LXXXI. THE PROPERTIES OF COLLOIDAL GUM BENZOIN.

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INTRODUCTION.

IN 1920 a new precipitation test for the cerebro-spinal fluid was introduced by Guillain, Laroche and Lechelle [1920]. This test is carried out in a manner similar to the Lange colloidal gold test [Lange, 1912]. A colloidal solution of Sumatra gum benzoïn¹ is added to an equal volume of salt-solution containing varying amounts of cerebrospinal fluid. In certain of the tubes, flocculation and sedimentation of the 'benzoïn' takes place, and from this, indications are said to be obtained of the nature of the disease. The zones of precipitation vary in position but more particularly in intensity, and are difficult to explain. It was with the hope of obtaining some knowledge of the mechanism of these precipitations that an investigation of the colloidal properties of 'benzoïn' sol was undertaken.

Macheboeuf [1921] and Guillain, Laroche and Macheboeuf [1921] have determined that the charge on the particles in the sol is negative, but otherwise very little information is available on the subject.

In view of the fact that the behaviour of gelatin has been so elaborately studied and that this material could be obtained in relatively pure form by Loeb's [1922, 1] method this protein was first used in order to gain information as to the effect of proteins on 'benzoïn.' The interesting fact was discovered, that in great dilution gelatin caused precipitation of the sol. This property, it has been found, is not peculiar to gelatin, and a similar precipitation occurs in presence of very dilute solutions of other proteins, *e.g.* haemoglobin, of the proper concentration. Neisser and Friedemann [1904] and Bechhold [1904] had shown that small amounts of gelatin rendered mastic precipitable by amounts of sodium chloride which, alone, were incapable of causing precipitation. Similar effects had also been noted by Walpole [1913] regarding the interaction of acid, gelatin and mastic sol or an emulsion of oil in water. Zsigmondy in 1901 discovered that proteins had a definitely protective action against the precipitation of solutions by electrolytes while, according to Zunz [1904], certain proteoses are, on the other hand, capable of causing precipitation. The 'benzoïn' sol therefore showed itself to be somewhat remarkable

¹ Afterwards in this paper referred to as 'benzoïn.'

in that it is precipitated by gelatin, without acid or salt, and, accordingly, this point has been more fully investigated. It is, however, to be noted that Gann [1917] was able to demonstrate precipitation by gelatin of gold sols which had an acid reaction but not of sols which were alkaline and considered that the hydrogen ion concentration was an important factor. In a recent summary of experimental results Pauli [1922] has stated that pure proteins, free from electrolytes, have no protective power but, on the other hand, cause precipitation of colloids.

From the study of the effect of proteins in precipitating the sol, we have been led to investigate its precipitation by electrolytes. It is well known that the power of electrolytes to cause precipitation of a colloid depends largely on the valency. This is well shown by results obtained by Tartar and Gailey [1922] who found that in the case of mastic sol precipitation by acid always occurred at a p_H of about 2.6, independently of the actual acid used, or the actual amount added. At any p_H greater than 2.6, no precipitation occurred. We have obtained exactly similar results with a 'benzoin' sol, so that a protective effect of the acid anion would not appear to occur. Tartar and Gailey have also shown, using the same mastic sol, that in precipitation by salts, the p_H of the salt solution is of great importance and may largely account for differences attributed to a special protective effect of the anion. With, for example, sodium acetate, the p_H is high, and high concentrations of the salt are required to precipitate, but if the p_H is brought to a constant value by adding the acid corresponding to the salt, *e.g.* acetic acid in the case of sodium acetate, the same equivalent amount of salt is effective. We have treated the question from a slightly different point of view, as we have found the amounts of a standard electrolyte, sodium chloride, required to precipitate at various hydrogen ion concentrations, and compared the amounts of other salts necessary to precipitate with the amount of sodium chloride required at the p_H produced by the new salt. Our results, it will be seen, confirm those of Tartar and Gailey.

Closely connected with the precipitating effect of gelatin on 'benzoin' is its protective power. This protective action of gelatin is of course well known and has been extensively investigated, particularly with regard to the gold sol. From our observation on the effect of gelatin on 'benzoin' it will be seen that considerably higher concentrations of gelatin are required to protect than to precipitate, confirming the statement made by Neisser and Friedemann.

The obvious suggestion is that with increasing concentrations of gelatin, increasing amounts are absorbed on the 'benzoin' particles. If the gelatin is positively charged, the negative charge on the 'benzoin' particles should decrease, until the zone of precipitation occurs. With more gelatin, the particles now coated with gelatin ought to be positively charged and they should commence to show protection. Full protection should set in when sufficient gelatin is absorbed to form a film round the particles, that is, at higher concentration than is sufficient to cause precipitation. If, however, the gelatin is negatively

charged, no such zone of precipitation ought to occur. According, however, to the work of Loeb [1922, 1] gelatin is positively charged on the acid side of the isoelectric point ($p_H = 4.7$), and negatively charged on the alkaline side of the isoelectric point. Hence, on the alkaline side of the isoelectric point, no precipitation ought to occur. It was, therefore, of the greatest interest to investigate the effect of change of the p_H on the precipitating and protecting effect of gelatin. The details will be found in the experimental part of the paper, but it may be said that, in general, the results to be expected are obtained. In particular, the zone of precipitation of 'benzoin' by gelatin suddenly disappears when the p_H exceeds 4.7. On the other hand, if oxyhaemoglobin, which behaves similarly to gelatin in precipitating 'benzoin,' is used, the zone persists until the p_H is over 6.

The experimental work which follows naturally divides itself into four parts:

1. General.
2. Precipitation of the sol by electrolytes.
3. Precipitation of the sol by gelatin.
4. Protection of the sol by gelatin.

I. GENERAL.

The 'benzoin' sol used by Gullain, Laroche, and Lechelle [1922], is prepared from an alcoholic extract of Sumatra gum benzoin, 10 g. of powdered crude Sumatra gum benzoin and 100 cc. of absolute ethyl alcohol, being allowed to stand at ordinary temperature for forty-eight hours. 0.3 Cc. of the filtered extract, which can be kept indefinitely, is added drop by drop with vigorous stirring to 20 cc. of distilled water at 35°. The white milky sol obtained is cooled to room temperature when it is ready for use. As, however, it was not homogeneous even to the naked eye but possessed a scum of fine particles, slight modifications were introduced in order to obtain a uniform sol for experimental purposes. After a few experiments it was found that the sol, if prepared at 55° instead of 35°, could be filtered readily through a folded No. 1 Whatman filter paper, whereas the sol prepared at 35° did not filter readily and yielded a very weak filtrate. Accordingly, the sol used in the following experiments was prepared by adding, at 55°, 0.3 cc. of the alcoholic extract drop by drop to 20 cc. of water, with vigorous stirring, care being taken to drop the extract directly into the liquid without touching the side of the vessel. The fluid obtained was filtered at once through a folded filter paper, and the filtrate used after cooling to room temperature. In the initial stages of the work, fresh sol was prepared for each experiment, but later it was found that this was not necessary as the properties of the sol did not change on standing for a few days.

The sol so prepared is slightly less sensitive to precipitation by sodium chloride than is the original, as shown by the following table. 1 Cc. of sol is

added to each of a series of small test-tubes, each containing 1 cc. of a solution of sodium chloride. The tubes are immediately shaken and allowed to stand overnight at room temperature. The strengths of the solutions of sodium chloride in the various tubes are given.

Table I.

Effect of mode of preparation on sensitiveness of sol to salt precipitation.

Concentration of NaCl	0.01 %	0.02 %	0.03 %	0.04 %	0.05 %	0.06 %
Sol prepared at 35°, unfiltered	0	2	4	4	4	4
„ 35°, filtered	0	0	0	0	2	4
„ 55°, unfiltered	0	0	2	3	4	4
„ 55°, filtered	0	0	0	0	4	4

The degree of precipitation in the tubes in these and in the following experiments is denoted by numbers:

4 = complete precipitation;

3 = almost complete precipitation, a slightly opalescent fluid being left;

2 = partial precipitation, the fluid being milky;

1 = slight precipitation, some of the precipitated sol being visible at the foot of the tube, but little change being observable in the liquid left. Sometimes differences in opacity were observed, without definite flocculation or sedimentation, but usually these have been neglected.

The test-tubes were of hard glass, and before being used in any experiment were thoroughly cleaned by standing at least sixteen hours in sulphuric acid-dichromate mixture, followed by washing out each one separately about twelve times in tap-water, after which they were immersed for some time in tap-water and then in freshly distilled water. They were touched throughout only with clean nickel tongs. They were then allowed to drain in crates lined with filter paper.

Unless otherwise stated, the tubes were well shaken after the various solutions had been added, and were then allowed to stand overnight, so that they were read the following day.

The sol, as prepared for these experiments, is an apparently homogenous, milky fluid, of great stability. No apparent settling takes place even after standing for weeks. The sol is definitely acid, 0.3 cc. of *N*/50 NaOH being required to neutralise 10 cc. of it (using methyl red as indicator). When seen under the ultra-microscope, the 'benzoin' particles appear of fairly uniform size, in vigorous Brownian movement, and the observation of Macheboeuf [1921] that they are negatively charged has been verified repeatedly. A very rough count indicated that the diameters of the particles were of the order 0.3μ ; precise estimations were not attempted. They may be seen with the ordinary dark ground illumination apparatus.

The determination of hydrogen ion concentration in the following experiments was carried out colorimetrically using the Clark-Lubs sulphonaphthalein series of indicators, an electrometric apparatus not being available [Clark,

1920]. This meant that determinations could be made only in the supernatant fluid, after precipitation had occurred, as it did not appear possible to make readings in the presence of the sol. It is unlikely, however, that the hydrogen ion concentration of the supernatant fluid differs from that of the mixture before precipitation, as it is shown later that in the case of precipitation by calcium chloride or by silver nitrate, the amount of calcium or silver carried down with the precipitate is at most very small. Now a change of 0.1 in the p_H means a change of about 25 % in the actual hydrogen ion concentration, and so it is very improbable that hydrogen ions are absorbed by the precipitate in quantities sufficient to cause any significant change in the p_H .

Readings were at first made with buffer mixtures, but it was found that very good readings were obtained by the use of the coloured plate given in the book by Clark [1920]. With practice good and consistent readings are obtained, and most of the readings recorded have been obtained by this method.

II. PRECIPITATION OF THE SOL BY ELECTROLYTES.

Acids. Tartar and Gailey [1922] found that the precipitation of mastic sol by acids was largely if not entirely, dependent on hydrogen ion concentration, precipitation occurring when the p_H of the solution was less than 2.6 or 2.8. The following experiment shows that 'benzoin' gives a similar result.

To 5 cc. of acids of various strengths was added 5 cc. of 'benzoin' sol. After precipitation was complete the p_H was determined in the supernatant fluid of the tube which showed complete precipitation in the presence of the smallest amount of acid.

The results obtained were as follows:

Hydrochloric acid	2.6
Acetic	2.8
Sulphuric	2.6
Oxalic	2.8
Citric	2.8

It will be seen that, within the experimental error of the methods employed, precipitation by acids depends on the hydrogen ion. The mineral acids appear to be slightly less effective than the organic acids, but it is very doubtful if the difference has any significance. In any case, it is usually the ions of the organic acids that are said to exert antagonistic and protective action [cf. Taylor, 1920], as much larger amounts of equivalent solutions of the sodium salts of these acids are required to precipitate than of sodium chloride. They certainly seem to exert no protective action against precipitation by the hydrogen ion. It would, in fact, appear that organic anions as such do not protect, but that, as Tartar and Gailey suggest, the anomalous behaviour of their salts is due to alkalinity.

To investigate this further a large number of experiments has been carried out. Determinations have been made of the minimum amounts of various salts which, in a volume of 1 cc., were required to produce precipitation of 1 cc.

of 'benzoin' sol. The maximum amount failing to produce precipitation is also recorded. In Table II are recorded the percentage strength of the solutions added and also the amount of salt in these solutions calculated in terms of gram equivalents $\times 10^6$ (b and b_1 in Table II).

Table II.

Precipitation by electrolytes.

	Maximum salt concentration not giving precipitation		Minimum salt concentration giving precipitation		p_H of supernatant fluid
	a = actual concentration of salt solution	b g. Eq. $\times 10^6$ per cc.	a_1 = actual concentration of salt solution	b_1 g. Eq. $\times 10^6$ per cc.	
	%		%		
NaCNS	0.025	3.1	0.05	6.2	4.2
NaBr	0.05	4.9	0.075	7.3	4.2
$\text{Na}_2\text{Fe}(\text{CN})_6\text{NO} \cdot 2\text{H}_2\text{O}$	0.075	5.0	0.125	8.4	4.2
$\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$	0.14	8.7	0.15	9.3	—
$\text{NaI} \cdot 2\text{H}_2\text{O}$	0.125	6.7	0.175	9.4	4.2
NaIO_3	0.15	7.6	0.2	10.1	4.2
NaCl	0.04	6.8	0.06	10.2	4.2
NaClO_3	0.075	7.0	0.125	11.7	4.2
NaNO_3	0.05	5.9	0.1	11.8	4.2
NaClO_4	0.125	10.2	0.2	11.3	4.2
$\text{Na}_2\text{Fe}(\text{CN})_6 \cdot 12\text{H}_2\text{O}$	0.15	11.5	0.225	17.3	5.4
NaF	0.05	11.9	0.875	20.8	4.5
$\text{Na}_2\text{C}_2\text{O}_4$	0.2	29.8	0.25	37.3	5.4
Na_2 tartrate $\cdot 2\text{H}_2\text{O}$	0.3	26.3	0.5	43.8	5.4
NaNO_2	0.15	21.7	0.3	43.5	5.6
Na acetate $\cdot 3\text{H}_2\text{O}$	1.0	73.5	2.0	147.1	6.8
Na_3 citrate $\cdot 5\frac{1}{2}\text{H}_2\text{O}$	1.5	126.1	2.0	168.1	7.4
NaCN		No precipitation up to 5 % NaCN			
$\text{K}_2\text{Cr}_2\text{O}_7$	0.005	0.34	0.015	1.0	3.8
$\text{K}_2\text{Fe}(\text{CN})_6$	0.0625	5.7	0.075	6.8	4.0
KIO_3	0.1	4.7	0.15	7.0	4.2
KBr	0.0875	7.3	0.1	8.4	4.2
K_2SO_4	0.05	5.7	0.075	8.6	4.2
KI	0.1	6.0	0.15	9.0	4.0
KCl	0.06	8.0	0.07	9.4	4.2
KNO_3	0.05	4.9	0.1	9.8	4.0
KClO_3	0.1	8.2	0.125	10.2	4.2
KCNS	0.075	7.7	0.125	12.9	4.2
$\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$	0.15	14.2	0.2	19.0	5.2
KF $\cdot 2\text{H}_2\text{O}$	0.14	14.8	0.18	19.1	4.3
K_2 oxalate $\cdot \text{H}_2\text{O}$	0.2	24.1	0.3	36.1	5.2
K_3 citrate $\cdot \text{H}_2\text{O}$	1.0	92.5	2.0	185.0	7.2

It will be seen that the figures obtained for salts with univalent cations were not constant. This is in agreement with the experimental results of others. Measurements were made of the p_H of the supernatant fluid in the tubes which just showed complete precipitation. It was then found that there was a marked relationship between the p_H of the solution and the amount of the salt required to produce precipitation of the solution.

This agrees well with the results of Tartar and Gailey [1922] for mastic sol. They added equivalent amounts of various salts to the sol and then determined the p_H to which the mixtures required to be brought in order to cause precipitation. It was found to be the same in all cases. In view of this it became of interest actually to determine the effect of p_H on precipitations

of 'benzoin' by sodium chloride. Solutions of alkali and acid of various strengths were prepared and 0.5 cc. of these added to each of a row of tubes. To each tube in the various rows were then added 0.5 cc. of sodium chloride solution of increasing strength and then 1 cc. of 'benzoin.' After precipitation was complete, the p_H of the supernatant fluid was measured in the tube showing complete precipitation with the smallest amount of sodium chloride in each row (see Table III). The amount of sodium chloride present was

Table III.

Effect of p_H on precipitation by NaCl.

Concentration of NaCl

		4 %	2 %	1 %	0.5 %	0.25 %	0.125 %	0.06 %	0.03 %	0.015 %	0.007 %	0.0037 %	0.0019 %
Concen- tration of hydrochloric acid	N/400	—	—	—	—	4	4	4	4 (p_H 2.8)	4	4	4	4
	N/800	—	—	—	—	4	4	4	4 (p_H 3.2)	3	2	0	0
	N/1600	—	—	—	—	4	4	4 (p_H 3.4)	4	2	0	0	0
	N/3200	—	—	—	—	4	4	4 (p_H 3.6)	2	0	0	0	0
	N/12800	—	—	—	—	4	4	4 (p_H 3.8)	0	0	0	0	0
	Distilled water	—	—	—	—	4	4	4 (p_H 4.1)	0	0	0	0	0
	N/12800	—	—	—	—	4	4	4 (p_H 4.4)	0	0	0	0	0
	N/6400	4	4	4	4	4	4 (p_H 4.4)	1	0	0	0	0	0
	N/3200	4	4	4	4	4	4 (p_H 4.6)	0	0	0	0	0	0
	N/2500	4	4	4	4	4	4 (p_H 4.6)	0	0	0	0	0	0
Concentration of sodium hydroxide solutions	N/2000	4	4	4	4	4 (p_H 5)	0	0	0	0	0	0	0
	N/1600	4	4	4	4	4 (p_H 5)	0	0	0	0	0	0	0
	N/1400	4	4	4 (p_H 6.3)	2	0	0	0	0	0	0	0	0
	N/1200	4	4	4 (p_H 6.4)	0	0	0	0	0	0	0	0	0
	N/800	4	4	3 (p_H 6.8)	0	0	0	0	0	0	0	0	0
	N/600	4	4 (p_H 7.2)	0	0	0	0	0	0	0	0	0	0
	N/400	4 (p_H 7.4)	3	0	0	0	0	0	0	0	0	0	0
	N/200	4 (p_H > 9.5)	0	0	0	0	0	0	0	0	0	0	0

calculated. It was found that the amount of sodium chloride required to produce precipitation increased with the increase in p_H . It is, therefore, necessary in order to compare the efficiency of salts as precipitants to do so at the same p_H . In Fig. 1 curves have been drawn representing the amount of sodium chloride required at various p_H . With this is recorded a curve in which the amount of different sodium salts required to produce precipitation is plotted against the p_H of the supernatant fluid in the tube where complete precipitation just occurs.

It will be seen that though the correspondence of the curves is not quite exact, the p_H may at least account for most of the discrepancies between the various salts.

In the case of sodium citrate (p_H 7.4) the figure found is 192, whereas at that p_H the amount of sodium chloride required is 250. The difference is probably not more than the experimental error, which is large because of the small degree of buffering of the sodium chloride solutions. Moreover, the colorimetric estimations are only approximate.

One or two interesting points which arise from our experiments may be commented on here.

With ferric chloride difficulties in estimating the minimum amount of salt required to precipitate were encountered, because two zones of precipitation occurred. In the zone at higher concentrations (0.1 % solution) the p_H is 2.8, a degree of acidity which could of itself produce precipitation. In the second

Fig. 1. Minimum concentrations of salts required to precipitate when added to equal volume of 'benzoin' sol.

×. Concentrations of sodium chloride, the p_H being adjusted by addition of acid or alkali.

⊗. Concentrations of various salts as detailed in Table II. p_H is that of supernatant fluid after precipitation of 'benzoin.'

Curve A represents concentrations of sodium chloride just sufficient to cause precipitation at various p_H .

Curve B is drawn from results obtained with various salts other than sodium chloride.

zone of precipitation (at 0.0015 %) the precipitation is possibly due to colloidal ferric hydroxide as in the intermediate region no precipitation occurs and the 'benzoin' particles have a positive charge. Similar results were obtained by Neisser and Friedemann [1904] with 'mastic' sol.

With aluminium sulphate, however, between concentrations of 10 % and 0.00007 % no such second zone appeared as in the case of ferric chloride.

Indeed aluminium sulphate precipitates the sol at all concentrations greater than that at which it first produces precipitation, and causes no reversal of sign of charge on the particles. Such reversal of sign in the case of ferric chloride is possibly due to adsorption of colloidal iron hydroxide on the particles. At the low concentration used, most of the ferric chloride will be hydrolysed, and so will probably be present as ferric hydroxide sol. In that case the phenomena observed will be similar in nature to the precipitation observed with gelatin, and it is being investigated at greater length.

It is to be noted that no precipitation was obtained with mercuric chloride in concentrations up to 10 %, presumably because of the low degree of ionisation of this salt. Similar results have been previously obtained using other sols, and this very strongly indicates the ionic nature of the precipitation.

With sodium cyanide and potassium cyanide no precipitation was obtained. This is no doubt due to the strong alkalinity of the solutions.

Experiments were carried out with silver nitrate and also calcium chloride solutions as precipitants of 'benzoin' in order to determine any change in the salt concentration in the supernatant fluid after precipitation of the sol.

Calcium chloride solution (approx. 0.06 %) was added to an equal quantity of the sol. After standing overnight precipitation of the sol was complete. The clear supernatant fluid was pipetted off. The amount of calcium present was estimated by the usual method—precipitation as oxalate and titration of the oxalic acid with potassium permanganate solution. In 150 cc. of the supernatant fluid was found 0.0131 g. of calcium. An estimation of the calcium present in 150 cc. original solution gave 0.0259 g. Ca, or, diluted one in two, 0.0130 g. So that, within the limits of experimental error the determinations were identical.

Similar results were obtained when silver nitrate was used as the precipitating salt, the silver in this case being estimated volumetrically.

The amount of the precipitating ion removed during the process of precipitation was apparently too small to be detected by the methods. As noted above (p. 639), these results give a basis for the practice of measuring the p_H of the supernatant fluid as the p_H at which precipitation actually takes place.

Effect of Concentration of Sol on Precipitation by Electrolytes.

Burton and Bishop [1920] and Burton and McInnes [1921] have formulated the following laws:

- (1) In the case of univalent ions, the amount required to cause precipitation increases as the solution is diluted.
- (2) In the case of bivalent ions, no such increase is observed.
- (3) In the case of tervalent ions, the amount required is approximately proportional to the concentration of the solution.

Weiser and Nicholas [1921] have, however, cast doubt on the general validity of these laws, a doubt which is apparently shared by Kruyt and van der Spek [1919]. We have determined the minimum concentrations of sodium

chloride, copper sulphate and aluminium sulphate solutions required to produce precipitation when added to 'benzoin' sols of the following strengths:

(1) 'Benzoin' of double¹ the usual strength (*i.e.* 3 cc. of alcoholic extract added to 100 cc. of water).

(2) 'Benzoin' of double the usual strength, diluted 1 in 2 with distilled water.

(3) 'Benzoin' of double the usual strength, diluted 1 in 20 with distilled water.

The results are given in Table IV, and the amount of sodium chloride required in the case of 'benzoin' of the usual strength is given for comparison.

Table IV.

Effect of concentration of sol on precipitation by kations of different valency.

Nature of sol	Concentration of solution which produces precipitation when added to an equal volume of sol		
	NaCl %	CuSO ₄ %	Al ₂ (SO ₄) ₃ %
'Benzoin,' ordinary strength	0.05-0.06		
" double "	0.04-0.05	0.03-0.05	0.007-0.015
" " " diluted 1/2	0.05-0.08	0.04-0.06	0.007-0.015
" " " " 1/20	0.1-0.2	0.08-0.1	0.015-0.03

It would appear that these laws are not here applicable.

In all cases, an increased concentration of salt is required to precipitate diluted solutions.

Dilute sols are also apparently slightly less sensitive to precipitation by hydrogen ions. Direct experiment showed a slight but constant difference between the p_H required to precipitate a concentrated solution and that effective for a dilute one. Whereas a double strength 'benzoin' sol is precipitated when a p_H of 2.8 is reached, the same sol diluted 1 in 20 requires a p_H of 2.6 to precipitate it. This shows that the greater sensitiveness of the stronger solution to salts is not due altogether to its higher p_H , although there is actually a slight difference in the p_H of the concentrated and dilute solutions. The following experiment in which the salt content is kept constant and the p_H varies, also shows that this difference in p_H is not sufficient to account for the difference in sensitiveness to electrolytes. 1 Cc. of a dilute sol (double strength diluted 1 in 10) was added to each of ten tubes, each containing 1 cc. of sodium chloride of the concentration (0.06 %) required to produce precipitation in an ordinary sol at a p_H of 4.2. The sodium chloride solution contained acetic acid of strengths 1 %, 0.5 %, 0.25 %, etc., in the various tubes so as to vary the p_H . Precipitation appeared in those tubes having a p_H of 3.6 or less and not in the others. The dilute sol, therefore, appears definitely less sensitive to electrolyte precipitation than the normal one, which is precipitated by an equal volume of 0.06 % sodium chloride at a p_H 4.2.

¹ This 'benzoin' sol double the usual strength was used so that, when diluted 1 in 20, the resulting liquid was still opaque enough to show precipitation readily when that had occurred.

III. PRECIPITATION OF 'BENZOIN' BY GELATIN AND OXYHAEMOGLOBIN.

As mentioned in the introduction it has been found that gelatin at certain dilutions produces precipitation of 'benzoin' sols. A 1% solution of iso-electric gelatin was prepared by the method described by Loeb [1922, 1]. On ignition this was found to leave an ash weighing 0.08% of the dry gelatin. A series of dilutions of gelatin in distilled water was prepared varying from 1/10,000, 1/20,000 to 1/5,120,000. Each tube contained 1 cc. of gelatin solution and to this was added 1 cc. of 'benzoin' sol. Precipitation occurred in the tubes containing gelatin solution 1/320,000 and 1/640,000 and in the tubes containing gelatin solutions above and below these concentrations no precipitation occurred.

A series of experiments was carried out to study the effect of variation of p_H on the zone of precipitation. For the purpose a series of dilutions of gelatin, hydrochloric acid and sodium hydroxide were prepared. To each of a series of tubes were added 0.5 cc. of gelatin solution of various strengths, 0.5 cc. of the appropriate acid or alkali solution and finally 1 cc. of 'benzoin.' Readings were made when precipitation was complete and the p_H was estimated in the supernatant fluid. The figures in Table V represent the concentrations of acid or alkali and of gelatin in the mixture before addition of the 'benzoin.' The concentrations originally prepared were double that recorded.

Table V.

Precipitation of 'benzoin' by gelatin.

		Concentration of gelatin	1/2500	1/5000	1/10,000	1/20,000	1/40,000	1/80,000	1/160,000	1/320,000	1/640,000	1/1,280,000	1/2,560,000	1/5,120,000	No gelatin
Concentration of sodium hydroxide	N/800	0	0	0	0	0	0	0	0	—	—	—	—	—	—
	N/1600	0	0	0	0 (-)	0 (-)	0	0	0	—	— (-)	—	—	—	—
	N/3200	0	0 (+)	0	0 (+)	1 (?+)	4	4 (p_H 4.7)	1	4 (p_H 4.1)	1	0	0	—	0
	N/12800	0	0 (+)	0	0 (+)	0 (+)	0 (+)	0	0	3	4 (p_H 4.1)	0	0 (-)	—	0
	N/51200	0	0	0	0	0	0	0	0	3	4 (p_H 4.1)	0	0	—	0
Concentration of hydrochloric acid	H ₂ O	0	0	0	0 (+ +)	0	0	0 (+)	2	4 (p_H 4.1)	4 (p_H 4.1)	0	0	—	0 (-)
	N/51200	0	0	0	0	0	0	0	1	4 (p_H 4.1)	4 (p_H 4.1)	1	0	—	0
	N/12800	—	—	—	—	0	0	0	1	4 (p_H 3.8)	1	0	0	—	0
	N/3200	—	—	—	0 (+ + +)	0	0 (+ +)	0	1	4	4 (p_H 3.6)	2	0	0	0
	N/1600	—	—	—	—	—	—	0	1	4	4 (p_H 3.5)	3	0	0	0
	N/800	—	—	—	—	—	—	0	1	4	4 (p_H 3.2)	4	3	0	0
	N/400	—	—	—	—	—	—	0	1	4	4 (p_H 2.8)	4	4	4	4
	N/200	—	—	—	—	—	0	0	0	1	—	—	—	—	—
	N/100	—	—	—	—	—	0	0	1	2	—	—	—	—	—
	N/40	—	—	—	—	—	0	1	3	4	—	—	—	—	—
	N/10	—	—	—	0	0	1	4	4	4	—	—	—	—	—

(+) and (-) indicate sign of charge on the particles.

It may be noted that in these experiments as in those on precipitation by electrolytes, the p_H recorded is that of the supernatant fluid. The 'benzoin' solution is itself acid, and so the p_H of the gelatin solution before the 'benzoin' is added is of no value in giving the p_H at which the precipitation actually occurs. The p_H of the supernatant fluid suggests itself as the best measurement of the p_H at which precipitation occurs.

Fig. 2 shows the p_H plotted against concentration of gelatin. It will be seen that as the acidity is increased a decrease occurs in the concentration of gelatin at which precipitation takes place until a p_H of 2.8 is reached when the zone

Fig. 2. Illustrating effect of p_H on precipitation of 'benzoin' by gelatin. (N.B. The concentration of gelatin given is that of solutions before addition of equal volume of 'benzoin' sol.)

effect disappears, the acid itself precipitating the 'benzoin' solution, the gelatin now appearing in the rôle of a protective colloid. On increasing the p_H on the other hand the precipitating zone is found in higher concentrations of gelatin. As the isoelectric point of gelatin is reached (p_H 4.7) the zone broadens, but when this is passed no further precipitation of the 'benzoin' is observed.

Precipitation of 'Benzoin' by Oxyhaemoglobin.

In view of the interest attached to the precipitation of 'benzoin' by gelatin, more particularly with regard to the importance of the isoelectric point, and its predominance in the effect, it appeared to be very desirable to investigate in a similar way, a protein having a markedly different isoelectric point. Serum globulin and serum albumin were both investigated; precipitation took place at a p_H up to the isoelectric point, and beyond this, there was no precipitation. This, however, will be discussed at greater length in the succeeding paper. Oxyhaemoglobin is of greater interest as its isoelectric point differs from that of gelatin and is given as 6.6, and, therefore, these zones of precipitation should not exist at p_H exceeding 6.6, but should exist up to about that point.

A solution of oxyhaemoglobin was prepared by washing well with 0.8 % sodium chloride solution, red blood cells obtained from citrated sheep's blood, so that the washings finally gave no test for protein. The cells, which weighed moist 8.01 g., were added to distilled water, and the resulting oxyhaemoglobin solution dialysed for over thirty-six hours in a collodion sac against running tap-water. The final volume of oxyhaemoglobin solution was 85 cc. This is of course not quite pure oxyhaemoglobin solution but, particularly in high dilutions, the amount of impurity present is probably very small.

An experiment exactly similar to the experiment with gelatin described on p. 645 was carried out with this solution. The results are given in Table VI. It may be remarked that variations in different experiments were

Table VI.

Precipitation by Oxyhaemoglobin.

	Concentration of oxyhaemoglobin	1/625	1/12·5	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/25600	1/51200	1/102400	1/204800	1/409600
Concentration of sodium hydroxide	N/200	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	0
	N/400	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	0
	N/800	4	2	0	4 (pH 6·3)	2	0	0	0	0	0	0	0	0	0	0	0	0
	N/1000	4	4	2	4 (pH 6·4)	2	0	0	4 (pH 6·1)	0	0	0	0	—	—	—	—	—
	N/1200	4	4	4	4	4 (pH 6·2)	2	2	4 (pH 6·4)	0	0	0	0	—	—	—	—	—
	N/1400	4	4	4	4	0	0	0	4 (pH 5·7)	4 (pH 5·6)	0	0	0	—	—	—	—	—
	N/1600	4	4	4 (pH 5·8)	0	0	0 (+)	0	4	4 (pH 5·8)	0	0	0	0	—	—	—	—
	N/3200	—	—	—	—	—	0	0	0	0	4 (pH 4·8)	4	0	0	—	—	—	—
	N/6400	4	1	0	0	0	0	0	0	0	0	4 (pH 4·8)	2	0	0	0	—	—
	N/12800	—	—	—	—	—	—	—	0	0	0	4 (pH 4·4)	0	0	0	0	—	—
Distilled water	—	—	—	—	—	—	—	—	0	0 (+)	0	4 (pH 4·2)	4	4	0	0 (-)	—	—
Concentration of hydrochloric acid	N/3200	—	—	—	—	—	—	—	—	0	0	0	4 (pH 3·8)	4	0	0	0	—
	N/1600	—	—	—	—	—	—	—	—	—	0	0	4 (pH 3·4)	4	0	0	0	—
	N/800	—	—	—	—	—	—	—	—	—	—	0	4 (pH 3)	4	4	3	0	—
	N/400	—	—	—	—	—	—	—	—	—	—	0	2	4 (pH 2·8)	4	4	4	0

It will be seen that the zone of precipitation which at a p_H of 3.8 appears in the tube containing the oxyhaemoglobin solution diluted 1/12,800 gradually moves to the left with increasing p_H until the p_H rises to about 6.64, when

Fig. 3. Illustrating effect of p_H on precipitation of 'benzoin' by oxyhaemoglobin. (N.B. The concentration of oxyhaemoglobin is given in terms of an original solution prepared as described in the text, and is the concentration before addition of an equal volume of 'benzoin' sol.)

precipitation occurs at 1/800. A zone of precipitation at this p_H occurs with all concentrations of haemoglobin solution down to 1/6.25. The absence of any precipitation beyond the isoelectric point and presence of it up to the isoelectric point is very marked.

The effect of alteration in the reaction of the gelatin or oxyhaemoglobin in precipitation of 'benzoin' is a peculiar one. There is a general change in the position of the zone of precipitation with change of p_H . This is readily understood if it be assumed that the primary effect of change of the p_H is on the protein. In accordance with the work of Loeb gelatin exists on the acid side of the isoelectric point as a positively charged kation, on the alkaline side as an anion. If the effective precipitating agent is the positively charged gelatin ion it can be understood that little or no precipitation will occur on the alkaline side of the isoelectric point. The alteration in the position of the zone of precipitation is probably due to the fact that an optimum concentration of the gelatin is required for precipitation. This amount will be found at lower concentrations as the p_H decreases, as a greater part of the gelatin exists with a positive charge at the higher acidity.

That there may be some effect on the sol must be granted as acid in excess causes its precipitation, while excess of alkali dissolves it. But that this effect is not the primary one is shown by an experiment in which varying amounts of sodium chloride solution are added to the gelatin, instead of acid.

Table VII shows the result of such an experiment.

Table VII.

Influence of sodium chloride on the precipitation of 'Benzoin' by Gelatin.

The concentrations given are those of the sodium chloride and gelatin after mixing and before adding the 'benzoin.'

Concentration of sodium chloride	Concentration of gelatin												
	1/2000	1/4000	1/8000	1/16,000	1/32,000	1/64,000	1/128,000	1/256,000	1/512,000	1/1,024,000	1/2,048,000	1/4,096,000	Water
1.0 %	0	0	2	3	4	4	—	—	—	—	—	—	—
0.5 %	0	0	1	2	4	4	—	—	—	—	—	—	—
0.1 %	0	0	0	1	4	4	4	—	—	4	4	4	4
0.04 %	0	0	0	0	1	4	4	4	4	4	3	0	0
0.02 %	0	0	0	0	0	4	4	4	4	3	0	0	0
0.005 %	0	0	0	0	0	0	3	4	4	3	0	0	0
0	0	0	0	0	0	0	0	4	4	0	0	0	0

It will be seen that the effect of sodium chloride on the zone of precipitation of 'benzoin' by gelatin is merely to widen it. Sodium chloride at such concentrations presumably has little effect on gelatin. It has at least no such powerful influence in altering its properties as has acid or alkali. On the other hand, the effects of sodium chloride and of hydrochloric acid on the 'benzoin' solution in the absence of gelatin are qualitatively the same, they either precipitate it or increase its sensitiveness. Hence there seems to be little doubt that the action of the acid in the above experiment must be primarily on the gelatin, because, if it were on the 'benzoin,' we should expect an effect, similar to that of the salt. This of course is also borne out by the determining influence of the isoelectric point of the protein.

Effect of Concentration of the Sol on Precipitation by Gelatin.

Neisser and Friedemann [1904] and Biltz [1904] showed that a quantitative relationship held in the mutual precipitation of oppositely charged colloids, and that this is related to a neutralisation of the charge on the particles or to a reduction of the potential differences between the disperse phase and the continuous phase. If the precipitation of 'benzoin' (negatively charged) by gelatin (positively charged) is of this nature, change in the concentration of 'benzoin' ought to cause a similar change in the concentration of gelatin at which precipitation occurs. That this actually occurs is shown by an experiment, the results of which are given in Table VIII.

Table VIII.

Effect of concentration of sol on precipitation by Gelatin.

1 cc. of the gelatin solution was added to 1 cc. of 'benzoin' solution, the mixture shaken and allowed to stand overnight.

Concentration of gelatin solution $\times 10^4$	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
'Benzoin,' ordinary strength	0	0	0	0	4 (p_H 4.2)	4	0	—	—	—
" double	0	0	0	4	4 (p_H 4.2)	2	0	0	—	—
" " " diluted 1/2	0	0	0	0	4 (p_H 4.2)	4	0	0	—	—
" " " " 1/4	0	0	0	0	4	4 (p_H 4.4)	0	0	0	—
" " " " 1/20	0	0	0	0	0	2	4 (p_H 4.6)	0	0	0

It will be seen that a sol in a dilution of 1/20 of double strength (= 1/10 dilution of ordinary strength) requires gelatin 1/1,280,000 to produce maximum precipitation (p_H 4.6). Referring to Table V it will be seen that the maximum precipitation of a sol of ordinary strength with this p_H occurs in a concentration of gelatin of 1/160,000. Approximately, therefore, diluting the solution ten times reduces the optimum amount of gelatin to one-eighth of the amount required for the more concentrated solution, or, within the error of the experiment, the same actual amount of gelatin is required to precipitate the same actual amount of 'benzoin' at different dilutions.

Charge on the 'Benzoin' Particles.

For the purpose of observing the nature of the charge on the particles a Siedentopf-Zsigmondy slit ultramicroscope was employed. The ordinary cell was used and two fine platinum wires were introduced into this, one from each end in such a way that the end of each wire was about 2 inches from the part of the fluid observed. It was found that, to produce a definite movement, the 220-volt current was convenient, a switch, change over key and lamp being included in the circuit. The circuit was closed and the movements of the particles in the field observed immediately. The current was then reversed and a second observation made. It is essential that observations should be made immediately after closing the circuit so that interference by electrolyte disturbances may be avoided. It was found that this method was particularly useful where, as in the case of experiments with cerebrospinal fluid, only very small quantities (about 1 cc.) of liquid were available. In the crude form described, quantitative estimations were, of course, not possible but for quali-

tative determinations the arrangement was quite satisfactory. The results of such observations are indicated in Tables V and VI.

On the alkaline side of the isoelectric point of gelatin (p_H 4.7) the particles were invariably negatively charged in all concentrations of gelatin observed.

On the acid side of the isoelectric point in those concentrations of gelatin which are too great to cause precipitation, where in fact the 'benzoin' is protected, the charge on the particles is positive. The boundary between positively and negatively charged particles is within the zone of precipitation. For example, in Table V, where gelatin is diluted with distilled water, in dilutions of gelatin up to 1/1,280,000 the particles are negatively charged. At the gelatin dilution of 1/160,000 they have a slight positive charge which becomes more marked as the concentration of gelatin increases. Between these concentrations the zone of precipitation occurs.

An experiment was carried out to determine the effect of tannin in precipitating the mixtures of gelatin and 'benzoin' in the following way. Gelatin solution (1 cc. of 1/40,000 dilution) was added to 1 cc. of various strengths of sodium hydroxide solution. To each tube was added 2 cc. of 'benzoin.' This was allowed to stand overnight and precipitation observed and observation of the charges on the particles were made. The results are given in Table IX.

Table IX.

Effect of Tannin on Benzoin and Gelatin mixtures.

Sodium hydroxide	N/200	N/400	N/800	N/1600	N/3200	N/6400	N/12800	N/25600	N/51200	N/102400
Row I	0	0	0	0 (-)	2 (+?)	0 (+)	0	0	0	0
1 cc. of gelatin and 1 cc. of sodium hydroxide solution mixed, and 2 cc. of 'benzoin' added to the mixture. Read as in Row I.										
From each tube 1 cc. was taken and an equal volume of a 0.2 % solution of tannin added. The tubes were read after standing overnight as follows in Row II.										
Row II	0	0	4 (p_H 4.4)	4	4	4	4	4	4	4

The acidity of the tannin solution has slightly changed the p_H and therefore the electrical charges in the mixtures, but the negative colloid (tannin) is able to precipitate the mixture only at p_H 4.4 and below, under which conditions the particles are positively charged. The 'benzoin' alone is not precipitated by the tannin under the conditions of the experiment, and no visible precipitation is given by gelatin at the concentration employed.

From these experiments it would appear that the 'benzoin' particles combine with the gelatin with the result that their electrical condition is modified. The degree of the modification is dependent on the condition of the gelatin, which again depends on the reaction. Where the charge is neutralised or reduced precipitation occurs. Where, however, the negative charge is reinforced by the gelatin or changed to a positive charge of sufficient magnitude, the sol is protected against precipitation by electrolytes. These results are in substantial agreement with those obtained by Eggerth and Bellows [1922] on bacterial and other suspensions and by Northrop and de Kruif [1922, 1, 2] on bacterial suspensions.

IV. PROTECTION OF 'BENZOIN' BY GELATIN AGAINST
 PRECIPITATION BY ELECTROLYTES.

According to the views of Bechhold [1904], and Neisser and Friedemann [1903] the protection of a lyophile colloid by a lyophile depends probably on the formation of a covering of the former round particles of the latter. The result is that the behaviour of the lyophile particles is governed rather by the properties of the protective colloid than by its own. This accounts for the fact, rather striking at first, that colloidal 'benzoin' requires little more gelatin to protect it against a large concentration of salt than is required to protect it against a small quantity. This is shown in the following experiment. Varying solutions of gelatin (1 cc.) 1/100,000, 2/100,000, etc. to 1/10,000 were introduced into a series of test-tubes. To each test-tube was added 0.2 % sodium chloride (1 cc.) and the 'benzoin' (2 cc.). A similar experiment was carried out at the same time with 2 % sodium chloride, and the results are given in Table X.

Table X.

Protection by Gelatin against precipitation by NaCl.

Concentration of gelatin $\times 100,000$	1	2	3	4	5	6	7	8	9	10
Concentration of sodium chloride	0.2 %	4	4	4	0	0	0	0	0	0
	2.0	4	4	4	4	0	0	0	0	0

It may be noted here that if any such mechanism really does occur, the 'benzoin' particles are completely protected within a few minutes. This is proved by experiments in which to each of a series of ten test-tubes was added 0.5, 1.0, 1.5, 2, etc., up to 5 cc. of gelatin (1/10,000) and distilled water to bring the total volume in each tube to 5 cc. This was followed by the addition to each tube of 5 cc. of 'benzoin' sol, of twice the usual strength (0.6 cc. of alcoholic solution to 20 cc. of water). Six rows, each containing ten tubes, were arranged so that, corresponding to each mixture of gelatin and 'benzoin,' there were six test-tubes, and into each of these six test-tubes was put 1 cc. of the corresponding mixture of 'benzoin' and gelatin, each of the six rows at this stage being of course identical. Ten minutes after the mixture of the 'benzoin' and gelatin, 1 cc. of 2 % sodium chloride was added to each test-tube in the first row and 1 cc. of 0.2 % sodium chloride solution was added to each test-tube in the second row. Thirty minutes after the mixing of 'benzoin' and gelatin, addition was made to the third and fourth row of 1 cc. of salt solution of 2 % and 0.2 % respectively, and after $2\frac{1}{2}$ hours, similar addition to the tubes in the fifth and sixth rows respectively. The results are represented in Table XI, and it will be seen that protection of the 'benzoin' by the gelatin is apparently complete after 10 minutes. It is probable, however, that the protection is not instantaneous, as is shown by the fact that if the salt and gelatin be first mixed and the 'benzoin' then added, slightly different results are obtained, as is also shown in Table XI.

Table XI.

Rate of protection by Gelatin.

Concentration of gelatin $\times 100,000$	1	2	3	4	5	6	7	8	9	10
2 % NaCl added after 10 mins.	4	4	4	4	0	0	0	0	0	0
0.2 % " " 10 "	4	4	0	0	0	0	0	0	0	0
2 % " " 30 "	4	4	4	4	0	0	0	0	0	0
0.2 % " " 30 "	4	4	0	0	0	0	0	0	0	0
2 % " " 2½ hours	4	4	4	4	0	0	0	0	0	0
0.2 % " " 2½ "	4	4	0	0	0	0	0	0	0	0
'Benzoin' added to mixture of NaCl (0.2 %) and gelatin	4	4	2	0	0	0	0	0	0	0

As is to be expected, there is less protection when the 'benzoin' is added to the mixture of salt and gelatin, as in this case the salt is active as quickly as the gelatin, and effects precipitation before the protective power of the gelatin can fully exert itself.

Experiments were carried out on the amount of gelatin required to protect 'benzoin' of various strengths. A series of five rows each containing ten tubes is arranged. To each tube in each of the first four rows is added 1 cc. of a solution of gelatin, to the first, 1/100,000, to the second, 2/100,000, etc. To the test-tubes of the last row is added 1 cc. of gelatin of strength 1/20,000, 1/40,000, 1/80,000, etc., respectively. Then 1 cc. of 'benzoin' of a certain concentration is added to each tube. Single strength 'benzoin' is added to the tubes of the first row, double strength to the tubes of the second row, double strength diluted 1 in 2, 1 in 4, 1 in 20, to the tubes of the third, fourth and fifth rows respectively. All the tubes are shaken and after 15 minutes 2 cc. of 2 % sodium chloride solution is added to each tube. The results obtained after standing overnight are shown in Table XII.

Table XII.

Effect of concentration of sol on protection by Gelatin.

The concentrations of gelatin given in this table are the concentrations *before* mixing with the 'benzoin.' Sodium chloride (2 %) solution is added to the mixture of 'benzoin' and gelatin after 15 minutes.

Concentration of gelatin $\times 100,000$	1	2	3	4	5	6	7	8	9	10
'Benzoin,' ordinary strength	4	4	2	0	0	0	0	0	0	0
" double "	4	4	4	4	4	3	2	0	0	0
" " " diluted 1/2	4	4	4	0	0	0	0	0	0	0
" " " " 1/4	4	0	0	0	0	0	0	0	0	0
Concentration of gelatin $\times 10,000$	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
'Benzoin,' double strength, diluted 1/20	0	0	0	0	0	4	4	4	4	4

It will be noted in the first place that there is not much difference between the single strength 'benzoin' and the double strength diluted 1 in 2. This, of course, is to be expected. On the other hand, the various dilutions of the double strength showed marked differences in the amount of gelatin required to protect them. The double strength 'benzoin' is almost completely protected by 8/100,000 gelatin, giving a final concentration of gelatin in the experiment of $\frac{1}{4}$ (8/100,000) = 1/50,000—the factor $\frac{1}{4}$ being required, since 1 cc. of gelatin is diluted to 4 cc. by the addition of 'benzoin' and of salt, whereas

if the 'benzoin' be diluted 20 times protection occurs at a final concentration of about $\frac{1}{4}$ ($1/320,000$) = $1/1,280,000$, or about $1/25$ of the concentration required with 'benzoin' of 20 times the strength of that here used. In fact, the amount of gelatin required to protect is approximately proportional to the concentration of the sol. In interpreting the result it must be remembered that less salt is required to precipitate a concentrated sol than a diluted one, but, on the other hand, if excess of salt be used, as in this experiment, the amount of gelatin required to protect increases very slowly with increase in the actual concentration of the salt (see p. 651). It may be noted, however, that this effect may account for the small deficiency ($1/25$ instead of $1/20$) of gelatin that is required for the precipitation of the dilute sol compared with the concentrated one.

In view of the very great importance of the hydrogen ion concentration on the precipitation effect of gelatin on 'benzoin' it was of interest to investigate its effect on the protective power of gelatin. Solutions of hydrochloric acid, $N/100$, $N/200$, $N/400$... $N/16,000$, $N/64,000$, sodium hydroxide, $N/200$, $N/400$, $N/800$, $N/1400$, $N/3200$, and solutions of gelatin $1/500$, $1/1000$, $1/1600$, $1/3200$ were prepared. 0.25 Cc. of acid (or alkali), and the same amount of gelatin, were added to each one of a row of eight tubes, there being eleven rows, one for each strength of acid or alkali and one for water. 0.5 Cc. double strength 'benzoin' was then added to each tube, and readings made of those (marked * on the table) which showed incipient precipitation as indicated by increased opacity. After standing 10 minutes, 1 cc. of sodium chloride solution (2 %) was added to each tube and the tubes were allowed to stand overnight. The results obtained are shown in Table XIII.

Table XIII.

Effect of p_H on protection by Gelatin.

The concentrations given are the final concentrations after mixing 'benzoin,' gelatin and salt solutions.

Final concentration of gelatin $\times 1000$	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Final concentration of sodium hydroxide								
$N/1600$	0	0	0	0	0	4	4 (p_H 6.4)	4
$N/3200$	0	0	0	0	0	4	4 (p_H 5.2)	4
$N/6400$	0	0	0	0	1	4*	4* (p_H 4.8)	4*
$N/12800$	0	0	0	0	4	4	4* (p_H 4.6)	4*
$N/25600$	0	0	0	0	2	4	4 (p_H 4.4)	4*
Distilled water	0	0	0	0	2	4	4 (p_H 4.3)	4*
Final concentration of hydrochloric acid								
$N/51200$	0	0	0	0	1	4 (p_H 4.2)	4	4
$N/12800$	0	0	0	0	0	4 (p_H 4.1)	4	4
$N/3200$	0	0	0	0	0	4 (p_H 3.6)	4	4
$N/1600$	0	0	0	0	0	4 (p_H 3.3)	4	4
$N/800$	0	0	0	0	0	4 (p_H 3)	4	4*

The most interesting result of this experiment is that protection by gelatin is minimum at the isoelectric point. From the point of view that the particles of a protected colloid have many of the properties of the protective substance, this is easily understood. It is interesting to note, however, that Loeb [1923] has obtained similar results in the case of collodion particles coated with gelatin. These show a minimum of stability at the isoelectric point

of gelatin. According to Loeb this is due to the fact that gelatin is least soluble at the isoelectric point, and a similar explanation fits well the results obtained in the above experiment.

One further point is of interest. As shown above (p. 651), rather more gelatin is required to protect against precipitation by 2 % salt solution than against 0.2 % salt solution. The lower part of the table in the above experiment may be looked on as a series of experiments in protection against salt along with a definite amount of acid. It will be seen that up to a point, addition of acid does not increase the amount of gelatin required to protect. In fact, it diminishes it. The only interpretation of this result is that the acid renders the gelatin a more efficient protector, and this may be due to the gelatin anion being a more efficient protector than the uncharged neutral particle. In this case, it is not unreasonable to explain the effect of alkali on the protective power of gelatin as due to a similar increased efficiency of sodium gelatinate compared to unionised gelatin. Of course this comes well into line with the point of view of Loeb, the minimum protection at the isoelectric point being due to the small affinity for water of the isoelectric gelatin particle and the resulting small solubility. At the same time it must be remembered that alkali has itself a protective effect against precipitation by salt (see p. 641), whereas acid tends to precipitate. This may well account for the greater efficiency of gelatin as a protector in presence of alkali compared to acid, as is shown by the fact that, at a p_H of 5.2, 1/64,000 gelatin affords complete protection whereas at a p_H of 4.2 it does not, although the isoelectric point, $p_H = 4.7$, is nearer 5.2 than is 4.2.

DISCUSSION.

Colloidal solutions have for convenience been classified into two types—lyophobic colloids, such as gold sol or 'benzoin' sol which are precipitated by small quantities of salt, and are suggestive of very fine suspensions, and lyophilic colloids, on the other hand, such as gelatin solution or a solution of silicic acid which are precipitated only by large quantities of salt, and then redissolved again if the excess of salt is removed. The work of Loeb [1922, 2] has in fact shown that gelatin in certain respects may be regarded as forming a true solution, and that it behaves chemically as an amphoteric substance, combining with acid on one side of the isoelectric point ($p_H = 4.7$) and with bases on the other. The large size of the gelatin aggregate, however, prohibits it from passing through a collodion membrane, and this along with the strong attraction of the gelatin molecules for each other, aiding the formation of micelles, and tending to cause gelatin to swell rather than to dissolve, results in producing the colloidal properties of gelatin.

The 'benzoin' particles, on the other hand, have a negative charge, presumably due to the presence of some organic acid (benzoic acid) on the surface of the resin particles. These acid molecules will naturally ionise to some extent, thus producing a negatively charged particle and a slightly acid solution. The

stability of these particles is largely dependent on the electric charge. Loeb [1922, 2] who uses collodion particles shows that a dispersion of these becomes unstable if the potential difference between particle and liquid is less than about 16 millivolts.

On this basis, the experimental results we have obtained in the investigation of the precipitation of the 'benzoin' sol by gelatin are to be explained. With gradually increasing concentration of positively charged gelatin, there occurs a change in the charge of the 'benzoin' particles, and as the charge is reversed, there occurs the zone of precipitation. It is of great interest to note that the concentration of gelatin at which the zone of precipitation occurs depends essentially on the p_H . On the alkaline side of the isoelectric point, no precipitation occurs at all. The precipitation in fact would clearly seem to depend on the amount of positively charged gelatin present. As the p_H increases and the solution becomes more acid, an increasing proportion of the gelatin present exists as kation, and so the actual concentration at which the gelatin precipitates is decreased.

The work of Walpole [1913] is of interest. Working with an oil emulsion this observer found no precipitation with gelatin alone, but in presence of hydrochloric acid, a zone could be observed, very similar to that described with the 'benzoin' sol without hydrochloric acid. Walpole did not measure the p_H of the solutions, but gives only the normality of the acid employed. Hydrochloric acid, of normality 10^{-2} is alone sufficient to aggregate the emulsion. Between about 10^{-4} and 10^{-2} N precipitation takes place if gelatin be present in concentrations of 10^{-6} to 10^{-7} , the zone tending towards the region of more concentrated gelatin as the concentrations of acid decrease, until at about 10^{-4} N it broadens, and extends from about $10^{-4.2}$ to 10^{-6} . Our own results give a zone of the same general type, and so far confirm the findings of Walpole. In one respect, however, the experiments do not agree. Walpole finds that the line dividing the positively from the negatively charged particles in his diagram, in which the normality¹ of the acid is plotted against the concentration of the gelatin, does not coincide with the zone of precipitation. In our experiments, there was always a different charge on the particles on the two sides of the zone; both in the case of gelatin and of oxyhaemoglobin. Even the case of precipitation by ferric chloride solution (p. 642) which, similarly to gelatin precipitates at high dilutions only over a limited zone, the particles are charged oppositely at the different ends of the zone. In fact, as we have suggested above, this precipitation by ferric chloride probably bears more than a superficial resemblance to the precipitation by gelatin, as the ferric chloride is partially hydrolysed, and the ferric hydroxide exists in a colloidal condition.

Such a reaction between a lyophobic colloid and a lyophile sol is no doubt fairly general for lyophile sols of different kinds. For instance, 'benzoin' is precipitated not only by gelatin, but also by oxyhaemoglobin, serum globulin,

¹ Actually the exponents of normality and concentration respectively.

and serum albumin (see the following paper). The question arises whether the 'benzoin' sol has peculiar properties among lyophobic colloids that render it peculiarly liable to precipitation by such lyophilic sols. Reference has already been made to the oil emulsions, investigated by Walpole. It may be noted that Gann found that acid gold sols were precipitated by gelatin. On the other hand, Eggerth and Bellows [1922] in a study of the agglutination of bacteria by various proteins in presence of buffered solutions of constant salt content and varying p_H showed that the amount of protein required varied with the p_H of the solution. In the case of 'benzoin' sol, however, the conditions are so simple that it seems particularly adapted for investigation of the principles of such precipitations.

The dependence of the protective power of gelatin on the isoelectric point is less marked than that of the precipitating power, though it is fairly clearly shown in Table XIII, and is minimum at p_H 4.7, the isoelectric point of gelatin. Apparently, ionised gelatin is better able to protect than isoelectric gelatin, and this may be correlated with the greater solubility of salts in water than the undissociated molecule. For the gelatin ions, either positively or negatively charged, will probably have a greater affinity for water than the undissociated uncharged molecules, just as the benzoate ion has a greater affinity for water and is, therefore, more soluble than uncharged benzoic acid. In this way, the result may be brought into line with the views of Langmuir on the affinity of certain groups for water, and of Loeb [1923]. Loeb shows that the protective effect of gelatin is to be ascribed largely to its high affinity for water, in fact, to its high true solubility.

The results we have obtained in regard to precipitation by salts support the view that such precipitation is largely a matter of the ion of charge opposite to that on the colloidal particle. The anomalies are to be, at least largely, explained as due to the varying p_H . This is but another example of the many instances in colloid chemistry where difficulties are cleared up if due note is made of the hydrogen ion concentration.

CONCLUSIONS.

1. Precipitation of colloidal gum 'benzoin' occurs when it is added to an equal volume of gelatin of concentration about 1/500,000. The actual concentration of gelatin required varies with the p_H of the solution.
2. No precipitation occurs on the alkaline side of the isoelectric point of gelatin.
3. The charge on the particles is negative if insufficient gelatin to precipitate it has been added. If excess of gelatin has been added so that precipitation does not occur, the charge is positive. On the alkaline side of the isoelectric point of gelatin the charge is always negative.
4. Similar results are obtained with oxyhaemoglobin.
5. The power of gelatin to protect 'benzoin' against precipitation by sodium chloride depends on the p_H and is minimum at the isoelectric point.

6. Precipitation by an acid depends essentially on the concentration of hydrogen ions.

7. The amount of salt required to precipitate 'benzoin' increases with increase in the p_H . This, at least largely, accounts for the differing amounts of salts of univalent kations required to effect precipitation.

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