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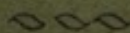
GLOBULIN

BY

J. MELLANBY

(From the Wellcome Physiological Research Laboratories)

1905



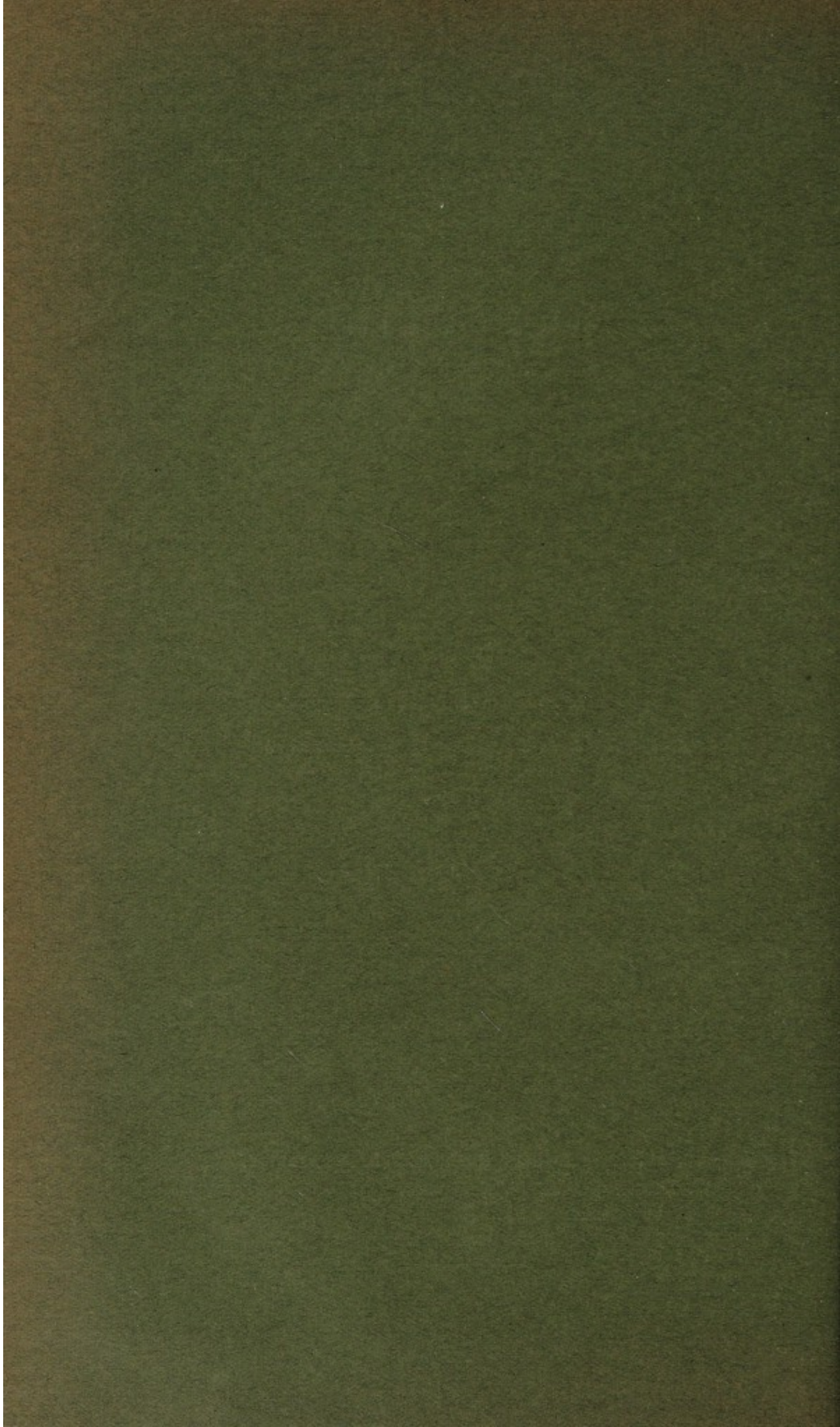
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GLOBULIN¹. BY J. MELLANBY. (Sixteen Figures in Text.)
(From the Wellcome Physiological Research Laboratories.)

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Definition. The word globulin as used in this paper indicates a proteid which is insoluble in water but is soluble in dilute solutions of electrolytes. The term does not imply any definite salt precipitation limits.

Method of preparation. A globulin dissolved in neutral salt solution is precipitated when the percentage of salt is diminished. This diminution may be effected in two ways: (*a*) by dialysis, (*b*) by diluting the proteid solution with water. The first method is tedious and unsatisfactory. The properties of globulin are materially altered by prolonged contact with water. The second method gives better results and was used in the preparation of the globulin described in the following pages. At the beginning of the work fresh sterile horse serum was used as the source of the globulin. The globulin obtained from this source did not

¹ The experiments detailed in this paper were concluded in September, 1904.

give constant results when dissolved in salt solutions. Later horse plasma from blood which had been prevented from clotting by means of potassium oxalate was used. Fresh sterile horse blood was mixed with sufficient potassium oxalate to prevent it clotting. The corpuscles were allowed to settle. This settling usually took place in three or four hours. The plasma was then drawn off and added to ten volumes of distilled water. A quantity of plasma globulin was precipitated, but the amount was greatly increased by adding to the plasma water mixture 10 c.c. of 10% acetic acid for each litre of plasma used. The precipitation was done in fifteen-litre glass aspirators. After the plasma had been added to the water and the precipitation increased with acetic acid, the mixture was well shaken and the aspirator supported with its neck downwards.

The precipitated globulin rapidly settled (the settling was usually complete in an hour) in the neck and on the bottom of the aspirator. The supernatant fluid was drawn off by means of a syphon and a fairly thick suspension of plasma globulin obtained. Usually about five litres of plasma were precipitated and about two litres of crude plasma globulin suspension obtained. This suspension contained about 1% of globulin and 1% of albumen. The globulin in the suspension corresponded in its properties to those ascribed to fibrinogen. It was fairly soluble in neutral salts and on the addition of calcium chloride clotted. This suspension of globulin if left for a considerable time slowly settled and a more concentrated suspension could be thus obtained. But it was found that the settling was hastened by slight heat. The plasma globulin suspension was therefore put into Winchester bottles and immersed in water at 40° C. At this temperature the particles of globulin rapidly coalesced and sank to the bottom of the vessels. A considerable increase in the concentration of the plasma globulin suspension was thus effected.

The concentrated suspension was now placed in a glass beaker and sufficient pure sodium chloride added to make the solution 0.6%. This salt caused the greater part of the globulin to go into solution. Calcium chloride was added to the mixture in the proportion of one to two thousand five hundred. The fluid in a variable space of time (usually about ten minutes) started to clot and this clotting proceeded at different rates in different plasmas. Whilst it was proceeding the mixture was whipped with a test tube brush. This prevented the clot from being quite solid and facilitated the subsequent expression of the unclotted liquid.

When the clotting was complete the remaining fluid was poured off and the clot well freed from liquid by adequate pressure. The liquid thus obtained, in spite of the large quantity of fibrin which had been removed, contained a considerable quantity of globulin in solution, and was used in the preparation of the globulin the properties of which are described in the following pages. From its method of production it is comparable to serum. It differs from it inasmuch that whereas horse serum usually contains about 10 % of albumen and 0.15 % of globulin, the above liquid contains 1 % of albumen and 4 % of globulin. The preparation of the liquid was rapid, about three hours elapsing from the time the plasma was added to the distilled water to its production. Consequently decomposition processes were reduced to a minimum.

The liquid containing the globulin in solution was added to 20 volumes of distilled water. The salt was thus reduced to about 0.03 % NaCl. The globulin was immediately precipitated as a dense white cloud. The particles rapidly coalesced and sank to the bottom of the vessel. The clear supernatant liquid was syphoned off and the remaining suspension concentrated by warming and allowing to stand for a short time.

The thick suspension was filtered on folded hardened filter papers. A very thick suspension could be thus obtained. Further purification was made by adding the filtered suspension to water and allowing it to settle, or by washing the precipitate on the filter papers with distilled water. In the experiments detailed below the quantity of soluble proteid from the original plasma, and the salt in the globulin suspension are negligible. In no case was the proteid dried.

Suspensions of globulin were used as early as possible after they were made. In the determination of the solubilities of globulin in salts the experiment, from the precipitation of the plasma with distilled water to the obtaining of saturated solutions in salts, was completed in a day. When a considerable degree of washing was necessary, as in the case of certain acid and alkali experiments, the operations were conducted so as to involve a minimum of chemical change.

At the outset it was found that the globulins obtained from different horses differed in their solubility properties. There is, therefore, a probability that the globulins of all horses differ in their physical properties. Such differences might have been due to differences in external influences during their preparation. But in order that no such criticism might be attached to the following experiments comparative experiments were always done on the blood of one horse.

The determination of the solubility of globulin in any solvent. A suspension containing about 2% of globulin was obtained as described above. This strength of suspension was found to be easily workable and of sufficient concentration to allow accurate results to be obtained.

5 c.c. of this suspension were placed in each of a series of test tubes. To these Y c.c. of water and X c.c. of solvent were added, the relation between Y and X being such that the total volume in each tube was 10 c.c. The globulin suspension, solvent-solution, and water were all at constant temperature. The test tubes were well shaken and placed in a constant temperature bath.

On the addition of the solvent to the suspension a certain quantity of globulin went into solution. After the tubes had been kept at a constant temperature for some time the undissolved globulin sank to the bottom, and a clear saturated solution of globulin in the required salt could be syphoned off. The amount of globulin in this solution was determined in the following way. If the salt did not crystallise with water and was not decomposed by heat, it was only necessary to dry a known number of c.c. in a weighed crucible. The weight of salt in the solution was known and the difference between the determined weight and this gave the quantity of globulin which had gone into solution.

If the solvent salt crystallised with water or was decomposed by heat, the determination of the proteid in the solution involved the coagulation of a known number of c.c., filtering, washing the coagulum clean with distilled water, drying and weighing.

In the case of the first class of salts the initial dryings were done in small porcelain crucibles holding rather more than 10 c.c. and weighing about 5 grms. These were heated on a copper plate, the temperature being regulated by the distance of the crucible from a bunsen burner placed under one end of the plate. After the excess of liquid had been driven off, the drying was completed in an air oven at 120°C . This temperature did not decompose the proteid and could be continued indefinitely without altering the weight of the proteid after it was dried. The coagula after washing clean on hardened filter papers were transferred to these crucibles and dried in the air bath.

1. THE SOLUTION OF GLOBULIN BY NEUTRAL SALTS.

A. *Solution by Sodium Chloride.*

(a) *The relation between the amount of salt added and the globulin dissolved.* 5 c.c. of a 2.8% suspension of globulin were placed into each of a series of test tubes. To these varying quantities of 2% NaCl and water were added to make the total volume 10 c.c. The solutions were allowed to saturate at 20° C. The weight of globulin dissolved was determined from the weight of a known number of c.c. of solution when dry. The experimental results are expressed fully below.

$$5 \text{ c.c. } 2.8\% \text{ globulin} = 1.4\% \text{ in } 10 \text{ c.c.}$$

NaCl 2% c.c.s added	H ₂ O added	C.c. of glob. solution dried	Wt glob. and salt	Wt of salt	Wt of glob.	Glob. in 100 c.c.	Salt in 100 c.c.
2.5	2.5	6	.1065	.03	.075	1.275	.5
2.25	2.75	7	.1125	.0315	.081	1.16	.45
2.0	3.0	6	.0800	.028	.052	.93	.4
1.75	3.25	6	.0680	.0210	.047	.78	.35
1.5	3.5	3	.0280	.009	.019	.63	.30
1.25	3.75	2	.0150	.005	.01	.30	.25

The results may be clearer when expressed as follows:—

NaCl in terms of eq. wt=1000	% of original globulin dissolved
85.5	91
76.5	83
68.5	66.5
60.0	55.5
51.0	45
42.6	35.6

The curve expressing the results graphically is given on Fig. 1. It may be noted that the curve is not a straight line and that therefore the amount of globulin dissolved is not a constant proportion of the amount of salt added.

(b) *The influence of the concentration of the original globulin suspension.* From a number of experiments it was found that the amount of globulin dissolved by any percentage of salt depended to a large extent on the concentration of the original globulin suspension. To determine what this relation was experiments were made in which the concentration of the original globulin suspension was varied. The following experiment embodies the results obtained.

Globulin from one horse was suspended in water in five strengths.

A	(1)	Concentration 3.04 %
	(2)	„ 1.68
	(3)	„ 1.06
	(4)	„ .84
	(5)	„ .71

The solubilities of these five concentrations of globulin in sodium chloride were obtained. The numerical results are given below and are depicted graphically on Fig. 2.

% NaCl	Amount of dissolved globulin in each 100 c.c.				
	1	2	3	4	5
.55	2.79	—	—	—	—
.5	2.53	1.3	.94	.73	.63
.45	—	1.1	.81	.67	.57
.4	1.74	—	.66	.56	.5
.35	1.40	.77	.54	.44	.38
.3	1.12	.64	.39	.345	.30
.25	.83	.44	—	—	—
Strength of original globulin suspension	3.04 %	1.68 %	1.06 %	.84 %	.71 %

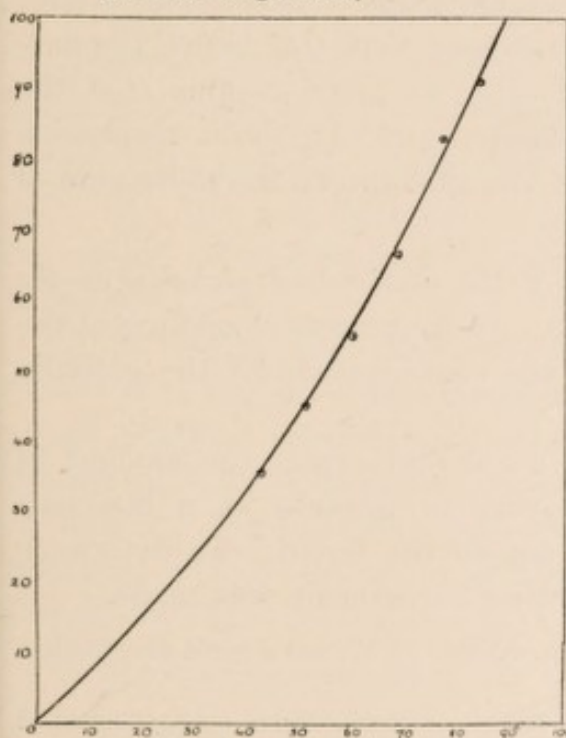


Fig. 1.

Fig. 1. Ordinates represent percentages of original globulin dissolved. Abscissæ represent NaCl present. 5.85 % NaCl=1000.

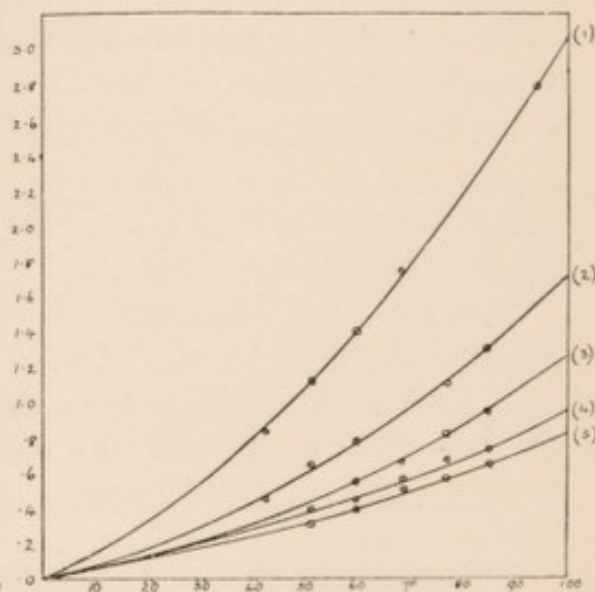


Fig. 2.

Fig. 2. Ordinates represent grams of dissolved globulin in each 100 c.c.s.

Abscissæ represent NaCl present. 5.85 % NaCl=1000.

If we examine the curves expressing these results we see that the amount of globulin dissolved by any given percentage of salt is directly proportional to the strength of the original globulin suspension.

The result is apparent if we examine the numerical values when the amount of globulin dissolved is expressed as a percentage amount of the original globulin.

NaCl in terms of eq. wt=1000	Percentage of original globulin dissolved				
	1	2	3	4	5
94	92	—	—	—	—
85.5	83.6	77.3	88.5	87.1	88.7
77	—	66.2	76.3	79.8	80.2
68.5	57.2	—	62.2	66.7	70.3
60	46.0	45.8	51.0	52.2	53.3
51.5	36.8	38.1	36.8	41.0	42.2
42.5	27.2	26.2	—	—	—

We may therefore formulate the law that the amount of globulin dissolved by a given percentage of salt is directly proportional to the strength of the original globulin suspension.

It may be noted that a given percentage of salt dissolves a little larger percentage of globulin with lower than with higher strengths of suspension. Small variations are undoubtedly due to experimental error but the whole trend of the results indicates that this is not the only cause. In considering the action of salts we must assume that the decreased solvent efficiency with higher strengths of globulin suspension is due to a slight inhibitory action of the globulin on the movement of the electrolytic ions.

(c) *The influence of the settling of the undissolved globulin on the amount dissolved in the different layers.* In the process of settling of the undissolved globulin in a test tube the concentration of the globulin increases in the various layers.

From the law formulated above we should expect the amount of globulin dissolved in the various layers to increase at a rate corresponding to its distance from the top of the liquid. To determine whether this was the case the following experiment was made.

A suspension of globulin containing sodium chloride (5 %) was allowed to settle in a 100 c.c. cylinder.

When the settling was complete a series of 10 c.c.s of clear solution were syphoned off from different levels without allowing any mixing to take place.

The amounts of solid in these layers were determined in the usual way.

The following results were obtained :

Layer	% of solid
1 top of cylinder	4.84
2	4.85
3	4.83
4	4.88
5	4.86
6	4.88

There is therefore no indication that the percentage of globulin in solution increases as we approach the undissolved globulin.

(d) *The distribution of the neutral salt between the dissolved and undissolved globulin.* In all the experiments previously described it has been assumed that the salt does not attach itself to any particular part of the system but is accurately distributed throughout the mixture. The following experiment was made to test this hypothesis.

50 c.c. of globulin suspension containing .5 % NaCl were allowed to settle. The clear layer was then syphoned off.

Both layers—the clear and the one containing the undissolved globulin—were coagulated and filtered.

(1) 10 c.c. of the filtrate from the coagulated clear layer were dried and gently ashed.

(2) 5 c.c. of the filtrate from the coagulated turbid layer were dried and gently ashed.

Weight of ash from A = .0490 grs.

“ “ “ B = .025 “

Theoretically the weight of ash from A should have been 0.05 grs. and from B 0.025 grs.

There is therefore no accumulation of salt in any part of the system when it is added to a globulin suspension so as to partially dissolve it.

(e) *The influence of the initial concentration of the added neutral salt.* We have seen how the amount of globulin dissolved by a given percentage of salt depends on the strength of the original globulin suspension.

A further point to be settled is how far the quantity of globulin dissolved at any time depends on the concentration of the solvent salt added. Sodium chloride and water were added to a series of tubes each containing 5 c.c. of globulin suspension, so that in every case the final volume was 10 c.c. and the percentage of NaCl .4. The only difference was that the concentration of the added salt, and consequently the amount of added water, varied.

The following results were obtained :

Globulin suspension	Water added	NaCl added	Globulin dissolved in 100 c.c. clear solution
5 c.c.	4.8	.2 c.c. of 20 %	1.93 grs.
5	4.6	.4 “ 10	1.94
5	4.2	.8 “ 5	1.96
5	3.8	1.2 “ 3.3	2.00
5	3.4	1.6 “ 2.5	1.95
5	3.0	2.0 “ 2.0	1.90
5	2.0	3.0 “ 1.33	1.96
5	1.0	4.0 “ 1.0	1.90
5	—	5.0 “ .8	1.96

It may be noticed that there are slight discrepancies in the various results obtained. But these are obviously due to experimental error. We may therefore conclude that the amount of globulin dissolved by any given percentage of salt does not depend upon the concentration in which the salt is added.

B. *A comparison of the efficiencies of various neutral salts to produce the solution of globulin.*

From the conclusions arrived at in the previous pages it is probable that purely physical forces are involved in the solution of globulin by neutral salts. To determine the nature of these physical forces and upon what they ultimately depend a number of comparable experiments were made with various neutral salts.

(a) *A comparison of sodium chloride, potassium chloride and ammonium chloride.* A pure suspension of globulin was made and solubility curves for NaCl, KCL and AmCl at 20° C. were determined. The following results were obtained:

	Salt in terms of the eq. wt.=1000	% of original globulin dissolved
NaCl 5.85 % = 1000	42.7	35.7
	51.2	45
	59.8	55.7
	68.2	66.5
	77.0	83
	85.3	91.5
KCL 7.45 % = 1000	47.0	40.7
	53.8	46.5
	60.3	56.0
	67.0	64.0
	74.0	72.0
	80.6	84.0
AmCl 5.35 % = 1000	44.8	33.5
	52.3	40.2
	60.0	51.2
	67.2	63.0
	74.8	71.5
	82.2	81.5

The curves expressing these results are given on Fig. 3. It may be noted that the curves for NaCl and KCL coincide and that the curve for AmCl is only a little higher at some points.

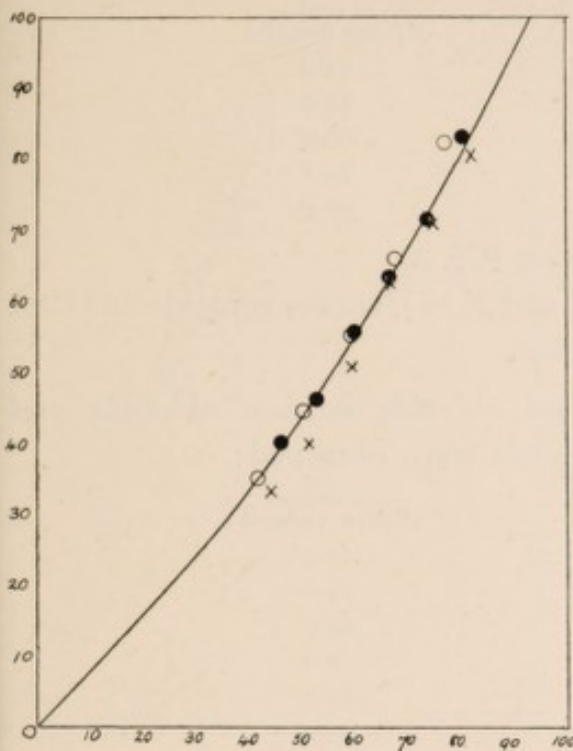


Fig. 3.

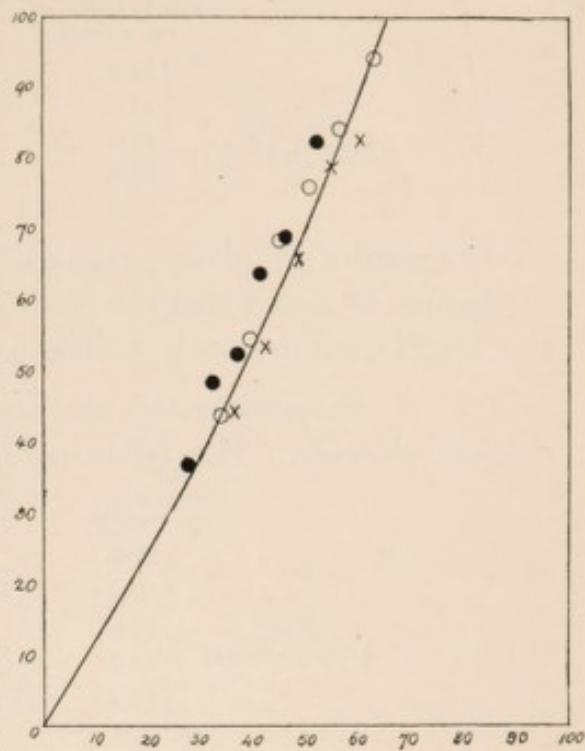


Fig. 4.

Fig. 3. Ordinates represent percentages of original globulin dissolved. In the abscissæ, equivalent weights of the salts=1000.

- represent points on NaCl curve.
- represent points on KCl curve.
- × represent points on AmCl curve.

Fig. 4. Ordinates represent percentages of original globulin dissolved. In the abscissæ, equivalent weights of the salts=1000.

- represent points on Na_2SO_4 curve.
- represent points on K_2SO_4 curve.
- × represent points on Am_2SO_4 curve.

(b) *A comparison of sodium sulphate, potassium sulphate and ammonium sulphate.* The following results were obtained:

Salt in terms of eq. wt=1000		% of original globulin dissolved
Na_2SO_4 7.1 % = 1000	33.8	43.5
	39.4	54.3
	45.0	68.0
	50.6	75.5
	56.3	84.0
	63.3	94.0
K_2SO_4 8.7 % = 1000	27.6	36.5
	32.2	48.0
	36.8	52.0
	41.3	63.5
	46.0	68.5
	51.7	82.0

	Salt in terms of eq. wt=1000	% of original globulin dissolved
Am_2SO_4 $6.6\% = 1000$	36.4	44.0
	42.5	53.0
	48.5	66.0
	54.5	78.5
	60.5	82.0

The results are given graphically on Fig. 4.

It may be noted that the Na_2SO_4 and K_2SO_4 curves coincide and that the Am_2SO_4 curve is only a little higher.

(c) *A comparison of magnesium chloride, barium chloride and calcium chloride.* The following results were obtained:

	Salt in terms of eq. wt=1000	% of original globulin dissolved
MgCl_2 $4.75\% = 1000$	29.5	46.5
	33.7	54.0
	37.8	63.0
	42.2	68.0
	47.4	78.5
BaCl_2 $10.4\% = 1000$	52.6	86.0
	23.1	49.0
	30.8	56.0
	34.6	61.5
	38.4	69.0
CaCl_2 $5.55\% = 1000$	43.2	72.0
	48.6	86.5
	25.3	40.7
	28.8	50.0
	32.5	58.0
	36.0	62.9
	40.5	60.0
	45.0	72.0

The curves are given on Fig. 5.

(d) *A comparison of barium chloride and potassium sulphate.*

	Salt in terms of eq. wt=1000	% of original globulin dissolved
BaCl_2 $10.4\% = 1000$	23.1	49.0
	30.8	56.0
	34.6	61.5
	38.4	69.0
	43.2	72.0
K_2SO_4 $8.7\% = 1000$	48.6	86.5
	27.6	36.5
	32.2	48.0
	36.8	52.0
	41.3	63.5
	46.0	68.5
	51.7	82.0

The curves expressing these results are given on Fig. 6.

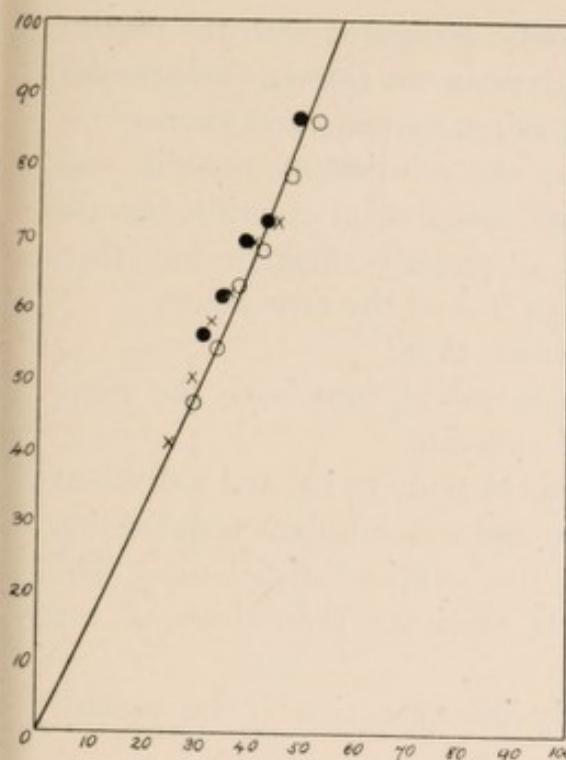


Fig. 5.

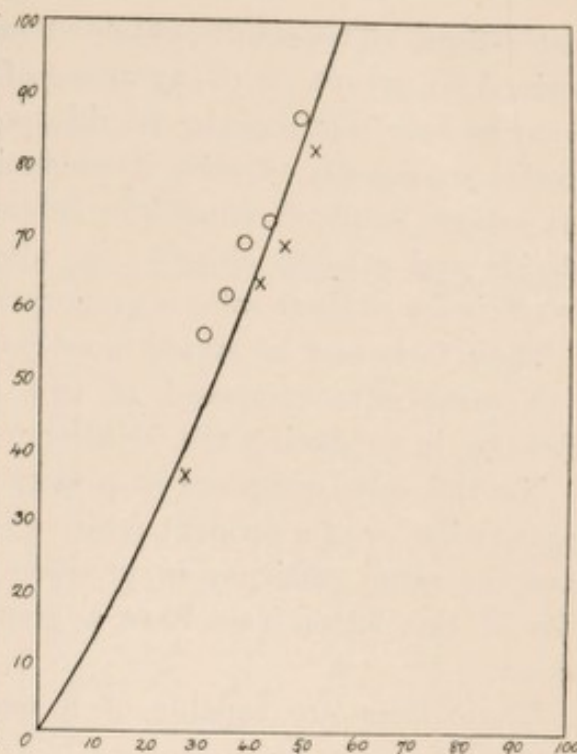


Fig. 6.

Fig. 5. Ordinates represent percentages of original globulin dissolved. In the abscissæ, equivalent weights of the salts = 1000.

○ represent points on MgCl_2 curve.

● represent points on BaCl_2 curve.

× represent points on CaCl_2 curve.

Fig. 6. Ordinates represent percentages of original globulin dissolved. In the abscissæ, equivalent weights of salts = 1000.

○ BaCl_2 points.

× K_2SO_4 points.

(e) *A comparison of ammonium sulphate and magnesium sulphate.*

	Salt in terms of eq. wt.=1000	% of original globulin dissolved
Am_2SO_4 $6.6\% = 1000$	37.8	33.5
	45.5	49.7
	53.0	67.0
	60.5	80
MgSO_4 $6.0\% = 1000$	25.0	28.0
	29.1	41.0
	33.3	47.7
	37.1	56.8
	41.6	65.0
	45.8	74.5

The curves expressing these results are given on Fig. 7.

The Experiments (a), (b), (c), (d) were done on the same sample of globulin under exactly similar conditions. Experiment (e) was done at

a later time. The results obtained may be compared with the results obtained for (a), (b), (c), (d) by means of Experiment (b). In these results it may be seen that sodium chloride, potassium chloride, and ammonium chloride are equally effective in producing the solution of globulin, and that sodium sulphate, potassium sulphate, magnesium chloride, barium chloride and calcium chloride are also all equally effective, but that the efficiency of these salts is greater than that of the salts above.

These facts may be stated more generally thus:

Neutral salts composed of two monovalent ions have the same efficiency in producing the solution of globulin.

Neutral salts composed of a monovalent positive ion and a divalent negative ion, or of a divalent positive ion and a monovalent negative ion have the same efficiency in producing the solution of globulin. The salts of this latter class have a greater efficiency than those of the former.

These facts are capable of a ready interpretation if we assume that:

(1) The solution of globulin by neutral salts depends upon the forces exerted by their free ions.

(2) That monovalent ions whether positive or negative are equally effective in producing this solution.

(3) That divalent ions whether positive or negative are equally effective in producing this solution but more so than monovalent ions.

To determine the numerical relation between the efficiencies of monovalent and divalent ions we may compare different solubility curves determined under the same conditions.

It is evident that the area $ABCD$ included between the solubility curve ABC , the ordinate AD , and the abscissa DC , is inversely proportional to the efficiency of the salt in producing solution. By comparing these areas for different salts we obtain a ratio of the efficiencies of monovalent and divalent ions.

(f) *A comparison of the area of the sodium chloride curve with that of the sodium sulphate curve (fig. 8).* The areas were determined by means of a planimeter.

NaCl curve = 24.4 sq. inches = 3×8.13 sq. inches,

Na₂SO₄ „ = 16.2 sq. inches = 2×8.1 sq. inches.

Therefore the ratio of the efficiencies of equivalent weights of sodium chloride and sodium sulphate is as two is to three. Or in terms of their molecular weights as two is to six.

(g) A comparison of the areas of the ammonium sulphate and magnesium sulphate curves (Fig. 7).

Am_2SO_4 curve = 19.6 sq. inches = 8×2.45 sq. inches,

MgSO_4 „ = 14.7 sq. inches = 6×2.45 sq. inches,

or

Eq. wt. Am_2SO_4 : Eq. wt. MgSO_4 :: 6 : 8.

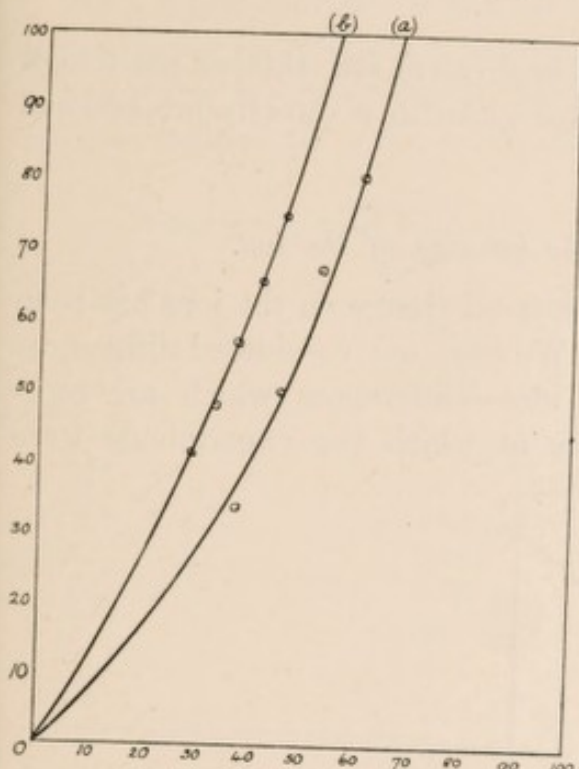


Fig. 7.

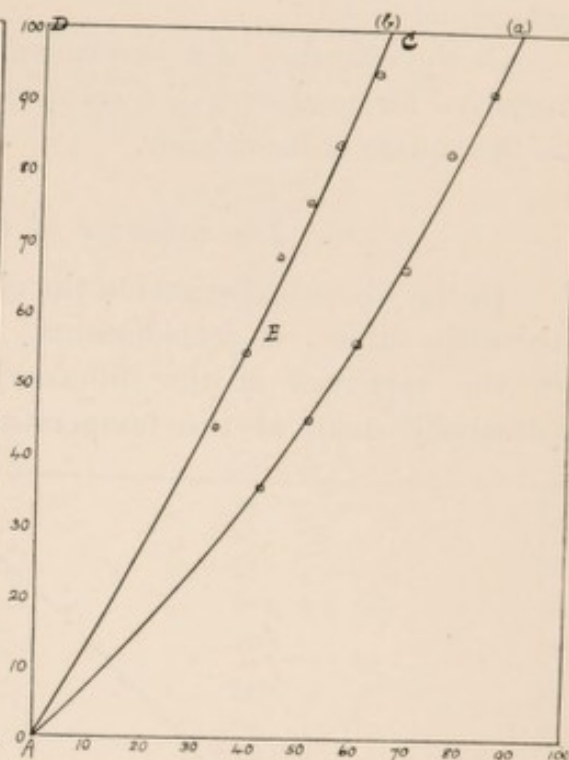


Fig. 8.

Fig. 7. Ordinates represent percentages of original globulin dissolved. In abscissæ, equivalent weights of salts = 1000.

(a) Am_2SO_4 curve.

(b) MgSO_4 curve.

Fig. 8. Ordinates represent percentages of original globulin dissolved. In abscissæ, equivalent weights of salts = 1000.

(a) NaCl curve.

(b) Na_2SO_4 curve.

Now we know that the efficiencies of sodium chloride, ammonium chloride, and potassium chloride are the same: also that the efficiencies of sodium sulphate, potassium sulphate, barium chloride, magnesium chloride, calcium chloride are the same.

If we express these facts numerically in terms of the molecular weights of the salts we see that

Efficiency $\text{NaCl} = \text{AmCl} = \text{KCl} = 2A$ (A is a constant).

Efficiency $\text{Na}_2\text{SO}_4 = \text{Am}_2\text{SO}_4 = \text{K}_2\text{SO}_4 = \text{MgCl}_2 = \text{BaCl}_2 = \text{CaCl}_2 = 6A$.

Efficiency $\text{MgSO}_4 = \frac{4}{3} \text{Am}_2\text{SO}_4 = \frac{4}{3} 6A = 8A$.

But we have determined that the efficiency of a salt is due to the summated efficiencies of its constituent ions; also that all monovalent ions whether positive or negative have the same efficiency, and that all divalent ions whether positive or negative have the same efficiency.

Therefore monovalent ions have an efficiency equal to A and divalent ions equal to $4A$.

Or the efficiency of a monovalent or divalent ion whether positive or negative for producing the solution of globulin is directly proportional to the square of its valency.

C. *The influence of the velocity of the ion.*

In the above experiments the electrical charge on the ions has been the main subject of consideration. We have not considered differences in the velocities of the different ions—differences which are comparatively small at the temperature at which the experiments were

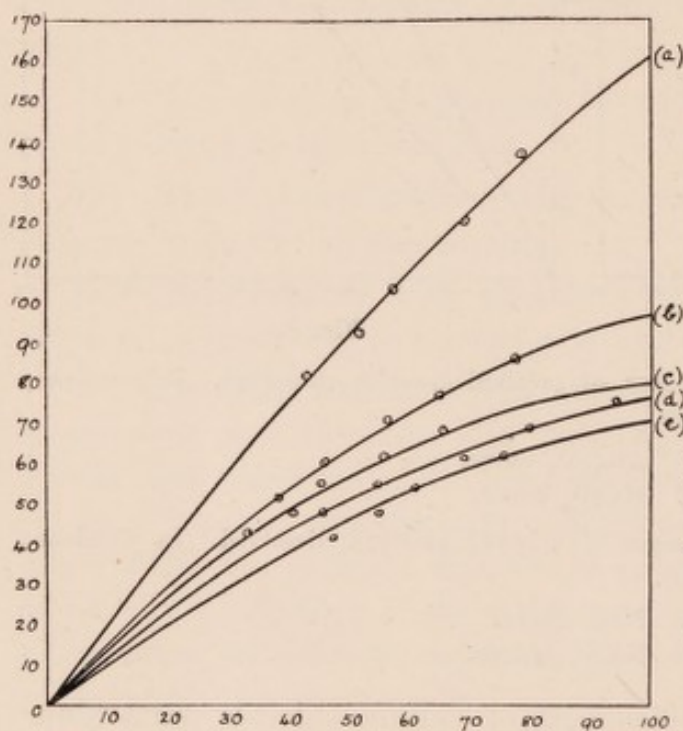


Fig. 9.

Fig. 9. Ordinates represent quantities of NaCl. Equivalent weight (5.85%) = 1000. Abscissæ represent percentages of original globulin dissolved.

In curve (a) temperature = 1.5°C .
 „ (b) temperature = 20.0°C .
 „ (c) temperature = 30.0°C .
 „ (d) temperature = 40.0°C .
 „ (e) temperature = 50.0°C .

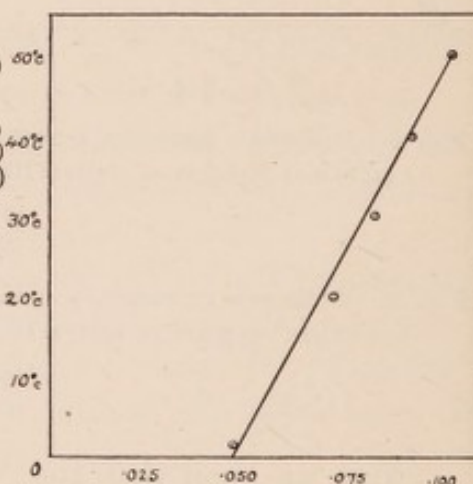


Fig. 10.

Fig. 10. Ordinates represent temperatures. Abscissæ represent reciprocal of areas.

done, but which to some extent would account for the small discrepancies observed in the experimental results.

To determine the influence of the velocity of the ion the solubility curves for the solution of globulin by sodium chloride at various temperatures were determined.

In a dilute solution of sodium chloride it is assumed that ionisation is practically complete and that increasing conductivity with increasing temperature is mainly due to the increased velocity of the ions. The results obtained are given below and are plotted on Fig. 9.

Temperature	NaCl in terms of eq. wt. (5.85%) = 1000	% of original globulin dissolved
1.5° C.	136.8	78.5
	119.0	69.0
	102.4	57.5
	92.0	52
	81.7	43
	68.0	37
20° C.	85.1	78
	76.6	66
	69.8	57
	59.6	46
	51.1	38.5
	42.6	26.5
30° C.	75.0	94.5
	68.0	66
	61.3	56
	54.5	45.5
	47.7	40.7
	41.0	30
40° C.	68.0	80
	61.3	69
	54.5	54.5
	47.7	49
	41.0	51
	34.0	32.5
50° C.	61.3	76
	54.5	61
	51.0	57.5
	47.7	55
	41.0	47
	34.0	36

The value of the temperature coefficient may be obtained by determining the areas enclosed by the different temperature curves. These areas are inversely proportional to the efficiency of the salt at that temperature. If therefore we take the reciprocals of these area values

we get comparative values for the efficiency of the sodium chloride at that temperature.

Temperature	Area in sq. inches	Reciprocal
1.5° C.	20.8	.048
20° C.	13.6	.073
30° C.	11.96	.083
40° C.	10.8	.092
50° C.	9.8	.102

The relation between the temperature and the reciprocal of the area is plotted on Fig. 10. We see that the temperature curve is approximately a straight line and that the temperature coefficient is about two per cent. per degree starting from 0° C. In 50° C. starting from 0° C. the efficiency of the sodium chloride for producing the solution of globulin has rather more than doubled.

The electrical conductivity temperature coefficient for sodium chloride is also about two per cent. per degree, and since the increased conductivity with increase of temperature in dilute solutions is due to the increased velocity of the ions, we are justified in concluding that the solution of globulin by electrolytic ions is directly proportional to the velocity of the ions.

D. *The relation between the ions of a neutral salt and the globulin.*

It has already been stated that the salt does not accumulate in any part of a globulin salt system. If we take a solution of globulin in neutral salt and determine its electrical conductivity, we find that it is the same as a similar solution of salt in pure water; in fact, that so far as the electrical nature of the ion is concerned there is no evidence of a close union with the proteid molecule.

E. *The theory of the mode of action of neutral salts.*

In the previous pages we have arrived at a number of facts regarding the solution of globulin by neutral salts.

The chief of these, stated briefly, are :—

1. The amount of globulin dissolved by a neutral salt depends upon the percentage present and not on the total quantity of salt.
2. The amount of globulin dissolved by a given percentage of neutral salt is directly proportional to the strength of the original suspension of globulin.



3. Solution of globulin by a neutral salt is due to its free ions. Ions with equal valencies, whether positive or negative, are equally efficient, and the efficiencies of ions of different valencies are directly proportional to the squares of their valencies.

4. The amount of globulin dissolved by a neutral salt is directly proportional to the velocity of its free ions.

5. The relation between the electrolytic ion and the proteid molecule is not of such a nature as to diminish the electrical conductivity of the salt solution.

These facts enable us to form a conception of the processes occurring in a salt globulin system.

We may assume that the equilibrium of a globulin salt system is of a dynamic nature—that the electrolytic ions are constantly in motion and the globulin molecules at rest, and that equilibrium is reached only when the same number of globulin molecules are dissolved as are precipitated in each unit of time. Also we may assume that an ion is a centre of force and that this force is directly proportional to the valency of the ion.

Let a monovalent ion exert a force F at a distance X from the centre of the ion.

An ion with a valency " n " will exert the same force at a distance nX .

For any ion the area of the sphere within which the ionic ~~force~~ potential is greater or equal to F is

$$2\pi (nX)^2 = 2\pi X^2 (\text{valency})^2.$$

If we now postulate that, when a molecule of globulin is subjected to an ionic force greater than or equal to F it goes into solution we may deduce that:—

(a) The amount of globulin dissolved is directly proportional to the strength of the suspension since in unit time a number of proteid molecules proportional to the strength of the suspension pass into the effective spheres of force of the ions.

(b) The solvent efficiency of an ion is directly proportional to the square of its valency since the area of the effective sphere of ~~force~~ potential is directly proportional to the square of its valency.

(c) The amount of globulin dissolved is directly proportional to the velocity of the ion since the number of molecules of globulin which pass

into the effective sphere of force in unit time is directly proportional to the velocity of the ion.

But there are some facts which prevent the entire acceptance of the theory of the dynamic nature of the equilibrium of a globulin salt system.

The determination of the amount of globulin dissolved by any given percentage of salt was made by adding the salt to the globulin suspension and allowing the mixture to settle. On the dynamic theory the concentration of dissolved globulin should increase from the top to the bottom of the mixture. But this was found not to be the case—the amount of dissolved globulin in the various layers was constant.

Although therefore the original equilibrium to be attained must be of a dynamic type, yet this must be particularly circumscribed and quickly take on a static form. In whatever way this stasis may arise the degree of fixation of the ions is not sufficiently great to prevent the free passage of an electric current—the conductivity of the salt solution is not altered by the presence of dissolved globulin within it.

There is also the observation that neutral salts are slightly more efficient in dissolving a given percentage of globulin when the strength of the original is small. This indicates a slight inhibitory action of the globulin molecules on ionic movements.

2. THE PRECIPITATION OF GLOBULIN FROM SOLUTION IN NEUTRAL SALTS.

A. *By neutral salts.* Some neutral salts, which, in small quantities dissolve globulin, in larger quantities precipitate it from solution. The neutral salts which are the most efficient precipitants of globulin are ammonium sulphate, magnesium sulphate, and sodium sulphate. These salts are all freely soluble in water and require to be added in large amounts to produce precipitation.

But that the quantity of salt able to be dissolved is not of importance is indicated by the fact that salts such as magnesium chloride or calcium chloride can be added to a globulin solution in quantities many times equivalent to a precipitating amount of ammonium sulphate without producing any apparent change.

When the percentage of salt in a precipitated globulin solution is diminished either by dilution with water or by filtering and adding water to the precipitate, resolution takes place, and the redissolved globulin has apparently all the properties of the original substance.

The solubility curves of the precipitate cannot be compared with those of the original substance since the salt clinging to it can be dialysed away only after a considerable time, and such a procedure alone alters the solubility properties.

The quantitative precipitation of globulin by ammonium sulphate and magnesium sulphate at constant volume and temperature was determined. A solution of globulin in the salt to be tested was made and additional salt was added to a known number of cubic centimetres in a series of test tubes so that varying amounts of precipitate were produced. The quantity of globulin not precipitated was determined by

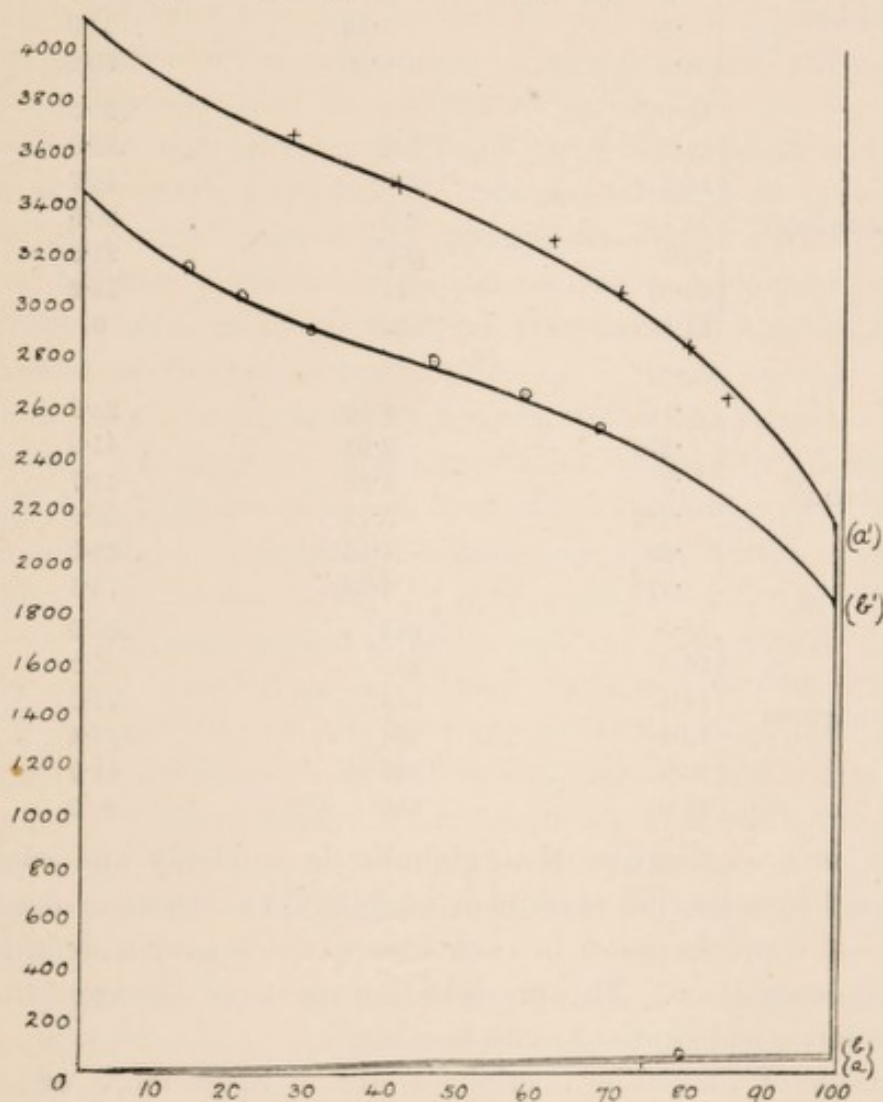


Fig. 11. In ordinates, equivalent weights of salts = 1000. Abscissæ represent percentages of original globulin dissolved.

- (a) represents MgSO_4 solubility curve.
- (a') represents MgSO_4 precipitation curve.
- (b) represents Am_2SO_4 solubility curve.
- (b') represents Am_2SO_4 precipitation curve.

coagulating and washing a known volume of the filtrate. The solubilities of the globulin in these salts were determined at the same time so that the solubility and precipitation curves are comparable. The results obtained are given numerically below and graphically on Fig. 11.

(a) *Magnesium sulphate and ammonium sulphate as solvents and precipitants of globulin. Temperature 20° C.*

	% Am_2SO_4	Salt in terms of eq. wt. = 100	Percentage of original globulin in solution
Solvent	.25	3.78	33.5
	.3	4.55	49.7
	.35	5.30	67.0
	.4	6.05	80.0
Precipitant	16.6	252	68.5
	17.5	265	55.3
	18.3	278	46.7
	19.16	290	30.5
	20.0	303	21.3
	20.83	314	14.2
	21.8	330	0
MgSO_4			
Solvent	.15	2.50	28.0
	.175	2.91	41.0
	.2	3.33	47.7
	.225	3.71	56.8
	.25	4.16	65.0
	.275	4.58	74.5
Precipitant	15.6	264	85.0
	16.8	284	80.0
	18.1	305	71.0
	19.4	326	62.4
	20.6	346	42.0
	21.8	366	28.0

There is no indication that globulin is suddenly and completely precipitated by a specific amount of ammonium sulphate or magnesium sulphate—the precipitation in each case extends over a definite range of salt concentrations. The precipitation curves of the two salts are of a regular form, and similar to one another.

Amounts of ammonium sulphate are a little more effective as precipitants than equivalent quantities of magnesium sulphate. In the case of ammonium sulphate we see that complete solution of the globulin exists between 0.6% and 11.8% of salt; with magnesium sulphate between 0.3% and 12.6%. If we take the solubility and precipitation curves of ammonium sulphate we see that the range

during which precipitation takes place is about twenty-six times as large as that which suffices for complete solution.

The influence of the concentration of the globulin on the precipitation curve was not determined. If the same law holds true for globulin as for albumen, then the more dilute the globulin solution, the less effective as a precipitant is any given percentage of salt. But this variation is small and requires albumen solutions of considerable strength to demonstrate it.

There is a slight temperature coefficient—the higher the temperature the less effective any given percentage of salt is as a precipitant.

Mode of action of neutral salts. The solution of globulin by a neutral salt depends upon forces exerted by its free ions.

Precipitation, therefore, may depend upon the absence of free ions, or upon some other physical or chemical change in the system. Solutions of neutral salts which precipitate globulin are good conductors of electricity and consequently contain a large number of free ions. Therefore precipitation by these salts must be initiated by some other physical or chemical change.

An hypothesis which is in accordance with experimental results is that precipitation depends upon a molecular combination between the salt and globulin, the compound so formed being stable only in excess of the combining salt. The idea of a molecular combination is suggested by the fact that the most effective precipitants are all sulphates. The character of the precipitated globulin and the observation that precipitation takes place over a definite range of salt concentrations renders it necessary to assume that the molecular compound is easily dissociated when the excess of salt is removed. If a stable molecular compound were formed, then, when precipitation took place, the globulin salt compound being removed from the sphere of action, the reaction should go on until a definite amount of globulin was precipitated by a definite amount of salt.

The equilibrium in a precipitated globulin salt mixture must be between the salt globulin compound, the molecules of salt, and the free ions of the salt. The amount of salt combined with the globulin is probably a very small proportion of the total salt present. Otherwise the percentage amount of globulin present would have a more marked influence on the precipitating efficiency of any given percentage of salt.

The antagonistic actions of the salt molecules and ions affords a satisfactory explanation of the results stated above. Ammonium

sulphate is a more effective precipitant than magnesium sulphate. On the other hand magnesium sulphate is a more effective solvent. Also the more concentrated a globulin solution the more effective any given percentage of salt is as a precipitant. The degree of concentration of the globulin affects the precipitating efficiency of any given quantity of salt in two opposite ways—by combining with the salt and by diminishing the velocity of the ions in increasing concentrations.

The first factor probably has very little influence—the amount of salt in actual combination being small—and being more than counteracted by the effect the globulin has on the velocity of the ions. That globulin has some effect in diminishing the velocity of electrolytic ions has been noted in the consideration of the solution of globulin. This increased precipitating efficiency of a given percentage of salt with increasing concentrations of globulin corresponds to the increased solvent efficiency with diminishing concentrations, and may be compared with the parallel phenomena of the solvent and precipitating efficiencies of ammonium sulphate and magnesium sulphate.

The diminished precipitating efficiency of any given percentage of salt with increased temperature is due to the increased temperature ionising more molecules and increasing the velocity of the ions.

The argument in favour of a molecular combination of salt and globulin only stable in a large excess of salt is materially strengthened by a consideration of the phenomena observed in the precipitation of globulin by metallic salts.

B. *By salts of the heavy metals.* Globulin is precipitated from solution in neutral salts by minute quantities of salts of the heavy metals. The precipitate so produced is only slightly soluble in neutral salts and requires acids or alkalies to adequately dissolve it. If coloured metallic salts be used, the precipitate has the characteristic colour of the precipitating salt, however long it may be washed. A quantitative determination of the precipitation of globulin dissolved in sodium chloride by zinc sulphate gave the following results.

A solution of globulin in .6 % NaCl was made.

5 c.c. of this were put into each of a series of test-tubes, and to these zinc sulphate dissolved in water containing .6 % NaCl was added so as to produce varying degrees of precipitation.

The precipitations were done at constant volume (10 c.c.), .6 % NaCl being added to the required amount in every case.

The solution of the zinc sulphate in .6 % NaCl is important; otherwise a considerable precipitation of globulin is produced by diminishing the original percentage of sodium chloride.

% ZnSO_4	ZnSO_4 in terms of eq. wt. = 100,000	% of original globulin precipitated
·005	6·2	12·6
·01	12·5	27·5
·0125	15	35
·02	25	43
·0225	28	45

From the above it may be seen that the statement that zinc sulphate is comparable to ammonium sulphate as a precipitant for proteids and is interchangeable with it in any precipitation is not true for globulin.

The precipitates produced in the two cases differ widely in their properties. Also comparing the above table with that given for the

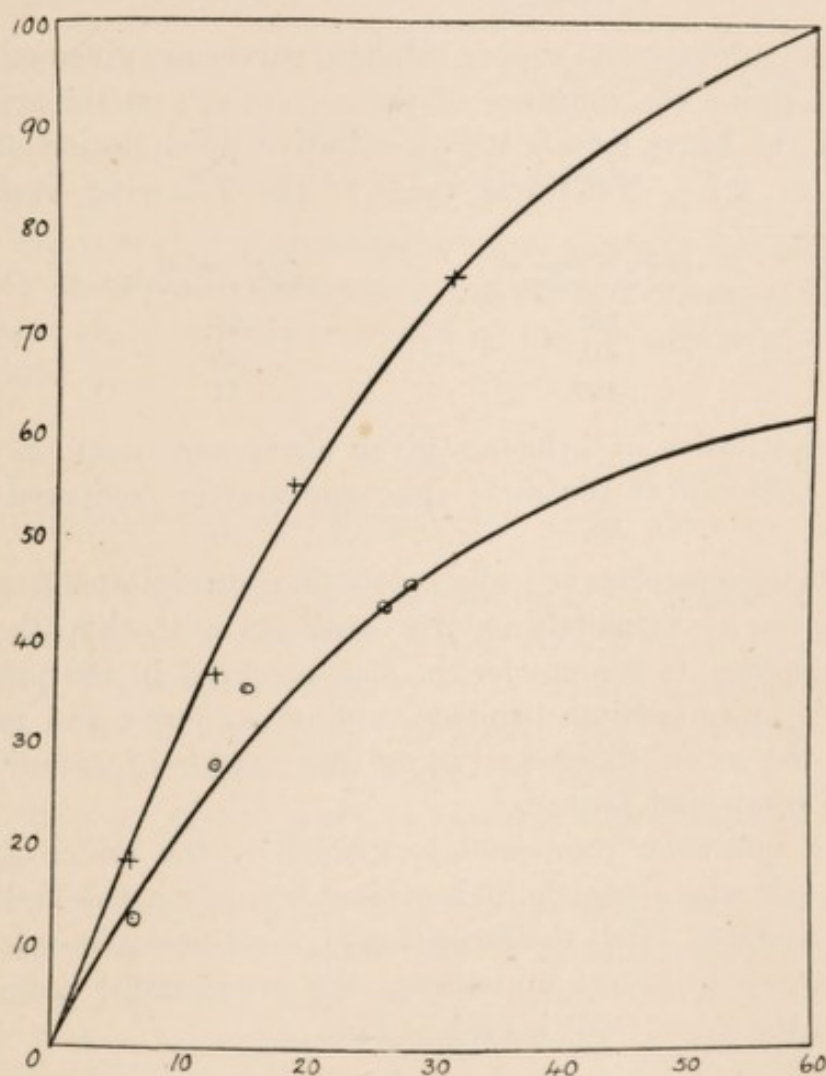


Fig. 12. Ordinates represent percentages of original globulin precipitated. In abscissæ, equivalent weights of salts = 100,000.

○ ZnSO_4 points.

+ CuSO_4 points.

precipitation of globulin by ammonium sulphate we see that 0.005% ZnSO_4 is as effective a precipitant as 19% Am_2SO_4 . If we express this in terms of the equivalent weights of the two salts we find that about three thousand times as much ammonium sulphate as zinc sulphate is required to produce the same degree of precipitation.

The quantitative precipitation of globulin dissolved in 6% sodium chloride by copper sulphate was determined. The following results were obtained.

CuSO_4 in terms of eq. wt. = 100,000	% of original globulin precipitated
6.25	18.2
12.5	36.5
18.75	55.0
31.25	75.0

The zinc sulphate and copper sulphate curves are given on Fig. 12.

To determine the influence of the solvent salt on the precipitation by salts of the heavy metals the quantitative precipitation of globulin dissolved in 2.7% NaCl was made. The following results were obtained:—

ZnSO_4 in terms of eq. wt. = 100,000	% of original globulin precipitated
120	23
310	35
430	44

The quantitative experiments given above were done on the same sample of globulin at the same time and may be compared with one another.

The phenomena observed when globulin is precipitated from solution by salts of the heavy metals may be explained on the hypothesis which has been assumed to account for the facts observed in the precipitation of globulin by ammonium sulphate, sodium sulphate, and magnesium sulphate, the main difference being the degree of stability of the molecular compound formed.

That a molecular compound is formed by the union of the precipitating salt with the globulin is evident from the colour and solubility of the precipitate. That the formation of this compound depends upon an equilibrium being set up between the precipitating molecules and the ions of the solvent salt is evident from

(a) The influence of the solvent salt on the precipitating efficiency of the metallic salt—an increase of the solvent salt from 6% NaCl to 2.7% NaCl demands an increase of zinc sulphate to ten times its previous amount.

(b) The existence of the precipitating salt in the filtrate showing that it is not all combined with the precipitated globulin.

(c) Copper sulphate being a more efficient precipitant than zinc sulphate although comparable to it in order of magnitude.

It may be noted in the curves for the precipitation of globulin by zinc sulphate and copper sulphate that at first the amount of precipitate produced is directly proportional to the amount of salt added. With larger quantities of salt this does not hold true—the curve bends round, showing that the additional salt is less efficient than the first quantity. This may depend on the presence of free ions in the precipitating salt assisting the ions of the solvent salt.

In the zinc sulphate experiment given above it was found that:—

·005 grs. of zinc sulphate precipitated ·3 grs. of globulin, or at the beginning of precipitation

·005 grs. $\text{ZnSO}_4 = \cdot 3$ grs. globulin.

Therefore

161 grs. $\text{ZnSO}_4 = 9700$ grs. globulin.

If we assume that a molecule of zinc sulphate combines with a molecule of proteid and that at the beginning of precipitation all the salt is combined the molecular weight of the globulin works out to be about 10,000.

3. THE SOLUTION OF GLOBULIN BY ACIDS AND ALKALIES.

Hardy has drawn attention to the precipitation of globulin dissolved in acids and alkalies by traces of neutral salts. The determination of the solubility of globulin in these solvents requires therefore a salt-free preparation. Globulin was prepared in the way previously described and was thoroughly washed by repeated suspension in water and filtration. By this method globulin suspended in water free from salt was obtained.

But that the globulin so prepared was associated with traces of salt is evident from the following facts. (a) A specimen free from ash was never obtained. (b) If a suspension of globulin were boiled and so coagulated it was found that the conductivity of the solution had appreciably increased after cooling to the original temperature.

The increase in conductivity was not due to the coagulation of the particles of globulin since it has been shown that these particles do not interfere with the free movement of electrolytic ions. The increase in

conductivity must have been due to the liberation of salt molecules when the globulin was coagulated.

(c) If acid or alkali were added to a suspension of globulin a clear solution was first obtained which in a variable space of time deposited a precipitate. This precipitate could be redissolved by adding an additional quantity of solvent.

It is probable that the association of traces of salt with globulin is of the nature of a molecular combination and that this combination is broken down when the proteid is coagulated or is dissolved in acids or alkalies.

The association of salt with globulin makes the determination of its properties when dissolved in acids or alkalies difficult. But since the solution of globulin, when in a fresh state of suspension, by acids or alkalies is practically instantaneous, and precipitation of the solution so obtained by salts requires a definite space of time, an approximate determination of its solubility in these solvents is possible.

In determining the comparative efficiencies of acids and alkalies very intimate suspensions of globulin in pure water were used, and for each acid or alkali several determinations were made. Approximate quantities of solvent required to dissolve a given amount of globulin were determined and then the final experiments were made in which the addition of the solvent was made in the shortest possible time. In this way the error introduced by reprecipitation of the globulin was reduced to a minimum.

As illustrating the necessity of determining the solubility of globulin in acids or alkalies in this way it may be stated that in a few minutes after complete solution of the globulin with a minimum quantity of solvent, a large quantity of globulin was reprecipitated and this reprecipitated proteid required a considerable additional amount of solvent to redissolve it.

The determination of the solubility of globulin in acids and alkalies was made by finding what quantity of solvent was required to dissolve

(a) A given amount of globulin when suspended in variable quantities of water.

(b) Variable amounts of globulin when suspended in the same quantity of water.

It was found that for any one specimen a given quantity of globulin always required the same amount of acid or alkali to dissolve it, and

that the quantity dissolved by a given amount of acid or alkali was independent of the strength of the original globulin suspension.

It was also found that there was no temperature coefficient—a given quantity of globulin required the same amount of acid or alkali to dissolve it whatever the temperature.

These two facts—the law of definite proportion between the solvent and globulin and the absence of a temperature coefficient—indicate that the solution of globulin by acids and alkalies is of the nature of a chemical combination.

The determination of the relative efficiencies of acids and alkalies for producing solution of globulin gave the following results in two separate experiments.

(a)	0.930 grs. of globulin were dissolved by	4.5 c.c. H_2SO_4	$\frac{N}{200}$
	"	"	"
	"	3.2 c.c. HCl	$\frac{N}{200}$
	"	"	"
	"	2.9 c.c. NaOH	$\frac{N}{200}$
	"	"	"
	"	2.0 c.c. KOH	$\frac{N}{200}$
	"	"	"
(b)	1.68 grs. of globulin were dissolved by	3.5 c.c. H_2SO_4	$\frac{N}{200}$
	"	"	"
	"	2.5 c.c. HCl	$\frac{N}{200}$
	"	"	"
	"	2.2 c.c. NaOH	$\frac{N}{200}$
	"	"	"
	"	2.3 c.c. KOH	$\frac{N}{200}$
	"	"	"

If we compare the efficiencies of the acids and alkalies in the experiments (a) and (b), taking the efficiency of hydrochloric as 100, we get:

(a) Efficiency	(b) Efficiency
HCl 100	HCl 100
H_2SO_4 71	H_2SO_4 71
NaOH 110	NaOH 113
KOH 110	KOH 109

That is the relative efficiencies of these solvents were about the same in the two experiments.

If we determine in each case the weight of globulin dissolved by a gram equivalent of hydrochloric acid we get the following results.

(a)	Gram equivalent of HCl=10500 grs. of globulin,
(b)	" " " = 7440 " "

From this we see that although the relative solvent efficiencies of the acids and alkalies used were the same in the two experiments, yet the weights of globulin dissolved by a gram equivalent of hydrochloric acid were widely different—indicating that in different animals of the

same species a wide difference in the size of the molecule is possible, although the constitutional type remains the same.

It may also be noticed that alkalies are more efficient solvents than acids, pointing to a more strongly marked acidic nature of globulin.

If we compare the relative efficiencies of the two acids and the two alkalies above we see that their efficiencies are of the same order as their relative avidities. This might have been anticipated from the chemical nature of globulin when suspended in water—its capacity to act as an acid or base. The avidity hypothesis is further supported by the fact that nitric acid has approximately the same efficiency as hydrochloric acid. But it is not supported by the relative efficiencies of organic acids such as acetic acid, oxalic and citric. If we take the efficiency of hydrochloric acid as a hundred these acids have an efficiency between thirty and forty. Their avidities are about 1% of that of hydrochloric acid.

4. THE PRECIPITATION OF GLOBULIN DISSOLVED IN ACIDS AND ALKALIES BY NEUTRAL SALTS.

The precipitation of globulin dissolved in acids and alkalies has been worked out by Hardy¹. He states that the active precipitating ion is of opposite electric sign to that of the colloid particles and that the amount of salt required to precipitate is a logarithmic function of the valency of the active ion. He has not, as yet, published the details of his experiments.

An attempt was made to verify those laws. The chief difficulty which prevented accurate results from being obtained was the reprecipitation of the globulin when dissolved in a minimal quantity of acid or alkali. This difficulty could be partially overcome by adding an excess of acid or alkali, but the criticism then remained that the conditions of the experiment were not known.

Also when an excess of acid or alkali was used to dissolve the globulin and prevent subsequent precipitation on adding neutral salts and allowing to stand, the precipitate produced only settled in those tubes in which precipitation was practically complete. Filtration was impossible since it may be assumed that the acid globulin salt system enters into a condition of dynamic equilibrium, and removal of the precipitated globulin upsets this state. Consequently only end points could be obtained. These factors effectively prevented accurate pre-

¹ This *Journal*, xxiv. pp. 158, 288. 1899.

cipitation curves of globulin dissolved in acid or alkalies by various neutral salts from being obtained.

From a series of experiments the following results were obtained.

(1) When globulin was dissolved in alkalies, magnesium chloride and magnesium sulphate were more efficient precipitants than potassium chloride and potassium sulphate.

(2) When globulin was dissolved in acids, potassium sulphate and magnesium sulphate were more efficient precipitants than potassium chloride and magnesium chloride.

(3) To produce equal degrees of precipitation the more efficient salt had to be added in quantity equal to about one-tenth of that of the less efficient.

If we analyse the above results we see that in these particular experiments the most efficient precipitating ion of globulin dissolved in alkalies is magnesium—a divalent positive ion; and of globulin dissolved in acids is the sulphate—a divalent negative ion.

In alkaline solution the globulin is electrically negative: in acidic solution electrically positive. And therefore in this experiment the more effective precipitating ion of the neutral salt had an opposite electric sign to that of the colloid particles. This is a particular instance of a portion of Hardy's general law¹.

In Hardy's general law no mention is made of the part played by the ions of the solvent otherwise than by determining the charge on the colloid particles. In the precipitation the character of the solvent has a marked influence in determining the velocity of precipitation. With sulphuric acid the velocity of precipitation is much more rapid than with hydrochloric acid, whatever the nature of the precipitating salt. Also precipitation is much slower from alkaline than from acidic solution. The ultimate amount of precipitation appears to be independent of the velocity.

The physical characters of the globulin are not materially altered by solution in acids or alkalies and subsequent reprecipitation by neutralisation or by adding neutral salts. These reprecipitated globulins have the same solubility curves in neutral salts as the original substance. These processes, therefore, do not involve any marked change in the proteid molecule.

An attempt to determine the relation between globulin dissolved in acids and alkalies and neutral salts was made by working in the reverse direction. From an *à priori* point of view it seemed legitimate

¹ This *Journal*, xxiv. p. 181.

to assume that if globulin dissolved in acid or alkali is precipitated by neutral salts, then globulin dissolved in neutral salts ought to be precipitated by acids and alkalies. That this assumption is incorrect is indicated by the fact that globulin dissolved in neutral salts is only partially precipitated by acids and not by alkalies.

5. THE PRECIPITATION OF GLOBULIN DISSOLVED IN NEUTRAL SALTS BY ACIDS.

The precipitation of globulin from solution in neutral salts by various acids was determined. For a known concentration of globulin suspension

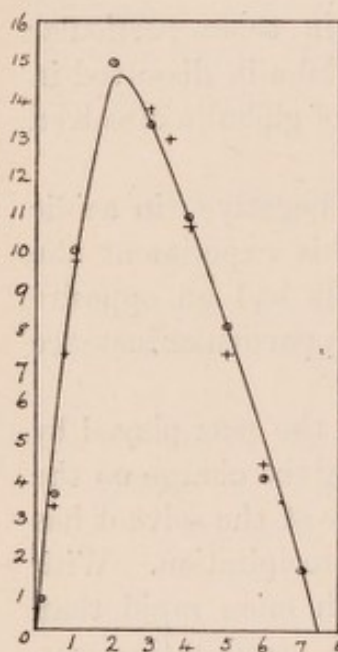


Fig. 13.

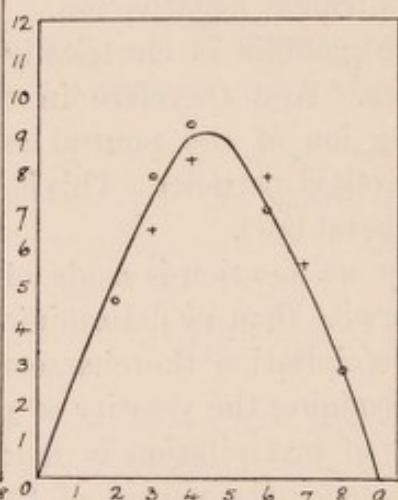


Fig. 14.

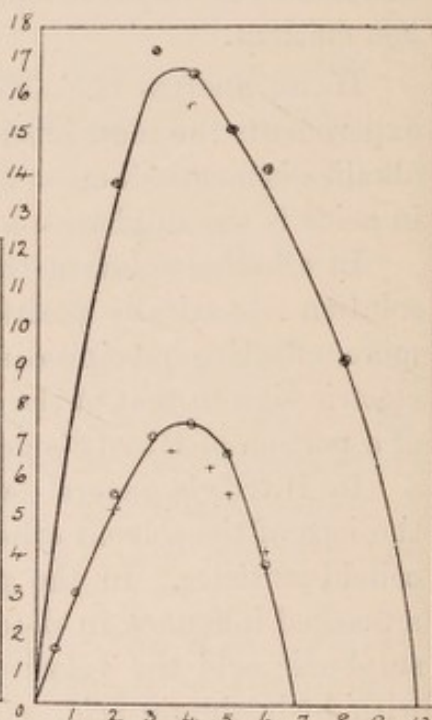


Fig. 15.

Fig. 13. Ordinates represent percentages of original globulin precipitated. Abscissæ represent quantities of acid. $\frac{N}{400}$ acid = 10. Globulin dissolved in .5% NaCl.

○ HCl precipitation points.

+ H₂SO₄ precipitation points.

Fig. 14. Ordinates represent percentages of globulin precipitated. Abscissæ represent quantities of acid. $\frac{N}{400}$ acid = 10. Globulin dissolved in .5% Na₂SO₄.

○ HCl precipitation points.

+ H₂SO₄ precipitation points.

Fig. 15. Ordinates represent percentages of globulin precipitated. Abscissæ represent quantities of acid. $\frac{N}{400}$ acid = 10.

Globulin dissolved in .3% MgSO₄: ⊕ HCl precipitation points (upper curve).

Globulin dissolved in .3% MgCl₂: ○ HCl precipitation points (lower curve).

+ H₂SO₄ precipitation points („ „).

saturated solutions of globulin in .3% MgSO_4 , .3% MgCl_2 , .5% Na_2SO_4 , .5% NaCl were made. To these solutions sufficient original salt was added to double the percentage, so that on adding water and acid to double the volume the original percentage of salt was obtained. This addition of salt is important to eliminate the precipitation error introduced by diminution of the percentage of salt when acid and water are added to constant volume.

The precipitations of the globulin dissolved in each salt by hydrochloric and sulphuric acids were worked out. The results are summarised below and are given graphically on Figs. 13, 14, 15.

Globulin in .5% NaCl

c.c.s of $\frac{N}{100}$ acid in each 10 c.c.		% globulin precipitated	c.c.s of $\frac{N}{100}$ acid in each 10 c.c.		% globulin precipitated
HCl	.3	.8	.5	3.22	
	.5	3.62	.75	7.25	
	1.0	10.05	1.0	9.7	
	2.0	14.95	2.0	14.9	
	3.0	13.3	3.0	13.7	
	4.0	10.85	3.5	12.9	
	5.0	8.05	4.0	10.6	
	6.0	4.02	5.0	7.25	
	7.0	1.6	6.0	4.42	

Na_2SO_4 .5%

HCl	2	4.65	H_2SO_4	3	6.52
	3	7.9		4	8.35
	4	9.3		5	8.85
	5	8.8		6	7.9
	6	7.0		7	5.6
	8	2.8			

MgCl_2 .3%

HCl	.5	1.47	H_2SO_4	.5	1.47
	1	2.92		2	5.12
	2	5.5		3	5.84
	3	7.0			
HCl	4	7.32	H_2SO_4	3.5	6.58
	5	6.6		4.5	6.22
	6	3.66		5.0	5.48
				6	4.02

MgSO_4 .3%

HCl	2	13.6
	3	17.1
	4	15.6
	5	15.0
	6	14.0
	8	8.95

From these results the following conclusions may be drawn.

(a) Precipitation by acids of globulin dissolved in salts is never complete.

(b) For the precipitation of globulin dissolved in any given salt hydrochloric acid has the same efficiency as sulphuric acid when equivalent quantities are compared.

(c) The amount of acid precipitation and its limits are not widely different for different salts.

(d) Quantities of acid which produce a maximal precipitation are comparable to the quantities that would be required to dissolve the original globulin.

These facts are difficult to explain on any simple hypothesis. That precipitation is due to an antagonism between the ions of the neutral salt and those of the acid is evident from the observations that increase of temperature or increase in the original percentage of salt to about double its amount entirely prevents precipitation.

But that the degree of antagonism is actively controlled by the amount of globulin present is apparent from the quantitative relations of the amount of acid required to produce maximum precipitation.

6. APPENDIX.

The solubility in neutral salts of globulin obtained from one horse affords strong evidence of the physical identity of the preparation used. This evidence is strengthened by a neutral salt fractionation of a preparation of globulin. The solubility curves obtained from the different fractions are identical.

But that the physical properties of preparations of globulin obtained from different horses differ in degree though not in kind is indicated by the fact that the solubility curves of different preparations do not coincide. The differences are usually too great to be accounted for by differences in preliminary treatment, although contact with pure water is found to have some influence on the solubility of the specimen. The different equivalent weights obtained from a determination of the solubilities of different specimens of globulin in acids and alkalies affords evidence of a variable molecular mass. These differences in molecular weight have been suggested to be due to associated nucleo-proteid. But from the method of preparation described above it is improbable that nucleo-proteid is associated with the final product.

The fibrinogen obtained from plasma by dilution with water and neutralisation with acetic acid is associated with this substance. But the subsequent treatment involving solution in neutral salt and coagulation will remove this undissolved nucleo-proteid moiety. Also the final product besides being readily soluble in neutral salts is completely dissolved by both acids and alkalies.

The different phenomena displayed by globulin when dissolved in neutral salts, acids or alkalies limit the number of experiments which may be made to definitely illustrate any one property.

Thus the determination of the solubility of globulin in a neutral salt demands that the salt used should belong to the alkali or alkaline earth group and be perfectly stable and fully dissociated in dilute aqueous solution. The consideration of the solution of globulin by salts of potassium illustrates these necessities. The following results give the solubility of a globulin preparation in KCl, KBr, KI, $K\bar{A}$, K_2SO_4 . These results are depicted graphically in Fig. 16.

Salt in terms of eq. wt.=1000		% of original globulin dissolved	Salt in terms of eq. wt.=1000		% of original globulin dissolved
KCl $7.45\% = 1000$	53.6	33.7	KI $16.6\% = 1000$	36.0	33.7
	67.1	46.5		42.0	37.5
	73.8	59		48.0	47.0
	80.6	71		54.0	61.0
	87.5	83		57.0	67.5
K_2SO_4 $8.7\% = 1000$	40.3	50	$K\bar{A}$ $9.8\% = 1000$	91.9	55.5
	46.0	57		97.0	58.5
	51.8	67		102.0	65.0
	57.3	79		112.0	71.0
	63.2	91		122.0	78.0
KBr $11.9\% = 1000$	50.3	37.0			
	59.0	46.5			
	67.2	60.0			
	71.3	72.0			
	75.5	77.5			
	84.0	91.0			

It may be noted that the curves for KCl and K_2SO_4 are related to one another according to the ionic law of valency stated above but that the curves for KCl, KBr, KI, and $K\bar{A}$ do not coincide. KBr is a more efficient solvent than KCl and KI more so than KBr, as has been already pointed out by Osborne¹; $K\bar{A}$ is less efficient than KCl.

¹ *Amer. Journ. Physiol.* xiv. 1905.

The increased efficiencies of KBr and KI are due to the hydrolysis of these substances in dilute aqueous solution. A very small amount of chemical change in substances composed of a weak acidic and a strong basic ion readily produces an increased solvent efficiency. The relative efficiencies of KBr and KI are in the same order as their chemical stability in aqueous solution.

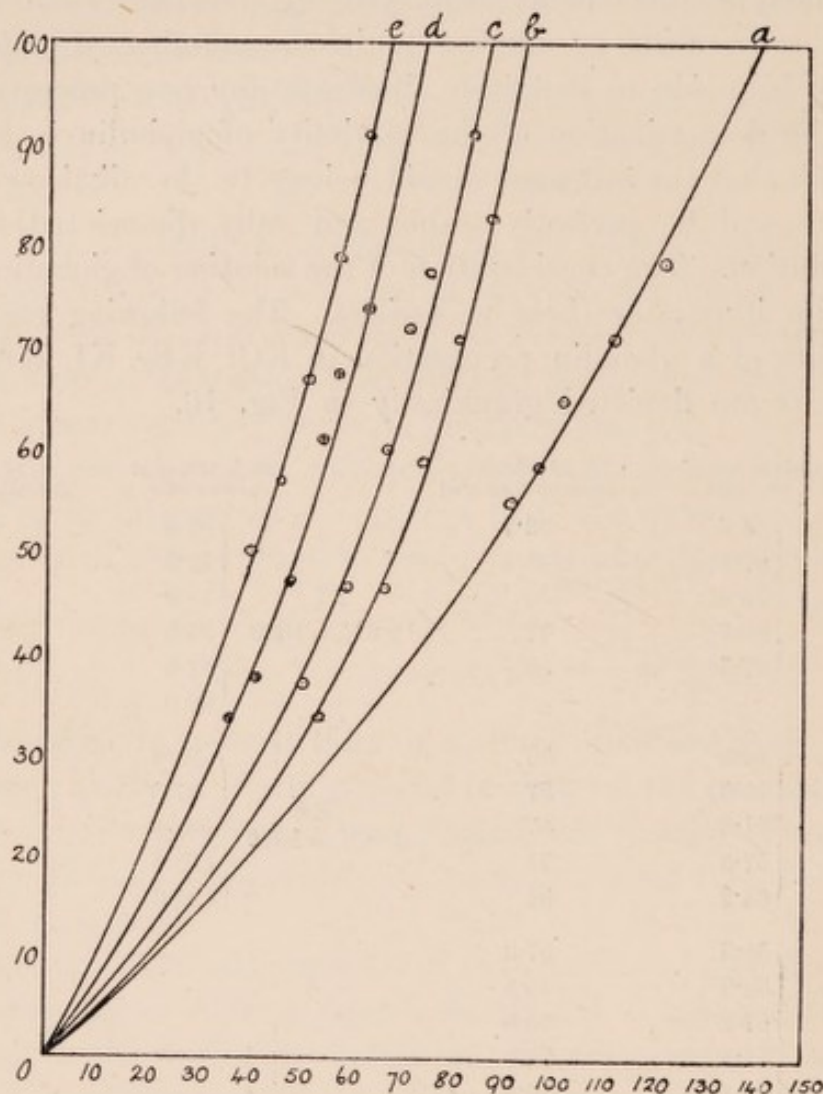


Fig. 16. Ordinates represent percentages of globulin dissolved. In abscissæ, equivalent weights of salts=1000.

a KA curve, *b* KCl curve, *c* KBr curve, *d* KI curve, *e* K₂SO₄ curve.

The decreased solvent efficiency of potassium acetate is due to its incomplete ionisation in aqueous solution.

The postulate that the neutral salts used must be perfectly stable and fully ionised in aqueous solution prevents the experimental demonstration of the ionic law with salts containing trivalent radicals.

The experimental work described above has involved many quantitative determinations. In this work I have received great assistance from Mr A. J. Ewins.

My thanks are due to Dr W. Dowson for the many facilities and opportunities which he has afforded me during the progress of this work.

SUMMARY.

I. Solution of globulin by a neutral salt is due to forces exerted by its free ions. Ions with equal valencies, whether positive or negative, are equally efficient, and the efficiencies of ions of different valencies are directly proportional to the squares of their valencies.

II. The amount of globulin dissolved by a given percentage of neutral salt is directly proportional to the strength of the original globulin suspension.

III. The precipitation of globulin from solution in neutral salts by neutral salts depends upon a molecular combination between the salt and globulin, the compound so formed being stable only in excess of the combining salt. Precipitation by salts of the heavy metals depends upon the formation of a stable salt globulin compound.

IV. Solution of globulin by acids or alkalies is of the nature of a chemical combination. The relative solvent efficiencies of strong acids and alkalies are of the same order as their chemical avidities.

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