the cylindrical mass of gelatin it may be disregarded since this is within the limits of unavoidable variation, due mainly to the formation of air bubbles in the mass and the error in measuring the height of the cylinder. The real additional swelling begins with an increase of 5 or 6 mm. in the height of the cylindrical mass of gelatin. The figures in Table I give this increase in the height of the cylindrical mass of gelatin in millimeters. The

<sup>3</sup> Loeb, *J. Biol. Chem.*, 1917, xxxi, 343.

TABLE I.  
*Action of Neutral Salts upon the Additional Swelling of Sodium Gelatin Produced by the Action of NaCl upon Gelatin.*

Additional swelling of column of gelatin perfused twice with 25 cc. m/8 NaCl (to produce sodium gelatin) and three times with 25 cc. of the following solutions.

Type of salt.	Nature of salt.	m/8	m/16	m/32	m/64	m/128	m/256	m/512	m/1024	m/2048	m/4096	m/8192	H <sub>2</sub> O	
		mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
I. Univalent cation, uni- valent anion.	NaCl.....	0	0	2.5	6	15	21	33.5	42	48	46	53	54	
	NaBr.....	0	2.0	2.0	5	12.5	17.5	27	43	43.5	51	49	54	
	NaNO <sub>3</sub> .....	1	?	4	7	12.5	19.5	29.5	39	43.5	44	48	55	
	Na acetate.....	1.5	1.5	3	6.5	15	22	31	38	45.5	44	53	60	
	NaCNS.....	1	3.5	4	7	12	21	31	40	46	53	53.5	57.5	
	LiCl.....	1	1.5	3.5	6	10.5	15	24.5	34.5	44.5	52	46.5	53.5	
	KCl.....	1	2	2.5	6.5	11.5	16	24	33	38.5	44	47	56	
	NH <sub>4</sub> Cl.....	2	2	2	5	13	23	22.5	39.5	48	47	47	56	
	II. Univalent cation, biva- lent anion.	Na <sub>2</sub> SO <sub>4</sub> .....	1	1.5	2.5	3.5	8	14	22.5	30	39	47	55	54
		Na <sub>2</sub> oxalate.....	2	2	2	3.5	6.5	14.5	24	29	35	46	50	56
Na <sub>2</sub> tartrate.....		2	2	3	5	5.5	13.5	23	32.5	43	46	52.5	56.5	
Na <sub>2</sub> malate.....		0	1	1	2.5	5	13	20	31	35	39	43	51	
Na <sub>2</sub> succinate.....		1	1	1	1.5	6	14.5	21	28.5	35	46	47.5	51.5	
Li <sub>2</sub> SO <sub>4</sub> .....		3	0	2	2.5	3.0	10	19.5	30.5	45.5	51	51	60	
K <sub>2</sub> SO <sub>4</sub> .....		1	1.5	0.5	3.0	6.5	11	20	29	40.5	42	50.5	54.5	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....		1.5	1.5	2	3	6	12	18	26	42	50	45	53	
K <sub>2</sub> oxalate.....		1	1	1.5	3.5	7.5	17	22.5	25	33.5	43	46.5	55	
(NH <sub>4</sub> ) <sub>2</sub> oxalate.....		0	1	1	2.5	5	11.5	22.5	27	36.5	48.5	49	54	
K <sub>2</sub> tartrate.....	0	0	1.5	1.5	7	13	19	28	37	42	46	53		
(NH <sub>4</sub> ) <sub>2</sub> tartrate..	2	1.5	2	5?	7	16.5	22	35.5	43.5	49.5	52	55		

III. Bivalent cation, univalent anion.	MgCl <sub>2</sub> .....	0	0	0	0	4	13	26	42	49	53	52
	CaCl <sub>2</sub> .....	0	0	0	0	1.5	8	24	39	48	51	47
	SrCl <sub>2</sub> .....	0	0	0	0	1	8	18	36	43	52	52
	BaCl <sub>2</sub> .....	0	0	0	0	1	10	21	40	45	51	50
IV. Bivalent cation, bivalent anion.	MgSO <sub>4</sub> .....	1.5	0	-1	0	1	7.5	22.5	38	48	55	63
	CaSO <sub>4</sub> .....				-3.5	2.5	8.5	18.5	37.5	43	47	55
V. Non-electrolytes.	Glycerol.....	43	42	40	41	41	41	43				42
	Cane sugar.....	51	51	50	51	51	51					48
	Ethyl alcohol.....	48.5	49.5	49.5	47	47.5	47					50.5



reader will notice that the increase between 5 and 7 mm. lies at  $m/64$  for all salts of Group I (type NaCl), while it lies at  $m/128$  for all salts of Group II (type  $\text{Na}_2\text{SO}_4$ ) regardless of the nature of the anion. This is the most fundamental of our results whereby the fact is established that we are dealing with simple chemical substitution phenomena.

Perfusion of Na gelatin with  $m/128$  solutions of the salts of Group I (type NaCl) causes an additional swelling of from 10 to 15 mm. in the height of the cylindrical mass of gelatin; the same quantitative effect is found for concentrations of  $m/256$  for salts of Group II (type  $\text{Na}_2\text{SO}_4$ ). All through the table it is obvious that the salts of the type  $\text{Na}_2\text{SO}_4$  give the same figures for additional swelling when their molecular concentration is one-half of that for Group I, type NaCl. This is true regardless of the nature of the anion or cation, as long as the latter belongs to the alkali group including  $\text{NH}_4$ .

When we perfuse the gelatin (previously treated with  $m/8$  NaCl) with solutions of non-electrolytes (sugar, glycerol, alcohol) of different concentration we get the same effect as when we use distilled water. This is one of the reasons why we are inclined to assume that the limiting concentration for the action of neutral salts upon swelling is that concentration which allows so many of the sodium gelatinate molecules to dissociate electrolytically that the additional swelling can begin. If our assumption is correct, the figures given in Table I might be utilized to calculate roughly the degree of electrolytic dissociation of the Na gelatin molecules in the solution of a salt of a given concentration, by taking the dissociation in  $\text{H}_2\text{O}$  as 100 per cent.

The reader will notice that we do not utilize the absolute amount of swelling as a criterion for our conclusions, but use only equal swelling in different concentrations of salt. The absolute swelling cannot be estimated by our figures since they include not only the water held inside the gelatin grains (the true swelling) but also the water held in the fine capillary spaces between the powdered granules which, however, may be only a comparatively small quantity.

It seemed desirable to see whether we could determine the limiting concentration for the swelling caused by neutral salts of Type I (NaCl) and Type II ( $\text{Na}_2\text{SO}_4$ ) a little more accurately.

For this purpose 2 gm. of powdered gelatin were perfused twice with 25 cc.  $M/8$  NaCl and then with solutions from between  $16 M/512$  to  $M/512$  of different salts of Types I and II. In this case we found again that the molecular concentrations of salts of Type I (NaCl) which just permit swelling are twice as great as those for salts of Type II ( $Na_2SO_4$ ), and that the limiting concentrations are approximately between  $3/512$  and  $4/512$  for Salts II ( $Na_2SO_4$ ) and between  $3/256$  and  $4/256$  for Salts I (NaCl) (Table II).

TABLE II.

More accurate determinations of the limiting concentration of different salt solutions upon the additional swelling of column of sodium gelatin.

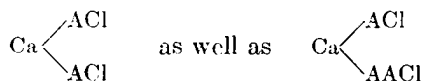
	$8M/256$	$7M/256$	$6M/256$	$5M/256$	$4M/256$	$3M/256$	$5M/512$	$4M/512$	$3M/512$	$2M/512$	$M/512$
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
NaCl.....	1.5	1	2	3.5	5	7		11.5		21.5	
NaNO <sub>3</sub> .....	1.5	3.5	2	2	3.5	8.5		13		23	
NaBr.....	2.5	2	3.5	3.5	5.5	8		14		24	
NaCNS.....	2	3	3	3.5	4	9		14		19.5	
LiCl.....	1.5	2	2	2	1.5?	5		10		18	
K acetate.....	2	1	4	4.5	3	10.5		16		26.5	
NH <sub>4</sub> Br.....	1	0	2.5	3	4.5	7		14		22	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	1	1	0	0	1	1	2.5	4.5	8	13	21
Na <sub>2</sub> malate.....	1		0	1	0	0.5	1	4	5.5	10	22.5
Na <sub>2</sub> tartrate.....	1	1	1	1	0	1.5	3.5	5.5	9	16.5	22.5
Na <sub>2</sub> oxalate.....	0	1	2	1	1	2	5.5	5.5	9	12	
K <sub>2</sub> SO <sub>4</sub> .....					1.5	1.5	4	5	3	13	

From these facts it follows that only the cation inhibits the swelling in this case and that univalent cations, Li, Na, K, NH<sub>4</sub>, all act in exactly the same way in the same concentration no matter what the nature of the anion is. *We can, therefore, use the limiting concentration of a neutral salt for the additional swelling of sodium gelatin as a rough method for determining the molecular concentration of the salt and for this reason we are entitled to state that the action of the neutral salts upon the swelling of gelatin is of a strictly stoichiometrical character.*

In all the experiments thus far reported we always used the same ion-gelatin compound; namely, Na gelatin prepared by

treating the gelatin with  $m/8$  NaCl. We have stated in a previous paper that we get the same results when we first treat the gelatin with some other neutral salt; *e.g.*, LiCl.

The effect of the neutral salts of the alkaline earth metals requires a special discussion. We have found that the salts of Mg, Ca, Sr, and Ba do not cause an additional swelling, and we are forced to the conclusion that such salts form either non-ionizable gelatin compounds or compounds of a different type from those which originate when salts of the alkali metals combine with gelatin. Pfeiffer and von Modelski<sup>4</sup> have shown that Ca forms with amino-acids and betaines compounds containing two or three atoms of amino-acid in the molecule; namely,



The idea suggests itself that similar products originate when the metals of the alkaline earths combine with proteins like gelatin and that these larger compounds (of the type calcium gelatin) do *not* cause any additional swelling. We will represent such com-

pounds under the symbol  $\text{Ca} \begin{cases} \text{gelatin} \\ \text{gelatin} \end{cases}$  while the compound really

formed may be of a more complicated or different nature than represented by our symbol and may also contain Cl. If it is the ionized gelatin which causes the additional swelling it follows that such compounds as calcium gelatin do not dissociate electrolytically or if they do so their ionization will result in the formation of a polymerized or otherwise more complex gelatin anion, which for some unexplained reason is not capable of swelling.

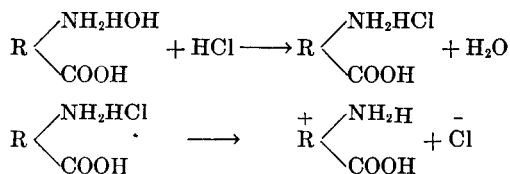
That the neutral salts  $\text{MgCl}_2$ ,  $\text{MgSO}_4$ ,  $\text{CaCl}_2$ ,  $\text{CaSO}_4$ ,  $\text{SrCl}_2$ , and  $\text{BaCl}_2$ , react with gelatin under the formation of definite metal gelatinates and that these compounds determine the negative influence upon swelling is indicated by the fact that the limiting concentration is identical for all these salts; namely, about  $m/512$  (Table I, Groups III and IV). This means that when we first perfuse gelatin with  $m/8$  NaCl to form sodium gelatin and then wash the NaCl away with solutions of salts of the alkaline

<sup>4</sup> Pfeiffer, P., and von Modelski, J., *Z. physiol. Chem.*, 1912, lxxxi, 329; 1913, lxxxv, 1.

earth metals, the additional swelling begins to appear when the concentration of the  $\text{CaCl}_2$ , etc., is about  $m/512$  or below. The fact that  $\text{CaCl}_2$  and  $\text{CaSO}_4$ , and  $\text{MgCl}_2$  and  $\text{MgSO}_4$  act alike confirms again the stoichiometrical character of the action of these salts on the gelatin.

## II.

Hardy made the discovery that proteins when treated with acid migrate to the cathode and when treated with a base to the anode, and this fact was first interpreted as a "colloidal" phenomenon due to the "adsorption" of the H or HO ion by the protein<sup>5</sup> or to the "transfer of the electric charges" of these ions to the protein. The writer pointed out that no such explanation was needed and that the phenomenon was a simple case of electrolytic dissociation due to the fact that the protein molecule is an amphoteric electrolyte, which in the presence of acid must dissociate as a base and become a cation, while in the presence of base it must dissociate as an acid and become an anion.<sup>6</sup> This chemical view has since been generally accepted but with an important alteration suggested by Hardy that when acid is added to a protein a salt is formed which dissociates into the anion of the acid added and a positive protein ion.<sup>7</sup>



Therefore, if we treat gelatin with acid, *e.g.*  $\text{HCl}$ , a salt (gelatin chloride) is formed which dissociates into a positively charged gelatin ion and a negatively charged chlorion. When a 1 per cent solution is made of gelatin previously treated with  $\text{HCl}$  the gelatin no longer migrates to the anode but to the cathode.

This new compound, gelatin chloride, which dissociates into

<sup>5</sup> The protein ion was supposed to be charged positively by the adsorbed H ion and negatively by the adsorbed HO ion.

<sup>6</sup> Loeb, *University of California Publications, Physiology*, 1902-04, i, 149.

<sup>7</sup> Hardy, W. B., *J. Physiol.*, 1905-06, xxxiii, 251.

<sup>+</sup>  
gelatin and Cl gives us an ideal chance to test our stoichiometric theory. If this theory is right we should expect that if we first treat powdered gelatin with dilute HCl, and follow this with three or four perfusions with various concentrations of neutral salts, we should expect the following results:

1. Neutral salts of the type NaCl (*e.g.* univalent anion, univalent cation) should permit additional swelling of gelatin chloride at the same degree of dilution which under the conditions of our experiments was again  $M/64$ .

2. Neutral salts of the type  $\text{CaCl}_2$  (*i.e.* alkaline earth metals and univalent anion) should cause additional swelling of gelatin chloride in molecular concentrations exactly twice as dilute as salts of the type NaCl; namely, from  $M/128$  down. The reader will notice that  $\text{CaCl}_2$  should act upon gelatin chloride exactly as salts of the type  $\text{Na}_2\text{SO}_4$  acted upon sodium gelatinate in the previous experiments reported in Table I.

3. Salts of the type  $\text{Na}_2\text{SO}_4$  (*i.e.* univalent cation, bivalent anion) should allow additional swelling of gelatin chloride in concentrations from  $M/512$  and below; in other words,  $\text{Na}_2\text{SO}_4$  should act upon gelatin chloride as  $\text{CaCl}_2$  did upon the sodium gelatinate in the previous experiments. It should form more complicated compounds symmetrical to those presumably formed by negative gelatin with Ca, expressible in the symbol  $\text{SO}_4$   $\left\{ \begin{array}{l} \text{gelatin} \\ \text{gelatin} \end{array} \right.$

corresponding to the symbol  $\text{Ca}$   $\left\{ \begin{array}{l} \text{gelatin} \\ \text{gelatin} \end{array} \right.$  discussed previously.

These predictions are fulfilled not only qualitatively but also quantitatively, as Table III shows.

In order to understand the results of this table it should be said that the preliminary perfusion with 50 cc.  $M/100$  HCl caused a considerable swelling, due to the strong ionization of the gelatin chloride formed and the low concentration of the HCl solution used for perfusion. When such a mass containing gelatin chloride was then perfused with a neutral salt above a certain critical concentration the ionization of the gelatin chloride was diminished and hence the gelatin gave off water and the swelling was reduced. Instead of causing an additional swelling the higher concentrations of the salt,  $M/8$  or  $M/16$ , etc., cause a shrinkage

TABLE III.  
Action of Neutral Salts upon the Additional Swelling of Gelatin Chloride Produced by the Action of HCl upon Gelatin.

Additional swelling of column of 2 gm. of powdered gelatin perfused twice with 25 cc. m/100 HCl (to produce gelatin chloride) perfused subsequently four times with 25 cc. of the following solutions.

Type of salt.	Nature of salt.	m/8	m/16	m/32	m/64	m/128	m/256	m/512	m/1024	m/2048	m/4096	H <sub>2</sub> O
		mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
I. Univalent cation, univalent anion.	NaCl.....	- 3.5	- 2	0	+ 4.5	12.5	21	23.5	27	30	31	31.5
	NaNO <sub>3</sub> .....	- 6	- 4	- 2.5	+ 4.5	10	17.5	25	31	33	29.5	38
	NaBr.....	- 8.5	- 3.5	- 2.5	+ 7.0	13.5	26	42	43	56	57	54
	NaCNS.....	- 7	- 5	- 2	0	+ 7.5	+ 13.5	26	25	29	27	33
	LiCl.....	- 6.5	- 5	- 1	+ 4	10.5	18.5	24	25	29	29	27
	KBr.....	- 6.5	- 4	+ 3.5	+ 6.5	15	20.5	35.5	33	50	41.5	37
	NH <sub>4</sub> Br.....	- 8	- 5	0	+ 5.5	16.5	22	24	28.5	37	36	35
	Na acetate.....	- 7	- 5	- 2	- 2	- 4.5	0	+ 7.5	14	21	49	32
	Na <sub>2</sub> SO <sub>4</sub> .....	- 8.5	- 8.5	- 10	- 8	- 4.5	+ 2.5	+ 20	+ 24	40	40.5	
	Na <sub>2</sub> oxalate.....	- 7	- 8	- 10	- 7.5	- 9.5	- 7	+ 2.5	+ 22	22	26	89
II. Univalent cation, bivalent anion.	Na <sub>2</sub> tartrate.....	- 4	- 6.5	- 7.5	- 8.5	- 6.0	+ 1.0	+ 3.5	+ 23	24	28.5	51
	Na <sub>2</sub> malate.....	- 12	- 8	- 9.5	- 7.5	- 6.5	+ 1.5	- 4?	+ 16	36	47	50
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	- 6	- 8	- 6.5	- 5	- 3	0	+ 3	+ 16	23	24.5	35
	Mg(NO <sub>3</sub> ) <sub>2</sub> .....	- 7	- 7.5	- 6	- 2.5	+ 4	+ 14	22	34	43	44	40
III. Bivalent cation, univalent anion.	CaBr <sub>2</sub> .....	- 9.5	- 8.5	- 8	- 3.5	+ 5.5	16	28.5	37	44	67	64.5
	Sr(NO <sub>3</sub> ) <sub>2</sub> .....	- 6.5	- 7.5	- 5.0	- 3	+ 1.5	+ 8.5	16	27.5	30	35.5	39
	BaCl <sub>2</sub> .....	- 6.5	- 5.5	- 5.5	- 2.0	+ 4.0	+ 8.5	+ 19	31	32	34	60
	Sr acetate <sub>2</sub> .....	- 8.5	- 8	- 10	- 10.5	- 8.5	- 10	+ 9	+ 20	33	35.5	49
	MgSO <sub>4</sub> .....	- 7.0	- 7.5	- 4	- 2	- 5	0	+ 4.5	+ 13	26	31	38

indicated by the negative sign in the table. This shrinkage ceases with that degree of dilution of the salt solution which no longer diminishes the original degree of electrolytic dissociation of the gelatin chloride, and with the next degree of dilution the *additional* swelling of the gelatin begins, indicated in the table by a plus sign.

We have arranged the salts in the same groups as in Table I. The first group includes the salts with univalent cation and anion. The additional swelling of gelatin chloride begins in the same dilution (with the exception of Na acetate which will be discussed later) as was the case for the sodium gelatin in Table I; namely, at  $m/64$ . Second, it can be seen that the additional swelling begins in Group III (type  $\text{CaBr}_2$ ) for our gelatin chloride at exactly twice the critical dilution as for Group I; namely, at  $m/128$ . This proves that regardless of the nature of the cation the additional swelling begins at exactly the same concentration of the (univalent) anion, and the value is the same for different univalent anions, for the simple reason that if a gelatin chloride is treated with a nitrate, gelatin chloride is transformed into gelatin nitrate.

Finally, the bivalent anions behave toward gelatin chloride as do the bivalent cations toward sodium gelatin; namely, they begin to permit additional swelling at a concentration of  $m/512$ . We assumed that this behavior was due to the formation of larger complexes of gelatin symbolized in the form  $\text{SO}_4$  gelatin<sub>2</sub> or  $\text{SO}_4$  gelatin<sub>3</sub>, which apparently do not ionize.

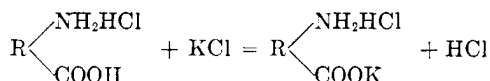
The acetates, Na acetate as well as  $\text{Sr}(\text{acetate})_2$ , behave like the bivalent anions, for some unexplained reason possibly connected with hydrolytic dissociation.

Wherever shrinking effects of sulfates, oxalates, etc., upon the swelling of colloids actually occur, we are probably dealing with cases of protein salts of the type gelatin chloride, which are transformed into salts of the type of protein sulfate, this latter type being as incapable of swelling as salts of the type calcium gelatin.

It might be argued that the statements of colloid chemists concerning the specific effects of anions are correct when applied to gelatin chloride. Thus the shrinking influence of the sulfates and acetates might be claimed as a confirmation of their views. Such a statement would overlook, first, the fact of the qualitatively

and quantitatively identical action of sulfates, oxalates, tartrates, malates; second, of the identical action of Cl, Br, NO<sub>3</sub>, CNS; and third, of the identical action of Li, Na, K, NH<sub>4</sub>, Mg, Ca, Sr, and Ba. The truth is that the influence of neutral salts upon the additional swelling of gelatin chloride is essentially of a stoichiometrical character, though for some unknown reason acetate acts abnormally.

We have assumed that gelatin chloride reacts with neutral salts only by exchanging its anion. Hardy and Pauli<sup>8</sup> have suggested a different reaction; namely,



If such a reaction takes place it is difficult to understand why CaCl<sub>2</sub> does not act in the same way upon gelatin chloride as it does upon sodium gelatinate; namely, by reducing its critical dilution (where additional swelling begins) to m/512, which is not the case. The fact that MgSO<sub>4</sub> behaves like Na<sub>2</sub>SO<sub>4</sub> also suggests that only the anion of the neutral salt reacts with the gelatin chloride and that the cation does not participate in the reaction.

*We, therefore, see that the limiting concentration of a neutral salt for the additional swelling of gelatin chloride could be used as a rough method for determining the molecular concentration of the salt. This is only another expression for the fact that the influence of neutral salts upon the swelling of gelatin is of a purely stoichiometrical character.*

### III.

When we treat gelatin with NaOH, sodium gelatinate is formed. This swells considerably, presumably on account of its high degree of electrolytic dissociation into a negative gelatin and a positive sodium ion. When we put such gelatin into a neutral salt solution that part of the swelling which is due to ionization of the protein (the "additional" swelling) should be suppressed as soon as the concentration of the salt solution reaches a certain critical value.

We assumed that when we treat gelatin with a neutral salt with bivalent cation, NaCl or K<sub>2</sub>SO<sub>4</sub>, sodium or potassium gelatinate is

<sup>8</sup> Pauli, W., *Fortschr. naturwiss. Forschung*, 1912, iv, 223.



formed with a high ionization constant; while the anion of the neutral salt, Cl or SO<sub>4</sub>, does apparently not combine with the gelatin; or if it combines does not participate in the electrolytic dissociation. We have a chance to put this view to a test, since, if it is correct, gelatin treated previously with a neutral salt should behave like that treated previously with NaOH.

2 gm. of powdered gelatin were perfused twice with 25 cc. M/100 NaOH to form sodium gelatinate. The mass underwent a certain amount of swelling, the *initial* swelling, which will not be considered in the following figures. The mass was then perfused three times with 25 cc. of solution of different neutral salts, from M/8 to H<sub>2</sub>O, to find out the limiting concentration where the different salts permit the beginning of the additional swelling. We notice that for Group I (type NaCl) this limiting concentration where the effect becomes positive is M/32; the salt with a weaker acid, Na acetate, and a weaker base, NH<sub>4</sub>Br, forming exceptions in an opposite sense—possibly due to hydrolytic effects. The limiting concentration for salts of Group II, type Na<sub>2</sub>SO<sub>4</sub>, is one-half of the preceding, namely M/64; for salts of Type III (CaCl<sub>2</sub>) and for Type IV it is M/256.

The relative values for these three groups are the same as for sodium gelatinate produced by the action of NaCl upon gelatin namely, NaCl: Na<sub>2</sub>SO<sub>4</sub>: CaCl<sub>2</sub> = 1: 2: 8.

The absolute values for the limiting concentration of these different salts for additional swelling are about but not quite twice as high for the sodium gelatinate produced by NaOH as for sodium gelatinate produced by NaCl.

#### *Theoretical Remarks.*

Our experiments were carried on with two types of gelatin salts, sodium gelatinate and gelatin chloride. The former is produced by the action of NaOH or NaCl, the latter by the action of HCl upon gelatin.

The reaction between the type sodium gelatinate and neutral salts takes place by the exchange of the cations; the anions of the neutral salts apparently not entering into the reaction with the gelatin. The reaction between gelatin chloride and neutral salts consists in an exchange between the anions, the cations apparently not participating in the reaction.

TABLE IV.  
*Action of Neutral Salts upon the Swelling of Sodium Gelatin Produced by the Action of NaOH upon Gelatin.*

Additional swelling of column of gelatin pertused twice with 25 cc. m/100 NaOH to produce sodium gelatin, and then three times with 25 cc. of the following solutions.

Type of salt.	Nature of salt.	m/8	m/16	m/32	m/64	m/128	m/256	m/512	m/1024	m/2048	m/4096	H <sub>2</sub> O
		mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
I. Univalent cation, univalent anion.	KBr.....	- 3	- 2.5 + 1	+ 9	+ 17	25	34.5	40	45	52	59	39
	Na acetate.....	- 1.5 + 1	+ 6.5 + 11	16.5	21.5	28.5	31.5	31.5	31.5	30.0	36	36
	NH <sub>4</sub> Br.....	- 4.5	- 8.0 - 2.0 + 1.0	5.5	12.5	12.5	51.0	55	64	59	46.6	63.5
	LiCl.....	- 2.5	- 1.0 + 3	10.5	12.0	18.5	29	49	39	37.5	43	
	KCNS.....	- 2.5	0 + 6.5	13	25.5	30.5	39	38				
	LiNO <sub>3</sub> .....	- 2	0 + 3	+ 10.5								
II. Univalent cation, bivalent anion.	Na <sub>2</sub> oxalate.....	- 2	- 1.5 + 2.5 + 7	+ 15	24	34	41	35	42	43	50	43
	K <sub>2</sub> SO <sub>4</sub> .....	- 8	- 4 - 1 + 4	+ 18	19	28	34	35.5	35.5	42.0	39.5	39.5
	K <sub>2</sub> tartrate.....	- 5	- 9.5 + 1.5 + 6.5 + 11.0	19.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
	Na <sub>2</sub> succinate.....	- 12.0	- 5.5 - 3.5 + 2.5 + 10.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
III. Bivalent cation, univalent anion.	MgCl <sub>2</sub> .....	- 7	- 7 - 6	- 6	- 4.5	0	+ 11	19	28	29	29	59
	CaBr <sub>2</sub> .....	- 12	- 11 - 10	- 10	- 7	+ 3	+ 9.5	19	32	37	37	46
	SrCl <sub>2</sub> .....	- 8	- 6.5 - 6	- 2	- 2	- 2.5	+ 15.5	15.5	27	35	35	47
	BaCl <sub>2</sub> .....	- 5	- 6 - 2.5	- 6.5	- 2	0	+ 13.5	16	27	30	30	47
IV. Bivalent cation, bivalent anion.	MgSO <sub>4</sub> .....	- 13	- 10	- 8.5	- 6.5	+ 10	19	29.5	40	45	62	62
	CaSO <sub>4</sub> .....					- 2.5 + 2.5	11	23	32.5	40.5	40.5	40.5

The gelatin compounds with univalent cation or anion, *e.g.* Na gelatinate and gelatin chloride, are capable of swelling, while the gelatin compounds with bivalent anion or cation, *e.g.* Ca gelatinate and gelatin sulfate, are not. The swelling of the former two types is apparently due to ionization and we may infer that the antagonistic effect of the Ca upon sodium gelatinate and of  $\text{SO}_4$  upon gelatin chloride is due to the formation of compounds not or less capable of ionization. Such an inference, though supported by certain facts, must, however, still be proved more directly before it can be accepted.

#### SUMMARY.

The new method described in previous publications for the determination of the effect of neutral salts upon the swelling of powdered gelatin has been applied to sodium gelatinate prepared by the action of  $\text{M}/100$  NaOH and to gelatin chloride prepared by the interaction of  $\text{M}/100$  HCl upon gelatin.

1. It was found that the additional swelling of gelatin chloride, produced by the action of  $\text{M}/100$  HCl upon gelatin, is inhibited in the solutions of neutral salts of univalent metals and univalent anions (type NaCl) in concentrations above  $\text{M}/64$  and by salts with bivalent metal and univalent anions (type  $\text{CaCl}_2$ ) in concentrations exactly half as high, namely above  $\text{M}/128$ , no matter what the nature of cation or anion. Salts with bivalent anion (type  $\text{Na}_2\text{SO}_4$ ) have a limiting concentration of a much lower order; namely,  $\text{M}/512$ .

2. It was found that the additional swelling of sodium gelatinate (produced by the action of  $\text{M}/100$  NaOH upon gelatin) is inhibited in the solution of neutral salts of the type NaCl when the concentration is above between  $\text{M}/32$  and  $\text{M}/64$ , and of salts of the type  $\text{Na}_2\text{SO}_4$  when the concentration is half as high, namely above between  $\text{M}/64$  and  $\text{M}/128$ , no matter what the nature of the cation or anion. Salts with bivalent cation, type  $\text{CaCl}_2$ , inhibit additional swelling in concentrations above between  $\text{M}/256$  and  $\text{M}/512$ .

3. It was found that the additional swelling of sodium gelatinate formed by the action of high concentrations ( $\text{M}/8$  or  $\text{M}/4$ ) of NaCl upon gelatin is inhibited in the solutions of salts with univalent metal and univalent anion (type NaCl) in concentrations above  $\text{M}/64$ , and by salts with bivalent anion and univalent

cation (type  $\text{Na}_2\text{SO}_4$ ) in concentrations exactly half as high; namely, above  $M/128$ . The limiting concentration of salts with bivalent cation (type  $\text{CaCl}_2$ ) is  $M/512$ . The neutral salts, therefore, produce the same type of compounds with gelatin as the bases; namely, metal gelatinates which dissociate into a positive metal and a negative gelatin ion.

4. These facts show that the limiting concentration of neutral salts for the additional swelling is within the restrictions mentioned independent of the nature of the anion and cation of the salt and that this limiting concentration upon the swelling of gelatin could be used to calculate roughly the molecular concentration of the salt used. The influence of neutral salts upon the swelling of gelatin is therefore of a stoichiometrical character.

5. When neutral salts act upon sodium gelatinates apparently only the cation of the salt combines with the gelatin; and when neutral salts react with gelatin chloride only the anion will combine with gelatin but not the cation. When neutral salts act upon ordinary gelatin, metal gelatinates are formed which may or may not contain the anion of the salt in non- or little dissociable bondage.

6. Our experiments show that gelatin salts with univalent anion (type gelatin chloride) or with univalent cation (type sodium gelatinates) are capable of additional swelling, while salts of gelatin with bivalent anion (type gelatin sulfate) and with bivalent cation (type calcium gelatinates) are not capable of additional swelling. It has been suggested in the previous paper that this swelling of gelatin salts with univalent cation or anion is due to a high degree of electrolytic dissociation, while the lack of the power of swelling of the gelatin salts with bivalent cation or anion is due to the low degree of electrolytic dissociation of these compounds.

7. Phenomena of antagonistic salt action can be produced by the transformation of protein salts of the type sodium gelatinates which are capable of additional swelling into salts of the type calcium gelatinates not capable of additional swelling; or by the transformation of protein salts of the type of gelatin chloride capable of additional swelling into salts of the type gelatin sulfate not capable of additional swelling.