The proteids of serum / by W.D. Halliburton.

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by

W. D. Halliburton

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THE PROTEIDS OF SERUM.

By W. D. HALLIBURTON, M.B., B.Sc. (*Lond.*), *Sharpey Physiological Scholar, and Fellow of University College, London.* Plate V. Figs. 3 and 4.

(*From the Physiological Laboratory, Univ. Coll. Lond.*)

The proteids of serum are generally regarded as two in number. One of these has received the names Serum Casein[1], fibrino-plastic substance[2], Paraglobulin[3], and Serum Globulin[4]. The other is called Serum Albumin, or Serine[5].

The nomenclature which I shall adopt in this paper will be for the former Serum Globulin, for the latter Serum Albumin. These names are on the whole the most convenient.

The Globulin of Serum, or Serum Globulin, has been the subject of many researches, particularly those of Schmidt and Hammarsten.

The Albumin of Serum, or Serum Albumin, has perhaps not received its due share of attention: it was to the investigation of this latter body that this research has been in the main directed.

Concerning the chemical constitution of the proteids in general, and of these two in particular, little or nothing is known: they are however well separated from one another by their physical characters, which no doubt indicates some definite difference in chemical constitution. But with the more strictly chemical side of the subject this paper will not deal. I shall here treat only of some of the physical characters of the proteids of serum.

[1] Panum, *Arch. f. Pathol. Anat.* Bd. IV. Heft 3, 1851; also in Virchow-Hirsch *Jahresbericht für* 1869. Bd. I. s. 91. Concerning the question of the existence in the serum of a serum casein there will be but little to say. Hammarsten's researches on Paraglobulin (Hammarsten, *Ueber das Paraglobulin. Pflüger's Archiv*, Bd. 17, 1878, p. 413 et seq., Bd. 18, 1878, p. 35 et seq.) seem to place beyond all doubt that a special serum-casein does not exist, at any rate normally in blood serum.

[2] Alex. Schmidt, *Arch. f. Anat. und Physiol.* 1861 s. 545, and 1862 s. 428.

[3] W. Kühne, *Lehrbuch d. physiol. Chem.* Leipsic s. 174. O. Hammarsten, *Arch. f. d. ges. Physiol.* Bd. XVII. s. 413, 1878.

[4] Weyl, *Zeitschr. f. physiol. Chemie*, Bd. I. s. 77, 1877.

[5] Denis, *Memoire sur le sang.* Paris, 1859.

The serum with which my work has been done has been chiefly blood-serum of mammalian animals. I have also submitted to examination the varieties of human serum which are generally known as pathological exudations.

These experiments, of which a preliminary notice has already appeared in the *Proceedings of the Royal Society*, may be arranged into two chief categories: first, those relating to the temperature of heat-coagulation of the proteids; and secondly, those relating to the action of certain salts upon them.

Concerning the Temperature of Heat-coagulation of the Proteids of Serum, especially of Serum Albumin.

The method by which the temperature of coagulation is generally ascertained is as follows[1]:—"A glass beaker containing water is placed within a second larger beaker also containing water, the two being separated by a ring of cork. Into the water contained in the inner beaker there is immersed a test-tube in which is fixed an accurately graduated thermometer, provided with a long narrow bulb. The solution of proteid of which the temperature of coagulation is to be determined is placed in the test tube, the quantity being just sufficient to cover the thermometer bulb. The whole apparatus is then gradually heated: the experimenter notes the temperature at which the liquid first shows signs of opalescence; he afterwards notes again the temperature at which a distinct separation of flocculent matter occurs."

This apparatus was found after repeated trials to possess two defects: first, the rise of temperature takes place with such extreme slowness that changes are apt to occur in the liquid under investigation during the experiment; secondly, it is exceedingly difficult to maintain the temperature constant for a considerable length of time. To obviate these difficulties a simple apparatus was devised by Professor Schäfer, which was found to be very easy to use. The accompanying figure gives a sketch of the apparatus.

F, Fig. 3, Pl. V. is a glass flask supported on a stand: down its neck is placed a test-tube, in which again is placed the liquid under investigation in sufficient quantity to cover the bulb of the thermometer put into it. The flask is kept filled with hot water, and this water is constantly flowing. It enters the flask by the tube *a* which goes down to the bottom of the flask, and leaves it by the tube *b* which is

[1] Gamgee's *Physiological Chemistry*, p. 15.

inserted as a T-piece into the upper part of the neck of the flask. This flowing water is kept hot by passing through a coil of tubing contained in a copper vessel C: this vessel contains water which is kept boiling by means of a large Bunsen burner. The water enters the coil of tubing from the tap T, is warmed by passing through the coil, and then leaves it by the tube a, which we have before seen enters the flask F: in its passage through the flask it warms the test-tube and its contents. The temperature of this water varies inversely to its rate of flow; this is easily adjusted by the screw tap T. By this means the temperature can not only be raised and lowered with considerable speed, but it can also be maintained constant without difficulty for a considerable length of time. The increase in the rate of heating thus obtained, provided it be not too great, does not interfere with the accuracy of the experiment, and is productive of great saving of time.

With the object of rendering the apparatus of still greater value, especially in the maintenance of a constant temperature, a slight modification was introduced by Professor Schäfer; this is shown by Figure 4. It consists in dividing the stream from the tap into two by means of a T-piece x: some of the water then goes to the coil to be heated: the rest passes on cold and rejoins the former stream by means of another T-piece y. By regulating the screw clamps s^1 and s^2 a mixed stream of hot and cold water can be sent to the flask: by mixing hot and cold in the right proportions a constant temperature can be maintained with great ease for a considerable length of time.

One additional precaution in the use of this apparatus has to be taken, and that is this:—In order that all parts of the liquid in the test-tube shall become heated at the same time, it is necessary to keep it well stirred by the thermometer during the progress of the experiment, or at any rate during a rise of temperature.

The temperature of heat-coagulation of serum globulin is stated by Hammarsten[1] to vary between 68° and 80°, but the average he puts as 75° C. The temperature of heat-coagulation of serum albumin is put down by Hoppe Seyler[2] as 70°—73° C. This is in each case the temperature at which the precipitate becomes flocculent, for the proteid enters into incomplete coagulation or a state of opalescence a few degrees below the temperature just mentioned.

But it was found that after heating serum to the higher of these

[1] Hammarsten, "*Ueber das Paraglobulin. Zweiter Abschnitt.*" *Pflüger's Archiv*, 1878. Vol. XVIII. p. 64.

[2] Hoppe-Seyler, *Handbuch d. phys. u. path. chem. Analyse.*

two temperatures, and filtering off the resulting coagulum, that the filtrate still contained coagulable proteid, which became a flocculent precipitate at a higher temperature.

A process of fractional heat-coagulation was therefore adopted to determine accurately the temperature of coagulation of this proteid with high coagulation temperature. The words fractional heat-coagulation almost explain themselves. A sample of serum is taken, heated until a flocculent precipitate appears: this is filtered off; the filtrate is then heated to a higher temperature until another similar precipitate appears: this is then filtered off; the filtrate is again heated, the process being repeated till the filtrate is free from proteid.

But in order to carry out effectually this apparently simple proceeding it is necessary to adopt several precautions, and first to ensure that the fluid under investigation should be as nearly as possible always of the same reaction. As will be seen later on, one of the most important factors in producing variations in the temperature of coagulation of a liquid is the amount of free acid present: up to a certain point, the greater the acidity, the earlier does coagulation occur. The liquid must not be alkaline; alkali albumin is then liable to be formed either wholly or partially, and thus coagulation does not occur. Neither must the liquid be exactly neutral, for it is a well-known fact that when a proteid separates as a heat-coagulum from a liquid, such separation is accompanied by an increase in the alkalinity, or decrease in the acidity, of that liquid. If the liquid is exactly neutral, a small amount of coagulation renders the liquid alkaline; alkali albumin is then apt to form as before. In order to obtain complete precipitation at any given temperature, it is necessary that the liquid be faintly acid; under these circumstances coagulation occurs without any apparent change in the proteids under investigation—at any rate no acid albumin is formed. It is well known that this body does not form so readily as alkali albumin. The flocculi are also larger and therefore easier to filter off.

A solution of 2% acetic acid was found to be the most convenient agent for so rendering the serum acid. It was dropped from a burette into the liquid; the reaction of the liquid being ascertained, after thoroughly mixing each drop, by dipping a glass rod into it, and then putting the drop adhering to the rod, on one of the delicate, glazed, violet-tinted litmus papers, prepared by Messrs. Townson and Mercer.

The proper quantity of this acetic acid, which had to be added to ensure the above-mentioned result, was soon recognised by experience

through the tint the liquid gave to the litmus employed; the quantity may be stated roughly as one drop of acetic acid of this strength, after neutrality had been reached, to three cubic centimeters of liquid: with the burette used, twenty-five drops went to the cubic centimetre. In addition to rendering acid in this way the original liquid, it is also necessary to render similarly acid the filtrate after precipitation of a proteid.

With regard to the length of time at which a liquid has to be kept at any given temperature to ensure complete precipitation of the proteid at that temperature, it has been found that five minutes is as a rule sufficient.

It is often necessary, when the precipitates are very dense, to repeat the experiments in ten, twelve, or even twenty specimens, to obtain a quantity of filtrate sufficient to go on working with. It is also convenient in many cases to hasten filtration by filtering under pressure. One more precaution has to be mentioned, and this is, not to mistake a dense opalescence for a precipitate. What is the meaning of the opalescence that precedes precipitation seems never to have received a sufficient explanation. It appears to be an intermediate stage between complete solution and complete precipitation. An opalescent solution however passes through a filter paper with its cloudiness not lessened at all; it passes through more slowly than a clear fluid, resembling somewhat a thin jelly; it will soak, though sluggishly, through a piece of filtering paper, but no solid particles remain behind. This opalescence, whatever be its explanation, gets denser and denser as the temperature of precipitation is approached; and sometimes when the temperature of the liquid is only about one degree below that of coagulation it is almost impossible to say whether coagulation has taken place or not; generally however one can see the flocculi by stirring; in doubtful cases, one must submit the fluid to filtration. If it comes through the paper unchanged, it is evident the temperature of complete coagulation has not yet been attained.

The process of fractional heat-coagulation was applied to serum in a twofold way: it was first applied to the serum, pure and simple. The proteids of the serum were afterwards separated into their two classes by means of Hammarsten's method—that is to say, the serum was saturated with magnesium sulphate; an abundant precipitate was thus produced. This, Hammarsten has shown to consist of serum globulin, while the serum albumin remains in solution; the precipitate, after being washed from all traces of serum albumin by means of a saturated

solution of magnesium sulphate, was then dissolved by adding distilled water. The water was enabled to dissolve the precipitate of serum globulin by means of the magnesium sulphate still adhering to it.

Thus two solutions were obtained: one, a solution of the serum albumin in saturated solution of magnesium sulphate; the other a solution of serum globulin in weak magnesium sulphate. To each of these the same process of fractional heat-coagulation was applied in the same way as has been described in the original serum.

As an example of the method and its results, the case of dog's serum will be first taken.

(1) By fractional heat-coagulation in a faintly acid solution, coagula were separated at the following points:

70°–1° C. 75° C. 77° C. 82° C.

All were white flocculent precipitates. The final filtrate contained no more proteid.

(2) The question then remained, were the precipitates falling at the two higher points of the nature of globulin or albumin? This was settled by saturating the serum with magnesium sulphate; the globulin was thus precipitated; it was washed and redissolved by adding distilled water. By heat-coagulation in a faintly acid liquid, coagulation was found to occur at 75° C., all the proteid in this solution coagulating at this temperature.

(3) After saturating with magnesium sulphate, and filtering off the so-precipitated globulin, the filtrate was also submitted to fractional coagulation; and precipitates fell at the following temperatures:

70°–1° C. 77°–8° C. 82° C.

Again all these were white flocculent precipitates. In dog's serum the albumin is then not a single proteid, but consists of three with different temperatures of coagulation.

(4) These results with dog's serum were compared with those obtained by a similar treatment of dog's plasma.

In the plasma, prevented from coagulating by mixing with an equal volume of saturated solution of sodium sulphate, and then rendered faintly acid as before, coagula fell at the following points: all being white flocculent precipitates.

56° C. 73° C. 75° C. 77°–8° C. 82° C.

(5) The precipitate produced in plasma by magnesium sulphate was dissolved by adding water; fractional heat-coagulation applied to this solution produced precipitates of which the following are the temperatures of coagulation:

56° C. 75° C.

(6) The filtrate after the separation of the above precipitate by magnesium sulphate was similarly treated, and coagula fell at

73° C. 77° C. 82° C.

(7) On comparing the results obtained from the serum with those obtained from the plasma, one notices certain differences. The most striking is the appearance of a precipitate in the plasma at the low temperature of 56° C. This is now known to be due to the presence in the plasma of the precursor of fibrin, viz.: fibrinogen. That such was the case was shown first by Hewson[1], and then by Fredericq[2]. This proteid is absent from the serum. The only other difference is that the temperature at which the lowest member of the albumin class separates is rather different in the case of the serum and plasma, being 70–1° C. in the former, and 73° C. in the latter. This however is due to the admixture in the plasma of a certain quantity of saturated solution of sodium sulphate, for by similarly diluting a portion of serum with this solution, the temperature at which the first coagulum fell is raised to 73° C.

The results of these experiments on dog's serum are then as follows:—in the same way that the plasmine of Denis has been long known to be separable into two proteids of the nature of globulin by heat-coagulation, so the serine of the same observer has now been shown to be in like manner divisible into three proteids, of the nature of albumin.

In the former case, the names the two proteids have received are respectively fibrinogen and paraglobulin. In the latter case, they have hitherto all been grouped together as serine or serum albumin. It will be seen immediately that this divisibility of the serum albumin of the dog is not peculiar to that animal, but that similar bodies are found in the serum of other animals: it is however not advisable to multiply terms, and I therefore propose to call these varieties of the albumin of serum, serum albumin α (coagulating at 70–73°), serum albumin β (coagulating at 77°–78°), and serum albumin γ (coagulating at 82°–85° C.).

The following tables will show at a glance the results obtained in the serum of the various animals that I have examined.

Table I. shows the results obtained with simple undiluted serum in certain animals.

[1] Works, edited by Gulliver, p. 26.

[2] Fredericq, *Recherches sur la constitution du plasma sanguin.* Gand, 1878.

TABLE I.

Temperatures of coagulation of the proteids contained in the serum by a fractional process.

Animal.	Serum Albumin. α.	Serum Globulin.	Serum Albumin. β.	Serum Albumin. γ.
Dog	70°–1° C.	75° C.	77°–8° C.	82°
Ox		73°	76°–8°	84°
Man	70°	73°–5°	77°	82°–3°
Monkey	72°	75°	77°	84°
Sheep		73°–74°	77°–8°	84°
Rabbit	72°	75°–6°	77°	83°
Pig	70°	73°	77°	84°–5°
Cat	71°	74°	77°	84°
Horse		74°–75°	77°	84°

Table II. shows the temperature of coagulation in the plasma of a few animals similarly treated.

TABLE II.

Temperature of coagulation of the proteids contained in the plasma by a fractional process.

Animal.	Fibrinogen.	Serum Albumin. α.	Serum Globulin.	Serum Albumin. β.	Serum Albumin. γ.
Dog	56° C.	73° C.	75° C.	77° C.	82° C.
Monkey	56°	72°	75°	77°	83°
Rabbit	56°	73°	75°	78°	84°

Table III. shows the temperature of coagulation of the solution obtained by redissolving the precipitate produced by magnesium sulphate in the serum and plasma of various animals.

TABLE III.

Temperature of Coagulation.

Animal.	Fibrinogen.	Serum Globulin.
Dog-Serum		75° C.
,, Plasma	56° C.	75°
Ox-Serum		75°
Man-Serum		74°
Monkey-Serum		76°
,, Plasma	56°	75°
Sheep-Serum		75°
Rabbit-Serum		76°
,, Plasma	56°	75°
Pig-Serum		75°
Cat-Serum		75°
Horse-Serum		75°

Table IV. shows the temperatures of coagulation of the series of proteids left in the filtrate after saturation with magnesium sulphate, and consequent precipitation of the globulin.

TABLE IV.

Temperature of Coagulation.

Animal.	Serum Albumin.		
	α.	β.	γ.
Dog-Serum and Plasma	70°–73° C.	77° C.	82° C.
Ox-Serum		77°	84°
Man-Serum	73°	77°	85°
Monkey-Serum and Plasma	69°	77°	84°–5°
Sheep-Serum		77°	84°
Rabbit-Serum and Plasma	73°	77°	84°
Pig Serum	72°–3°	77°	84°
Cat-Serum	73°	77°	84°
Horse-Serum		77°	84°

As will be seen from these tables the results are very uniform: there are slight differences of a degree or two in a few cases, but the average results are very similar. Excepting the case of human blood,

several specimens of serum in each of the above-mentioned animals were examined, and the results above are the averages obtained. Various circumstances influence the temperature of coagulation of a proteid: the most potent of these is the reaction; others are, the amount of sodium chloride present, and the duration and rate of heating. The influence of certain salts in very markedly lowering the temperature of coagulation will be presently alluded to.

There were however certain striking exceptions which it is desirable to allude to:—first in one specimen of monkey's plasma no precipitate beyond 77° C. occurred; this both in the simple plasma, and in that from which the fibrinogen and serum-globulin had been removed by magnesium sulphate. The total amount of proteids in this plasma was estimated: it was found to be 4·8 grammes in 100 cubic centimetres of plasma. This will be seen to be very small, the average amount of human serum being 7·6 %; in that of the horse 7·25; of the ox 7·5; of the rabbit 6·2. I can find no record of the amount of proteid present usually in monkey's blood, but it seems fair to imply that it would approximate to that contained in other vertebrate animals, especially those like man, closely related to it.

In other specimens of both monkey's plasma and serum that I examined, the total amount of proteids was greater than in that just alluded to, and in these all three varieties of serum albumin existed.

Again, in a few specimens, twice in the case of sheep's serum and once in the specimens of serum of the dog and ox that I have examined, a precipitate came down at a point higher than any of those mentioned; namely 87° C. This was quite exceptional. As a rule whatever mammal was the source of the blood, after precipitating at 84° C. the filtrate contains no more proteid: in these few instances this fourth precipitate appeared at 87° C.

Although I have now examined in this way a large number of specimens of mammalian serum, yet I have not yet subjected to analysis a number sufficient to afford me many of these exceptions. It would therefore be unfair to deduce any conclusions from some half-dozen exceptional cases.

Referring again to the tables already given, it will be seen that Tables III. and IV. are the most important: they show that serum globulin is probably a single proteid whereas serum albumin really consists of more than one. In the case of certain animals—man, monkey, dog, cat, pig, and rabbit—it can be differentiated into three proteids, while in the case of the ox, sheep, and horse only two of these

are present, namely those coagulating at 77° C. and 84° C. It is interesting to notice that these three animals belong to the Ungulata.

Tables III. and IV. are important because they show conclusively that the class of proteids to which these proteids with high coagulation temperatures belong is that of the albumins, not the globulins. The method by which the facts contained in these tables are arrived at is in the end the simplest. In the serum pure and simple the chain of proteids coagulating at 73°, 75° and 77° are so close together that it is a matter of difficulty to accurately separate them, unless the operator is well practised in the method. But after the middle member of this series has been taken out and put into another solution there is a difference of some four or five degrees between the first and second terms of the serum albumin series; then there can be no danger of precipitating the two together.

We must now return for a short time to the case of human serum. It is now a rare event to obtain human blood in any quantity; I have succeeded in obtaining only one specimen[1]. In this specimen the specific gravity of the serum was low (1015): the clot was small though of normal colour and consistence; the total quantity of proteids of the serum was also below the normal (6·5 %); and the serum contained ·62 % of urea (estimated by Haycraft's method)[2] which is very much above the normal; (as will be concluded from this description) the patient from whom the blood was drawn was suffering from Bright's disease. As has been already stated, the serum albumin was differentiated into three proteids analogous to those found in the serum of other mammals.

But although I have had only this one opportunity of examining human blood serum, yet I have had many opportunities of examining the various effusions into serous cavities; and the results obtained in these confirm abundantly the fact that human serum albumin is not a single body but consists of three proteids.

Table V. represents these results in a tabular form.

[1] For this I am indebted to Dr Henry Maudsley, Resident Medical officer, University College Hospital. I have also to thank others of my friends among the resident staff there for numerous specimens of pathological exudations to be referred to later on.

[2] Gamgee's *Physiological Chemistry*, p. 192.

TABLE V.

Solution of Globulins: precipitated by Mag. Sulph., washed and redissolved by water. Temperature of coagulation.			Solution of Albumins: i.e. after the precipitation of the Globulins by Mag. Sulph. Temperature of coagulation.		
Fluid.	Fibrinogen.	Serum Globulin.	Serum Albumin. α.	Serum Albumin. β.	Serum Albumin. γ.
Human blood-serum		75° C.	73° C.	77° C.	85° C.
Hydrocele Fluid	56° C.	75°	70°–71°	77°–8°	83°
Pleuritic Fluid		75°	73°–4°	78°	85°
Ascitic Fluid		75°	73°	78°	84°
Pericardial Fluid		74°	73°	77°	84°
Parovarian Fluid		75°	70°–73°	77°	82°

Some three or four specimens of each of the following fluids were examined, hydrocele fluid, pleuritic fluid and ascitic fluid. Hydrocele fluid was always found to contain fibrinogen, even though it was not spontaneously coagulable: ascitic fluid contained none: the pleuritic fluid had invariably coagulated spontaneously, and so, all the fibrinogen having thus gone to form fibrin, there was no precipitate at 86° C. Of pericardial and parovarian fluids one specimen only of each was obtained. In the case of pericardial fluid which came from a case of very acute pericarditis, a clot formed spontaneously as in the case of pleuritic fluid; in the case of parovarian fluid, the total quantity of proteids was as is usual in this fluid very small, being only one per cent. by weight. Still by careful fractional heat-coagulation, the serum albumin was differentiated into its three proteids.

Though I add these additional particulars about these fluids they only confirm what was previously well known regarding them; but I wish to draw attention to the last three columns in Table V. They show, to repeat, the fact that human serum albumin whatever its source consists of three proteids with different temperatures of coagulation. To sum up: the method of fractional heat-coagulation shows that the albumin of serum is not a single proteid, but consists of three separate proteids, coagulating at the temperatures of about 73°, 77°, and 84° C.

Concerning the amounts in which these are usually present in the serum I am not at present able to give any exact figures. The few quantitative estimations that I have at present made are not sufficiently numerous to enable any just conclusions to be drawn from them. In a

future paper I hope to be able to complete this part of the subject. At present I can only say that the amount of the proteids which coagulate at the higher temperatures is considerably less than of that coagulating at 73° C. The proteid coagulating at the highest temperature 84° C. is the least abundant of all, and in some cases forms but a faint cloud of precipitate.

In speaking hitherto of the precipitation or coagulation of these bodies I have intended that word to mean complete precipitation in a flocculent form which can be filtered off, the filtrate being clear. As may however be imagined, the filtrate is not always perfectly clear after one filtration; it often has to be passed through the same paper three or four times before this is the case. This occurs when the precipitate is a very finely divided one. I have in the tables already given not alluded to the temperatures at which opalescence first appears. Some particulars regarding this will be given in some tables later on. It may be mentioned in passing that my notes of the appearance of opalescence show but little difference from the already published statements of Hoppe-Seyler and Hammarsten on this subject: that is, in the case of serum albumin opalescence first appears between 60° and 65°, and in the case of serum globulin at about 68°C. In the case of the higher precipitates opalescence usually occurs one or two degrees before that at which a flocculent precipitate falls down. After saturation with magnesium sulphate the first appearance of opalescence is at a rather higher temperature than in the simple serum.

The fact that the reaction of the liquid is of considerable importance in the determination of the temperature of coagulation of any proteid contained in it has already been stated. It is now necessary to amplify this point, and to quote corroborative experiments.

Two solutions were prepared, one acid, the other alkaline. The acid chosen was oxalic acid. 73 grammes of pure oxalic acid were dissolved in a litre of distilled water.

The alkaline solution was made by dissolving 56 grammes of pure potassium hydrate in a litre of distilled water.

Thus two solutions are obtained which exactly neutralise one another when mixed in equal quantities.

The solutions of this strength were however found to be too concentrated to accurately determine the alkalinity of such a feebly alkaline liquid as serum.

Each was therefore diluted ten times; i.e. 45 cubic centimetres of distilled water were added to 5 cubic centimetres of the solution.

These diluted solutions were placed in burettes, in each of which twenty drops went to the cubic centimetre. It was then found that twenty drops of one solution exactly neutralised twenty drops of the other.

Each drop of the acid solution thus contained 0·000365 gramme of oxalic acid, and each drop of the alkaline solution an equivalent amount of potash, viz. 0·00028 gramme. To test the reaction of the liquid under investigation, two methods were employed:—one was to colour it with a small quantity of a neutral solution of litmus: the other was to use the test papers before mentioned. These were both employed for some time, but were found to give such closely similar results that in the later experiments the violet test papers were alone employed as being the less troublesome method.

Although I have not succeeded in tracing any definite progressive relationship between the acidity of a liquid and the temperature of coagulation of the proteids contained in it, it may be instructive to place upon record some of the observations and experiments I have hitherto made upon the subject, since they illustrate very clearly the general effect of reaction upon heat-coagulation. The difficulties of arriving at a more exact relationship between the two seem to be due to the varying amount of free alkali and of alkaline and neutral salts initially present in the serum.

1. The first experiment was one performed with a solution of globulin from horse serum, purified by repeated precipitation with magnesium sulphate, and then dissolved by the addition of water. In this way a faintly opalescent solution of serum globulin in weak magnesium sulphate was obtained.

It was found to be faintly alkaline: 5 cubic centimetres of the solution requiring three drops of the standard acid solution to neutralise it.

The following are the results of heat-coagulation:—

a. 5 c.c. of solution with initial alkalinity of 3 drops.

Opalescent 70° C.

Flocculent precipitate 76·8° C.

b. 5 c.c. of solution.

1 drop of alkaline solution added: thus alkalinity = 4 drops.

Opalescent 70° C.

Precipitate 77° C.

The precipitate was gelatino-flocculent and difficult to filter off.

c. 5 c.c. of solution.

2 drops of alkaline solution added; thus alkalinity = 5 drops.

Opalescent 72-3° C.

Precipitate 78° C.

This was at first an increase in opalescence: but after keeping the solution at the temperature of 78° C. for 5-10 minutes, it became a flocculent precipitate.

d. 5 c.c. of solution.

3 drops of alkaline solution added: thus alkalinity = 6 drops.
Opalescent 73° C.
Precipitate 80° C.

This also took a long time to form, and then was gelatinous in nature and difficult to filter.

In solutions with more than this amount of alkali added the formation of a precipitate occurred at still higher points; the precipitation of proteid was incomplete; a certain amount of alkali-albumin formed, which remained in solution. Moreover, on adding a larger amount of alkali, a precipitation of magnesia occurred which interfered with the observation.

2. The same solution of serum globulin was used:—

a. 5 c.c. of solution.

3 drops of acid solution added: liquid exactly neutral.
Opalescence 67°-8° C.
Precipitate 76·5° C.

This was filtered off: the filtrate found to be alkaline: alkalinity of 5 cubic centimetres = 5 drops.

b. 5 c.c. of solution.

4 drops of acid solution added; acidity = 1 drop.
Opalescence 69°-70° C.
Precipitate 75·5 C.

c. 5 c.c. of solution.

	Opalescence.	Precipitate.
Acidity = 2 drops.	69-70° C.	75° C.
= 3 drops.	68°-9° C.	74° C.
= 4 drops.	68°-9° C.	72-5° C.
= 5 drops.	68°-9° C.	70° C.

In all these cases precipitation of the proteid was complete.

d. The lowering of the coagulation point after this proceeded more rapidly. Mere addition of the acid at the temperature of the air (27° C.) rendered the liquid more opalescent.

5 c.c. were taken in each case.

	Opalescence increased.	Precipitate.
Acidity = 6 drops.	60° C.	67° C.
= 7 drops.	60° C.	64°-5° C.
= 8 drops.	55° C.	60° C.

The precipitation in all cases was complete. The filtrate after the coagulum had been filtered off was in the first of these three cases neutral, in the two latter it was faintly acid.

e. In the following cases the addition of acid caused considerable opalescence: this increased on standing. On heating the liquid a gradual increase of opalescence occurred until a precipitate fell. After filtering off the precipitate a considerable amount of proteid remained in solution, which was however precipitated by rapid boiling.

Acidity = 9 drops. Precipitate at 57° C.
Acidity = 10 drops. Precipitate at 53° C.

f. An addition of acid beyond the above amount causes, not only an opalescence, but on standing a small amount of flocculent precipitate falls at the temperature of the air (27° C.).

A solution of 2 per cent. acetic acid was found to act similarly.

3. The next experiment to be quoted was one performed with simple sheep's serum. In each case as before five cubic centimetres of the liquid were taken, a certain amount of acid added, and the temperatures at which opalescence and a flocculent precipitate first appeared were noted. It will be seen that in these experiments a larger amount of acid was required to produce as much lowering of temperature as was observed in the case of a solution of a pure proteid: the original comparatively large amount of alkaline salts was no doubt the cause of this.

Original Alkalinity: 5 c.c. require 20 drops of acid to effect neutralization.

a.	Alkalinity = 20 drops.	Opalescent 77° C.	Sets into a solid gelatinous mass	}	80°-82° C.
	,, = 15 drops.	,,	76° C.	,,	,,	80° C.
	,, = 10 drops.	,,	73° C.	,,	,,	78-9° C.
	,, = 0 (i.e. neutral)	,,	73° C.	,,	,,	78° C.

It is thus seen that while the liquid remained alkaline or neutral there was but little difference in the temperature of coagulation.

In case the above liquids should have been too concentrated some of the above solutions were in addition to the above diluted with an equal amount of water. The results then obtained were

Alkalinity = 15 drops. Opalescent 76° C. deeper at 80° C.

This became very dense on keeping it at that temperature some time, but flocculi never separated, even on boiling the solution.

Liquid neutral. Opalescent 74° C.: increased at 76°-7° C.; but even by boiling flocculi did not separate.

b. In the following two experiments in which the acidity of the five cubic centimetres was respectively 1 and 2 drops, the liquid appeared to test-paper to be still neutral: but that they were acid was shown by adding some neutral litmus solution to the liquid. In both the result was as follows:—

67° C. faint opalescence.
72° C. opalescence deepened.
77° C. opalescence still deeper.

84° C. opalescence still deeper.

99°-100° C. flocculi separate from an opalescent liquid.

c. Acidity = 3 drops.

67° opalescent.

75° opalescence increased.

77° opalescence increased: slight separation of flocculi after keeping the liquid at this temperature 10 minutes.

		Opalescent.	Precipitate.
d.	Acidity = 5 drops.	66°-7° C.	75° C. flocculi very fine.
	,, = 7 drops.	66°-7° C.	75° C. flocculi very fine.
	,, = 10 drops.	66° C.	74°-5° C. flocculi large: filtration easy.

The filtrate from the last case was neutral, clear, and contained abundance of coagulable albumin.

e. Beyond this point the lowering of the temperature of coagulation proceeded quickly. After filtering off the coagulum the liquid was found to be slightly acid in all cases.

Acidity = 15 drops. Opalescent 55° C.
Precipitate 65° C.

f. The addition of acid beyond this point caused a precipitate in the cold: i.e. at the temperature of the air (25° C.).

Acidity = 20 drops: opalescence gradually increased till 60° C. when the first flocculent precipitate fell.

Acidity = 25 drops. The first precipitate fell at 56-7° C.

4. In this experiment sheep's serum was again employed. It was saturated with magnesium sulphate; the serum globulin was thus precipitated and filtered off and the filtrate was used. Five cubic centimetres were as before taken in each case; the initial alkalinity of that quantity was equal to 17 drops of standard solution.

It is well known[1] that acetic acid, when added to the solution of proteid saturated with a neutral salt, precipitates that proteid from the solution completely and in an insoluble form.

Tartaric and citric acids are noted as acting similarly[2]. To this list I may now add oxalic acid.

Therefore in determining the coagulation temperature of a proteid in such a solution, one is prevented from adding an excess of acid by seeing the precipitate which such an excess causes. Up to the point of neutrality and a little beyond it addition of acid produces no precipitate; beyond this it does produce a precipitate.

[1] Gamgee's *Physiological Chemistry*, p. 13.

[2] *Elements of Physiological and Pathological Chemistry*. T. C. Charles, M.D. London, 1884.

The following are the results obtained by heat-coagulation in the solution before described.

	Opalescence.	Gelatino-flocculent precipitate.
a. Alkalinity = 17 drops.	80° C.	85° C.
= 7 drops.	80° C.	85° C.
Liquid neutral	80° C.	83°-4° C.

In all cases the flocculi separated from an opalescent liquid.

	Opalescence.	Gelatino-flocculent precipitate.
b. Acidity = 1 drop.	80° C.	83°-4° C.
= 2 drops.	80° C.	83°-4° C.

These solutions were very faintly acid: their acidity in fact was not shown by test papers, though it was by the addition of litmus to the solution.

	Opalescence.	Precipitate.
c. Acidity = 5 drops.	77° C.	80° C.
,, = 10 drops.	73° C.	77°-8° C.

The gelatinous character of the flocculi was here lost; filtration was easy. The temperature of coagulation is in this particular specimen seen to be rather higher than in the average of cases quoted in Table IV.

d. Beyond this point the rate of lowering proceeded quickly.

Acidity = 17 drops.

Opalescence 55° C.

Precipitate 65° C.

e. The addition of acid beyond this point produced a permanent precipitate in the cold.

The conclusions to be drawn from these experiments, which are simply given as illustrations, are:

1. If the liquid is alkaline, the precipitation, if one is produced at all, is not a flocculent one, and its separation is incomplete. The temperature at which it separates varies but little with the amount of alkalinity.

2. If the liquid is only very faintly acid (5 c.c. having an acidity = 2 or 3 drops of the standard solution), much the same effect is obtained.

3. In liquids exceeding this in acidity up to a certain point precipitation is complete, and filtration easy, the filtrate being clear: the point of heat-coagulation falling slightly the greater the amount of free acid present.

4. In liquids more acid still (in the above instances beyond 10 or 15 drops of acid to the 5 c.c.) the point of heat-coagulation falls rapidly, until at last precipitation occurs in the cold.

5. In solutions of a pure proteid similar effects are produced by smaller amounts of acid than where other salts are present, as in serum.

With regard to the literature of this subject of heat-coagulation both Hammarsten[1] and Fredericq[2] place the temperature of coagulation of serum globulin at 75° C. Hammarsten states that the temperature of coagulation of this body may vary between such wide limits as 68° and 80° C. I have however not found the limits to be as wide as this. Taking care to have the reaction of the liquid as nearly as possible always the same, I have found variations between 74° on the one hand and 77° C on the other: but in the vast majority of instances, I have noted the temperature of coagulation of serum globulin as 75°C.

The observer upon whose authority the coagulation temperature of serum albumin is stated as 70°-73° is Hoppe-Seyler[3]. Fredericq[4] however seems to have been the first who performed the experiment in serum which had been saturated with magnesium sulphate, and from which the thus precipitated serum globulin had been removed. He obtained the very remarkable result that coagulation began in such a liquid a little above 40° C., and by the time 50° C. was reached nearly the whole of the serine had been precipitated. Schäfer[5] repeated the experiment, but found that after he had completely removed the globulin with magnesium sulphate, he did not obtain a coagulum until the temperature had reached 70° C., the liquid becoming opalescent at 65° C. He effected complete saturation with the salt by attaching the bottle containing it and the serum to an eccentric connected with a steam-engine, and in this way had the fluid shaken up with the salt for at least three hours. He demonstrated that simply shaking the vessel with the hand was not sufficient to produce complete saturation: and in serum thus imperfectly saturated, and in which consequently some serum globulin still remained in solution, he obtained results which coincided with those of Fredericq—viz. a flocculent precipitate at 40° C. He therefore explained Fredericq's results, by supposing that Fredericq had worked with similarly imperfectly saturated serum. Professor Schäfer confirmed this view that this precipitate at 40° C. was really globulin in several ways, of

[1] *loc. cit.* [2] *loc. cit.* [3] *loc. cit.*

[4] Fredericq. "Recherches sur les substances albuminoïdes du sérum sanguin." *Arch. de Biologie*, Vol. I. 1880.

[5] Schäfer, *Journal of Physiology*, Vol. III. pp. 181—2.

which the most striking was the following: he collected this precipitate which occurred at 40° C. and found that it was soluble by the addition of water, from which solution it was again precipitable by saturating with magnesium sulphate.

It is also possible to explain Fredericq's results by supposing that he had too much free acid present, but on the whole Professor Schäfer's explanation seems the more reasonable. It is confirmed in a striking manner by some observations of Hammarsten[1] on the action of sodium chloride on serum. One of the results of Hammarsten's experiments was that sodium chloride only precipitates serum globulin incompletely, not completely as Schmidt[2] had previously stated. After having made this statement, and supported it by experiments, Hammarsten continues as follows:—"The amount of the precipitation of the paraglobulin from the serum by excess of finely powdered salt depends very much on the temperature.

"With sodium chloride added to complete saturation, at a temperature of 17°-20° the filtered blood serum always becomes cloudy by warming it to 37°-40° C., and if this fluid is cooled it becomes gradually clear again."

"Perfectly clear horse serum was saturated with sodium chloride: the clear filtrate concentrated in vacuo till an abundant quantity of salt had crystallised: then it was again filtered, and the filtrate heated pretty quickly to 40° C. At 30° C. the fluid became opalescent, and by 37·5° C. there was an abundant flocculent precipitate. The fluid was then placed in a cold chamber, and after three hours the precipitate had completely redissolved. It was again precipitated by heating the liquid to 37·5° C." Hammarsten then gives details of a similar experiment in which he saturated the serum with sodium chloride at 10° C. and obtained a precipitate by heating the filtrate to 18° C. He goes on to say that there is no doubt that this precipitate is serum globulin; he washed it with salt solution at a temperature of 40° C. and dissolved it by adding water. In this fluid he got a precipitate either by saturating it with magnesium sulphate or by dialysis.

Hammarsten also insists upon the difficulty of completely saturating serum with sulphate of magnesia, and upon the importance of arriving at complete saturation in order to obtain a complete precipitation of the globulin.

[1] Hammarsten. "Ueber das Paraglobulin. Erster Abschnitt." *Pfluger's Archiv*, Bd. 17, 1878, pp. 424—426.

[2] Alex. Schmidt. "Untersuchung des Eierweisses und des Blutserums durch Dialyse." Ludwig's *Jubelband*, pp. 99—100.

I need hardly say that I have profited by Professor Schäfer's experience. In all my experiments I saturated the serum with magnesium sulphate thoroughly; that is, by having the serum shaken with excess of the salt for at least three hours. It is in this way that I account for never having obtained a result similiar to Fredericq's. The results of Schäfer's and Hammarsten's experiments just quoted clearly show that serum-globulin is less soluble in warm serum nearly saturated with magnesium sulphate, or completely saturated with common salt, than in the same solution in the cold.

Concerning the Precipitation of the Proteids of Serum by means of certain Salts.

In this part of my paper, as in the first part, the proteids to which I shall have chiefly to allude are those included under the name serum albumin. It was Denis[1] who first studied the action of certain salts upon the constituents of the serum and plasma. It was however previously known to Hewson that coagulation could be prevented or hindered by mixing with the blood a certain proportion of neutral salts. Denis used sulphate of soda, and this salt has been generally employed since for the same purpose.

Two other salts were also used by Denis: one was chloride of sodium, the other sulphate of magnesia. By saturating the plasma with powdered common salt he obtained a precipitate of a proteid substance which he called plasmine: this has since been shown to consist of the now well-known bodies, fibrinogen and serum globulin. He found also that by saturating serum with sulphate of magnesia, he obtained the precipitation of a proteid substance which he considered to be the fibrin which had been redissolved in the serum.

Schmidt[2] studied the action of sodium chloride on serum, and concluded that by saturating serum with this salt he obtained a complete precipitation of the serum globulin. According to Heynsius[3] and Hammarsten[4], Schmidt was in error in this statement. Hammarsten showed however that with magnesium sulphate one can obtain a complete precipitation of the serum globulin.

Concerning these two latter salts I shall have but little to add to what is already known. With the first of the three, namely sulphate of soda, I have obtained some very interesting results.

[1] Denis, *loc. cit.* [2] Schmidt, *loc. cit.*

[3] Heynsius, "Ueber serum Albumin und Eieralbumin und ihre Verbindungen." *Pfluger's Archiv*, Bd. XII. [4] Hammarsten, *loc. cit.*

In addition to this I have extended my observations to the action of various other salts upon the proteids of the serum.

Having shown that by the process of fractional heat-coagulation the albumin of serum can be differentiated into three proteids, my next object was to find what other differences, if any, exist between them. I sought therefore to find some means by which they could be precipitated from serum without their undergoing coagulation. It was thought possible that in the same way as serum globulin is precipitated in an unchanged form by means of magnesium sulphate, so certain of these proteids might be similarly precipitable by certain other salts. To this end I have examined the action of a large number of salts upon the serum. Among them, several exist which do precipitate the albumin of serum, much as magnesium sulphate precipitates serum globulin: but all the salts that act so, act equally upon all varieties of serum albumin. By this method then it is not possible to differentiate the proteids included under the name albumin of serum, or at least I have not succeeded in doing so. There are however still many more salts to be examined with this view, and in any case the results that I have hitherto obtained are of sufficient interest to put upon record.

The list of salts, the action of which on the serum proteids I have examined, is as follows:—Magnesium Sulphate, Sodium Sulphate, Sodium Chloride, Sodium Nitrate, Sodium Acetate, Sodium Carbonate, Sodium Phosphate, Potassium Sulphate, Potassium Chloride, Potassium Chlorate, Potassium Nitrate, Potassium Carbonate, Potassium Phosphate, Potassium Iodide, Potassium Acetate, Sodio-Magnesic Sulphate, Ammonium Chloride, Ammonia Alum, Calcium Chloride, Barium Chloride, Silver Nitrate, Mercuric Nitrate, Lead Acetate, and Copper Sulphate.

Before taking these seriatim it will be convenient to allude to the method adopted in the investigation of the action of these salts.

In the first place the serum was saturated with one of them; if any precipitate formed, it was examined to determine its nature, that is to say, it was first examined to see if it were a proteid; if a proteid, if it were soluble in water. Of this solution the properties were tested in various ways, by heat-coagulation, by dialysis, by the action of other salts upon it being the chief. Not only was the precipitate examined in this way but also the filtrate from which the precipitate had been separated; this liquid also was treated by the methods of heat-coagulation, dialysis, and by further saturation with other salts.

By repeated experiments I confirmed Hammarsten's statement that magnesium sulphate was a sure agent for separating the serum globulin

completely from serum. I therefore felt justified in making this the basis of a second set of experiments. I knew that after I had filtered off the precipitate produced by saturation with this salt the only proteids the filtrate contained were of the nature of albumin. It was this filtrate that I saturated with all the other salts mentioned in the above list. Hence in the examination of serum albumin, I adopted a method of double saturation, or saturation with two salts: the first of these being magnesium sulphate.

In the examination of the effect of saturation with any particular salt my method was therefore as follows:—first to saturate the simple serum with that salt and examine the result: secondly to saturate with the same salt, serum from which the globulin had been removed, that is which had been previously saturated with magnesium sulphate.

In certain other cases I examined the effect of smaller quantities of these salts upon the serum; that is, the salt was not added to saturation.

It will be convenient in this connection to discuss the rules that govern solution of a solid in a liquid, and especially to discuss generally the question of double saturation[1].

The solution of a solid in a liquid is usually attended with a fall of temperature arising from the conversion of sensible into latent heat. In the case of anhydrous salts, however, these are converted into definite hydrates, and so there is a rise of temperature.

The manner in which the solubility of a solid in a liquid is related to the chemical composition of the two has not yet been reduced to any definite laws. As a general rule, it may perhaps be stated that solids dissolve with the greatest facility in those liquids which they most resemble in composition. Still similar salts of closely related metals often differ very much in their solubility in a liquid: as an example, the relative solubility of magnesium sulphate and barium sulphate may be quoted.

Pulverisation and stirring increase readiness of solution. Solubility increases with rise of temperature. There are however exceptions to this rule. For each temperature there is a certain limit called the *point of saturation;* the rate of increase is different for different substances, and is not reducible to any law.

It is unnecessary to allude to the supersaturation of water by salts like sodium sulphate and sodium carbonate which crystallize with more than one proportion of water, since in my experiments I have always saturated at the temperature of the air, and not employed any extra heat at all.

[1] Watt's *Dictionary of Chemistry*, Vol. v. p. 348, Art. "Solution."

When two or more salts dissolve in water and no new salt separates out by double decomposition, the quantity of each held in solution by a given quantity of water is generally, but not always, less than if either salt were dissolved separately. The quantities contained in such a solution are generally the same whether the two salts be dissolved simultaneously or if the water be first saturated with one, then with the other; crystals of the salt with which the salt was first saturated then separate out when the second salt is added. For instance, if a saturated solution of sodium chloride be shaken with solid ammonium chloride the liquid takes up large quantities of the latter, and cubes of sodium chloride crystallize out. Another frequent relation is the difference as to which salt is used first for saturation; as an example take ammonium chloride and potassium nitrate. The following table represents the proportions of these salts taken up by 100 parts of water at a temperature of $18\frac{3}{4}^{\circ}$ C. in the three following cases:—

α. A quantity of water saturated with ammonium chloride is shaken with excess of potassium nitrate.

β. A quantity of water saturated with potassium nitrate is shaken with excess of ammonium chloride.

γ. The water is saturated with the two salts simultaneously.

	α.	*β.*	*γ.*
Ammonium chloride	37·98	44·33	39·84
Potassium nitrate	37·68	30·56	38·62

The bearing of these remarks on my experiments must now be pointed out:

(i) As a rule the crystalline, not the anhydrous, form of a salt was used: when the anhydrous salt was used the fact will be stated.

(ii) The fact that the solubility of closely allied salts in water differs so much, is analogous to, though it does not explain, the other very different results in the precipitation of proteids that are obtained with chemically similar compounds: e.g. sodium sulphate and potassium sulphate.

(iii) Though no artificial heat was employed to aid the process of saturation, yet the temperature of the air made considerable difference in the rate of solution; in summer it was much quicker than in winter.

(iv) In the washing of precipitates obtained by double saturation it is necessary to use water saturated with the same salts as has been used to obtain the precipitate. The method I adopted in the preparation of these doubly saturated solutions was to take the saturated solution

of one salt and then add to it excess of the other. It is also necessary (as is shown by the example given above in the case of the ammonium chloride and potassium nitrate) to saturate the water with the salts in the same order as that in which the serum has been saturated, as the precipitate may be soluble in water saturated in the opposite order, and this often contains the salts in different proportions.

In solutions containing three salts still more complicated relations are observed; but it is not necessary here to enter into these as I have not yet employed, except in one or two cases, the method of triple saturation.

Having thus discussed the general methods I have adopted in these experiments, and the general principles of saturation and double saturation, it will now be convenient to take one by one the salts given in the above list.

1. *Magnesium Sulphate.* Concerning this salt I have but little to add to what Hammarsten has already written about it. As was previously stated in the first part of this paper, saturation is not completely effected by merely shaking the bottle containing the serum and excess of the salt with the hand for a few minutes. But after such a bottle has been shaken for about three hours saturation is complete; addition of more magnesium sulphate to the filtered fluid produces no more precipitate. In the summer when my later experiments were performed, a shorter time would no doubt have been sufficient: still no harm was done by having the mixture shaken for the full three hours, and therefore in all cases this was the period of time adopted. The machine used was an eccentric worked by one of Crossley's small gas-engines: to this the bottle or flask containing serum and the magnesium sulphate was firmly tied. It swung backwards and forwards one hundred times in a minute, and its excursion was fourteen inches. This machine was used not only for saturation with magnesium sulphate but also with each of the other salts used.

But although to obtain complete precipitation of the serum globulin it is necessary to completely saturate the serum in this way, yet to obtain an indication of the presence of a globulin such a long course of procedure is not necessary: and the easiest method of applying it as a qualitative test is as follows. Pour some saturated solution of magnesium sulphate into the bottom of a test tube, and on to the surface of this dense fluid pour the liquid suspected to contain globulin; this readily floats on the magnesium sulphate solution, and at the junction of the two fluids a ring of precipitate appears if globulin

is present. But although this test is a ready one, it is not very delicate. I have found that a liquid containing 1·07 grammes of serum globulin in 100 c.c. of water is the weakest that will give the test; roughly speaking, the strength of the solution must be over 1 per cent.

On referring to Table III. (p. 160) it will be seen that the precipitate produced in blood-plasma by saturating it with magnesium sulphate contains fibrinogen, while referring to Table IV. it will be seen that the filtrate, after the separation of the magnesium sulphate precipitates from plasma, contains none. Magnesium sulphate then not only completely precipitates serum globulin but it also completely precipitates fibrinogen in such a form that water redissolves it. Though no doubt this would be expected and even inferred from the general resemblance of these two bodies, and from the fact that sodium chloride acts very similarly on each, I cannot find the fact stated in any text-book or monograph. It may therefore now be taken as certain that precipitation of fibrinogen from a solution is brought about by saturation of that solution with magnesium sulphate.

2. *Sodium Sulphate.* In the consideration of this salt, that of another salt in the list given a few pages back must also be included. That salt is sodic-magnesic sulphate.

The action of sodium sulphate in hindering the coagulation of the blood has been long known, but it is not so generally known that this salt is also in part productive of the precipitation from the serum of certain of its proteid constituents.

Denis[1] makes the following statement:—"I have found a better means of isolating serine since the publication of my 'Etudes.' It consists of saturating at 50° with powdered sulphate of soda either plasma deprived of its plasmine, or serum from which the dissolved fibrin[2] has been removed by the aid of sulphate of magnesia. When the liquid has taken up at 50° all the sodium sulphate it can dissolve, serine is precipitated. It is necessary to filter it at the same temperature; it collects on the paper as a white precipitate, and is easily soluble in water."

This seems to have been entirely forgotten until the fact that sodium sulphate produces a precipitate of serum albumin from a solution already saturated with sulphate of magnesium was rediscovered

[1] Denis, *loc cit.* p. 39.

[2] By "dissolved fibrin" Denis meant what we now know as serum globulin.

by Professor Schäfer[1] in 1880. Schäfer did not however obtain complete precipitation of serum albumin by this salt.

My own results were as follows. When sodium sulphate was added to serum to saturation, and the mixture shaken for any number of times, there was no precipitate produced.

When serum which had been previously saturated with magnesium sulphate, and from which the so-precipitated globulins had been removed, was saturated with sodium sulphate there was an abundant precipitate produced. Half saturation produced no such effect, and the addition of very large quantities of saturated solution of sodium sulphate to serum already saturated with magnesium sulphate also produced no precipitate.

My earlier experiments coincided with those of Professor Schäfer: that is, after filtering off this precipitate a residue of coagulable proteid was present in the filtrate. But I found afterwards that the reason was, I had not shaken the liquid and the salt together long enough. The element of time is a very important factor in the precipitation of these bodies: and I found that after shaking the liquid (i.e. serum saturated with magnesium sulphate, the globulin being filtered off) with excess of produced sodium sulphate for a sufficient length of time, complete precipitation of the serum albumins takes place. What this length of time was I found by filtering the mixture at intervals of three hours, and then continuing the shaking if the filtrate contained any proteid; finally after nine hours' shaking on the average in March, and four to six hours' in July with the thermometer nearly at 30°C., a filtrate was obtained clear, colourless, syrupy in consistence, and absolutely free from proteid, as tested by the xanthroproteic reaction. It is not then necessary to raise the temperature to 50° as Denis did.

This result I have now confirmed over and over again. I have obtained it with the serum of the sheep, horse, ox, cat, rabbit, and dog, and also with hydrocele, pericardial, ascitic, and pleuritic fluids.

The precipitate which is left on the filter consists of the three serum albumins together; it can be dissolved by adding water. From this watery solution it cannot be precipitated by either magnesium sulphate or sodium sulphate, but by double saturation with the two salts it can be completely reprecipitated.

In this way the serum albumin can be obtained in a state of comparative purity: there is however a certain quantity of the salt

[1] Schäfer, *loc. cit.* p. 184.

used adhering to the white precipitate of proteid matter. On adding distilled water a solution of the substance is obtained with readiness. It is insoluble in water saturated with the two salts used.

If some of the solution obtained by adding distilled water be submitted to dialysis no precipitation takes place.

By a process of fractional heat-coagulation applied to this solution the three proteids of which serum has been shown to consist can be again separated: in the case of rabbit's serum, the temperatures of coagulation being 73°, 77° and 84°. In the case of sheep's serum it will be remembered that the serum albumin consists of two proteids only, coagulating at the temperature of 77° and 84° C. This is also found to be the case in the pure serum albumin prepared by the process of double saturation.

In these experiments the serum of the sheep and rabbit has been chiefly employed, the former being taken as an example of an animal whose serum albumin consists of two proteids, the latter as one whose serum albumin consists of three.

With a view of seeing in what order the serum albumins are precipitated by this process of double saturation, the following experiment was performed and repeated several times in the case of the sheep and rabbit.

(i) Sheep's serum was obtained perfectly clear, and then saturated with magnesium sulphate by shaking it with excess of that salt for three hours; the precipitate of serum globulin so produced was filtered off.

The filtrate was shaken for three hours with excess of sodium sulphate: at the end of this time an abundant precipitate so produced was filtered off and reserved for examination (*a*).

The filtrate was found still to contain much coagulable albumin, and so it was again shaken with excess of sodium sulphate for another three hours; again another precipitate was produced; this also was filtered off and reserved for examination (*b*).

The filtrate was found to contain still a small quantity of coagulable proteid, and so it was again shaken with excess of sodium sulphate for another three hours; at the end of this time, a small amount of precipitate was produced; this was filtered off and reserved for examination (*c*).

The filtrate was found to contain no proteid.

There were then three precipitates to be examined, collected at intervals of three hours: they were washed with water saturated with the two salts used until the washings contained no albumin; distilled water was then added, and so solutions of these bodies obtained.

The solution of *a* was of a light straw colour, perfectly clear, neutral: it

was rendered faintly acid, and then submitted to the process of heat coagulation, the result of which was that

at 73° C. there was opalescence,

at 76°-77° C. a flocculent precipitate; this was filtered off: the filtrate was clear, and neutral: it was again rendered faintly acid, and

at 81°-2° a flocculent pp. occurred; this was filtered off: the filtrate contained no proteid.

The solution of *b* was clear, colourless and neutral; it was rendered slightly acid. By heating it was found that at 77° C. a flocculent precipitate fell down; it was filtered off: the filtrate, clear and neutral, was found to contain no proteid.

The solution of *c* resembled that of *b* in being clear, neutral, and colourless: it was rendered slightly acid: and at 77° C. a slight amount of flocculent precipitate appeared, which being filtered off left a filtrate clear, colourless, neutral and containing no proteid.

(ii) With rabbit's serum, by similarly collecting the precipitates at intervals of three hours, three precipitates were obtained which we will again call *a*, *b*, and *c*. These were washed with water saturated with both salts, and then dissolved by the addition of water: the solution of *a* gave precipitates at 73°, 77° and 84° C.: of *b* at 73° and 77° C.: and of *c* at 73° only: the last precipitate being very small in quantity.

The conclusions that one draws from these experiments are, that double saturation with these two salts precipitates all forms of serum albumin, and precipitates them equally; and that the proteids with higher coagulation temperature being smaller in amount than those with lower coagulation temperatures require less time for their complete precipitation. Thus the proteids that separate in the later hours of the experiment consist wholly of the proteid coagulating at the lowest point (77° in the case of the sheep, 73° C. in that of the rabbit), since there is too much of this present for it all to come down in the first hours of the shaking, though a good deal of it also comes down then.

We may therefore state it as a fact that the double saturation of the serum by the two salts, magnesium sulphate and sodium sulphate, completely precipitates from it all the proteids contained therein. We have now to seek an explanation of this fact. Either salt alone will not precipitate serum albumin, but both together will. Two explanations seem possible: one is that the mere presence of magnesium sulphate enables sodium sulphate to precipitate serum albumin without the formation of any new compound; the other is that when magnesium sulphate and sodium sulphate come together, a new compound is formed,

and this it is which has the power of precipitating serum albumin. The first explanation is negative, and therefore vague and unsatisfactory; the second is more reasonable and satisfactory, and I will now endeavour to show that it is the correct one.

The formula for magnesium sulphate is $MgSO_4 . 7(H_2O)$; that is, it unites with seven molecules of water of crystallisation. It is a well-known fact[1] that magnesium sulphate forms with the alkaline sulphates double salts in which the alkaline sulphate takes the place of one molecule of water of crystallisation: thus $MgSO_4 . K_2SO_4 + 6H_2O$ is the potash double salt.

Here then is a ready explanation for the results obtained: when sodium sulphate is added to magnesium sulphate the double sulphate of soda and magnesia is formed, and this it is which precipitates the serum albumin. Here also we have a ready means of accounting for the long time that the precipitation of the serum albumin takes by this method; the substitution of one molecule of water by one of sodium sulphate is a slow process at the temperature of the air: hence the precipitation of serum albumin by it must be similarly slow.

Having thus theoretically obtained an explanation of the behaviour of these two salts, the next thing to do was to put it to the test of experiment: accordingly I obtained some of this double sulphate, and then tried what its effect on the serum proteids was.

Sodio-magnesic sulphate[2] ($MgNa_2 (SO_4)_2 . 6H_2O$) separates in rhombohedral crystals from the liquor obtained in the preparation of Epsom salts. It dissolves in three parts of cold water, is permanent in the air, decrepitates when heated, and gives off its water without melting. Being thus a commercial by-product it is not a difficult salt to obtain. Some solution of serum albumin was prepared; and this salt was added to saturation. It is a readily soluble salt, and saturation is effected in a few minutes by merely shaking the flask containing it and the liquid with the hand; an abundant precipitate is thus produced, which is found to be serum albumin; on filtering it off the filtrate is found to contain no proteid. Half saturating a solution of serum albumin with this salt produces no precipitate; when it is three-quarters saturated precipitation begins, but is not complete till complete saturation is attained. When added to serum to saturation, sodio-magnesic sulphate precipitates all the proteids therefrom, and the filtrate is free from proteids altogether.

[1] Roscoe's *Elementary Chemistry*, p. 192.

[2] Watt's *Dictionary of Chemistry*. Article "Sulphates."

Half saturation produces an abundant precipitate in the serum; this is found to consist wholly of serum globulin. But this method does not completely precipitate the serum globulin. That some still exists in solution may be shown in two ways; serum is half saturated with sodio-magnesic sulphate: this is done by mixing a certain volume of serum with an equal volume of saturated solution of the double salt: the precipitate so produced is filtered off; the filtrate is taken and either subjected to dialysis or saturated with magnesium sulphate. By either method a precipitate is produced, showing that globulin exists in solution in this filtrate.

After serum has been saturated with magnesium sulphate and the serum globulin filtered off, the filtrate may be saturated with the double sulphate of sodium and magnesium: in this way an abundant precipitate is immediately produced which is found to contain all the remaining proteids of the serum, that is all the serum albumins.

In this way we have at least one method of precipitating serum albumin in an uncoagulated condition. I shall presently have to show that this is not the only way.

Hoppe-Seyler[1] makes the following statement: "serum albumin is soluble in distilled water, and cannot be precipitated from this solution without undergoing chemical changes." This statement is no longer correct: it has long been known that potassium carbonate[2] when added nearly to saturation to a solution of any proteid will precipitate therefrom that proteid without coagulating it. This I have confirmed with regard to serum. When serum either before or after saturation with magnesium sulphate is saturated with potassium carbonate, a complete precipitation of the proteids occurs, the filtrate being clear, limpid, and free from proteid. The precipitate produced is readily soluble in water: this watery solution can be neutralized by acetic acid: the first effect is evolution of carbonic acid gas. After neutralisation the same series of proteids can be obtained by fractional heat-coagulation, as has been before dwelt upon.

Not only will potassium carbonate act in this way, but, as I shall presently show, potassium acetate and potassium phosphate act in a precisely similar manner. Moreover, by the process of double saturation with several pairs of salts, one of which we have already considered, the precipitation of serum albumin is also possible.

Hence Hoppe-Seyler's statement is abundantly contradicted.

[1] Hoppe-Seyler, *Physiologische Chemie*, III. Theil, p. 424. Berlin, 1879.

[2] Gamgee's *Physiological Chemistry*, p. 13.

Hoppe-Seyler[1] also states that serum albumin can be precipitated by means of several salts of the heavy metals but that it is at the same time coagulated. This I have confirmed with regard to copper sulphate, silver nitrate, lead sub-acetate, and mercuric chloride; the same result is obtained whether the solutions of these salts be added to serum simply, or whether that serum has been previously saturated with magnesium sulphate: namely a complete precipitation of all the proteids in a coagulated condition.

3. *Sodium Nitrate.* When serum is saturated with this salt an abundant precipitate of a proteid matter is produced. This precipitate was thoroughly washed with saturated solution of sodium nitrate until the washings contained no proteid: it was then dissolved by adding water. The resulting solution was faintly opalescent; the proteid in it coagulated entirely at 75° C., was completely precipitated by magnesium sulphate added to saturation, and by dialysis an abundant precipitate was produced. It was therefore concluded that the precipitate produced by saturating serum with this salt consisted of serum globulin. The next question was, Does sodium nitrate completely precipitate globulin? The answer is in the affirmative, but this method is not nearly such a ready one as Hammarsten's, that is, by magnesium sulphate. A solution of pure globulin prepared from horse serum was taken and sodium nitrate added to saturation; after 5 hours' shaking there was an abundant precipitate, but the filtrate contained a trace of proteid still; after 3 hours' more shaking, this trace was also precipitated, and the filtrate was free from proteids. Serum was shaken for 8—10 hours with sodium nitrate, and the resulting precipitate filtered off; the filtrate was submitted to dialysis and no precipitation occurred.

Not only does sodium nitrate precipitate serum globulin, but if the serum has been previously saturated with magnesium sulphate, and the globulin filtered off, the saturation of the filtrate with sodium nitrate causes an abundant precipitate; this is found to be serum albumin. It is soluble by the addition of water. Does it completely precipitate the serum albumins? The answer again is, Yes; but the process is an extremely slow one: eighteen to twenty and in some cases thirty hours' shaking is required to effect the precipitation. This result I have confirmed with the serum of the cat, rabbit, ox, and sheep, and with pleuritic fluid.

The following are the particulars of the experiment in the case of sheep's serum :—

[1] *loc. cit.*

Sheep's serum was shaken with magnesium sulphate, and the precipitate of serum globulin so produced was filtered off; the filtrate was shaken for eighteen hours with excess of sodium nitrate; the precipitate produced was collected at intervals in three lots; the final filtrate contained no proteid.

The precipitates were washed with water saturated with both salts and dissolved by adding water.

The first precipitate collected after three hours' shaking was washed and dissolved; by fractional heat-coagulation coagula were found to occur at 73°—4° and 83° C.

The second precipitate, collected after five hours' more shaking, was similarly found to coagulate at 77° C. The third, collected after ten hours' more shaking, was very small in quantity and coagulated at 77° C.

We here see, as in the case of double saturation with magnesium sulphate and sodium sulphate, that both the varieties of serum albumin in sheep's serum are precipitated equally by this method, only the precipitation of that coagulating at 83°C. is accomplished sooner because there is not present so much of it as of that which coagulates at 77°C. It will be seen in the above experiment that the first precipitate contained a proteid which coagulated at 73°—4°, not at 77°C. as one would expect: this lowering of the temperature of coagulation is due to the admixture of a certain amount of sodium nitrate with the liquid. That sodium nitrate has a very marked effect in lowering the temperature of coagulation of proteids, especially when present in large quantities, is shown by the following experiment:

Sheep's serum was first saturated with magnesium sulphate and filtered; the filtrate was saturated with sodium nitrate and shaken for three hours; by this means a large amount of the serum albumin was precipitated, but still a large amount remained in solution; the precipitate was filtered off, and the filtrate was found to be faintly alkaline. This was heated (no acid being added), and at

45° C. opalescence appeared,
55° C. abundant flocculent precipitate, soluble in water,
77° C. small amount of precipitate.

In a portion of this liquid rendered faintly acid in the usual way, the following took place on fractional heat coagulation:

60° C. opalescence,
65° C. flocculent precipitate,
77° C. slight amount of flocculent precipitate.

In another specimen diluted with an equal amount of water, the following was the result:

63° opalescence,
68° flocculent precipitate,
77° flocculent precipitate.

In a fourth specimen diluted with an equal amount of water and rendered faintly acid as usual, I obtained the following result:

65° opalescence,
73° flocculent precipitate,
79° flocculent precipitate, small in amount.

These experiments, and several more of a similar kind which might be quoted, are sufficient to show the influence that sodium nitrate has in lowering the heat-coagulation temperature of proteids. The very low temperature at which coagulation occurs in the undiluted, faintly alkaline liquid reminds one of the similar results obtained by Hammarsten and Schäfer in the temperature of coagulation of serum globulin when serum is imperfectly saturated with sodium chloride and magnesium sulphate respectively; these experiments have however been fully referred to in the former part of this paper. It seems that when serum saturated with magnesium sulphate is imperfectly saturated with sodium nitrate a similar condition occurs with regard to serum albumin; that is, it is less soluble in such a solution when hot, than it is when that solution is cold. It will be presently seen that potassium nitrate has also some effect in lowering the temperature of heat-coagulation.

The chief fact that these experiments with magnesium sulphate and sodium nitrate brings before us is, however, that double saturation of the serum with them will bring about in course of time a precipitation of the serum albumins. The question is how to explain this; and here I am not able to supply an answer as exact and conclusive as I was able to do in the case of the double saturation with magnesium of sodium sulphates. Analogy would suggest that a similar double salt is formed; that is, one or more molecules of sodium nitrate replaces one or more of the seven molecules of water of crystallisation that magnesium sulphate holds; and this double salt it is which precipitates the serum albumin. That this is the fact is confirmed by the immensely long time that double saturation with these salts takes to bring about complete precipitation of the albumins.

In the case of other pairs of salts which I shall have to allude to later on as producing precipitation of serum albumin, it will be convenient to state here once and for all that the formation of a new compound is what probably takes place in all cases, though at present I am not prepared to substantiate this assumption except in the case in which

I have already fully done so: namely in case of the sulphates of sodium and magnesium.

4. *Ammonia Alum.* This salt, which is the double sulphate of ammonium and aluminium, has like all the alums an acid reaction. When serum is saturated with it no precipitate is produced, but the serum becomes thick, treaclish in consistence, and faintly opalescent.

When the serum has been previously saturated with magnesium sulphate, and the globulin so precipitated has been removed, saturation with ammonia alum produces an abundant white precipitate; this can be filtered off, and the filtrate is free from proteid. This result I have obtained with the serum of the sheep, ox, and rabbit.

If the precipitate be not left too long in contact with the precipitant it can be redissolved by water. Mere shaking with the hand is sufficient to produce complete saturation with the salt, and therefore complete precipitation of the serum albumins. If this be filtered immediately, the precipitate can be entirely redissolved by water; if however it is left a long time in contact with the salt, the precipitate is thereby rendered partially insoluble in water. This therefore is a much quicker way of precipitating serum albumin than by either of the methods just described; there is but little difficulty in obtaining a completely soluble precipitate.

When this precipitate is redissolved by adding water, there is also dissolved at the same time much of the adhering alum; this renders this solution of the precipitate acid, and by fractional heat-coagulation in the case of rabbit's serum, coagula were found to occur at 68°, 77° and 84°C. The low temperature of the first precipitation was due to the acidity of the liquid. The liquid cannot be rendered less acid by means of an alkali, since the addition of an alkali to the liquid precipitates alumina. I therefore sought to get rid of the acid by means of dialysis.

Dialysis however was found to produce a very remarkable and puzzling result. The dialyser that I have employed in this and in other experiments was of the following kind[1]:—the ends of about six inches length of dialysing tube are closed with india-rubber corks, one of which is perforated by two glass tubes; of the two tubes, the longer must reach to the lower cork and be bevelled; the other must be cut off short: through this arrangement a current of water can be sent from the tap. This tube is placed in a narrow cylindrical vessel not much larger than the tube: in this vessel outside the dialysing tube is placed the liquid to be dialysed.

[1] Sanderson's *Practical Exercises in Physiology*, 1882, p. 60.

The solution of precipitate produced by alum was dialysed in this way for twenty-four hours, at the end of which time an abundant precipitate was produced. This was filtered off; the filtrate was still slightly acid, and contained only a trace of proteid. The precipitate was found to be proteid; it could not be globulin, since that had all been previously removed by magnesium sulphate; therefore it must have consisted of serum albumin.

Having obtained this result with the serum of the ox and rabbit I instituted a series of experiments to determine the explanation of the occurrence.

The precipitate produced in this way by dialysis was found to be insoluble in water, in weak acetic acid, and in saturated solution of alum. It was soluble in nitric acid: this solution was boiled and ammonia added: a deep orange colour was developed. This showed that the precipitate consisted of proteid. Two control experiments were performed; first, a weak solution of alum containing no proteid was similarly dialysed: no precipitate occurred; secondly, serum albumin dissolved in weak alum solution was allowed to stand, without being dialysed: no precipitate took place.

These experiments conclusively proved that the precipitate consisted of serum albumin though in an insoluble form, and that such precipitate is produced by dialysis.

The explanation was found by the following experiment:—ox serum was saturated with magnesium sulphate, and the precipitate of serum globulin so caused was filtered off. To this filtrate saturated solution of alum was added drop by drop; it was found that the addition of a small quantity of saturated solution of alum caused an abundant precipitate. The precipitation of the proteid was however not complete; the filtrate after filtering this off still contained proteid. The addition of more saturated solution of alum redissolved the precipitate. It is also soluble in water but with slight difficulty. If after adding the quantity of alum sufficient to produce a precipitate the precipitate was allowed to stand for five or ten minutes, it was thereby rendered much more insoluble; large quantities of saturated solution of alum were necessary to redissolve it, and even then the solution was opalescent.

The particulars of the experiment are as follows:

5 cubic centimetres of ox serum previously saturated with magnesium sulphate were taken, and to this was added saturated solution of alum from a burette.

In the burette used, thirty-six drops went to the cubic centimetre. In

round numbers 100 parts of water dissolve 5 parts of ammonia alum[1]; that is to say, a cubic centimetre of the solution contains ·05 grammes of alum, and a drop with the burette used, one thirty-sixth of this amount.

2—9 drops were added to five cubic centimetres of the liquid: no effect.

10 ,, ,, ,, ,, ,, : opalescence.

15 ,, ,, ,, ,, ,, : sets into an almost solid mass. This was allowed to stand some time, and after the addition of 12 cubic centimetres of the saturated solution an opalescent solution was formed.

When the alum solution was added quickly, three cubic centimetres added to the same amount of similarly treated serum as above (i.e. 5 c.c.) produced a precipitate which was quickly redissolved: when two cubic centimetres of alum solution were used the liquid still remained cloudy.

The result of this experiment is, that the addition of a minute quantity of alum to serum already saturated with magnesium sulphate produces an abundant precipitate of the serum albumin; this is redissolved by excess of alum, but again completely precipitated by saturation with the salt.

Hammarsten[2] obtained a somewhat analogous result with sodium chloride in the case of globulin. He found that if a very small quantity of common salt (from 0·03 to 0·7 per cent.) be added to a feebly alkaline solution of paraglobulin this body is precipitated: but on the further addition of the salt the precipitate redissolves, only to be again precipitated when the amount of sodium chloride exceeds about 20 per cent.

How can this fact however explain the precipitation by dialysis? When water is added to the precipitate of serum albumin produced by saturation with alum a solution is obtained; but the water dissolves not only the serum albumin but also much of the alum adhering to it. This solution is submitted to dialysis; by dialysing much of this dissolved alum passes off with the stream of water; this goes on until a very small quantity of alum is left, until in fact that quantity of alum is left which we have just seen is sufficient to precipitate serum albumin. The insoluble nature of this precipitate is accounted for by the long contact of it with the precipitant. That a small quantity of alum is still present is shown by the faintly acid reaction of the liquid, and also by the fact that addition of ammonia and ammonic sulphide produces a white precipitate.

[1] The exact numbers are 5·22 at 0° C.: 4·2 at 100° C. Watt's *Dictionary of Chemistry*.

[2] Hammarsten, *Op. cit. Zweiter Abschnitt.*

5. *Potassium Iodide.* When this salt is added to saturation to simple serum no precipitate is produced, but the liquid is thick and syrupy, much as it becomes by similar saturation with ammonia alum.

When this salt is added to saturation to serum from which the globulins have been removed by saturation with magnesium sulphate, and the mixture is shaken for three or four hours, an abundant precipitate is produced—not white and flocculent, but semitransparent and gelatinous in appearance. It can however be easily filtered off. It is readily soluble in water; from this solution by heat-coagulation the albumins comprised under the name serum albumin can be readily separated. The filtrate is clear, neutral, and generally faintly coloured by free iodine, which colouration increases by keeping. When this filtrate is rendered faintly acid and boiled, no precipitate occurs; by this test at least it contains no proteid. The xanthoproteic and Millon's tests cannot be tried in this liquid, because of the presence of potassium iodide. It is however probable that double saturation with magnesium sulphate and potassium iodide produces complete precipitation of serum albumin.

6. *Sodium Acetate.* When serum is saturated with this salt there is an abundant precipitate; when this is collected and examined it is found to be soluble in water; from this watery solution it can be re-precipitated by dialysis, or by saturation with magnesium sulphate; it also coagulates at 75°C. This precipitate therefore consists of serum globulin. Sodium acetate precipitates serum globulin completely; some four to six hours' shaking is necessary to ensure complete saturation, and so complete precipitation of the globulin. After this time, if the precipitate be filtered off, the filtrate can be proved to be free from globulin either by dialysis or by saturation with magnesium sulphate: in either case there is no precipitate.

A solution of pure horse globulin was made, and saturated with sodium acetate: after about ten hours' shaking this was filtered, and the filtrate found to be free from proteid.

When sodium acetate is added to serum from which the globulins have been precipitated by saturation with magnesium sulphate, and removed, there is no precipitate produced. Sodium acetate takes no part in the precipitation of serum albumin.

7. *Sodium Carbonate.* What has been said for sodium acetate may be repeated *mutatis mutandis* for sodium carbonate: it completely precipitates serum globulin from serum; but it takes no part in the precipitation of serum albumin therefrom. It is slower in its action

than sodium acetate; some twenty hours' shaking being necessary to ensure complete precipitation of the serum globulin.

When added to serum after saturation with magnesium sulphate there is an abundant white precipitate; this may be collected and washed. It consists entirely of magnesia and carbonate of magnesia, and contains no proteid whatever; the proteids are all in the filtrate.

8. *Sodium Chloride.* As is well known, this salt, when added to serum, produces an abundant precipitate in that liquid.

When it is added to serum from which the globulin has been precipitated by saturation with magnesium sulphate, and removed, it produces no precipitate.

Seeing how important is the element of time in the precipitation of these bodies I tried a few experiments to determine whether an exceedingly long time of contact of this salt with the serum might not effect complete precipitation of the serum globulin.

Schmidt makes no reference at all to the element of time. Hammarsten[1] says that he never filtered until the mixture had stood twenty-four hours, in other experiments he had the serum and salt shaken for periods varying from a quarter of an hour to six hours. He says a quarter of an hour's shaking is sufficient to ensure saturation, but admits that the longer the time he kept the salt in contact with the serum before filtering, the greater was the amount of precipitate he obtained.

In my experiments, I took first a solution of pure globulin prepared from horse's serum, and added excess of sodium chloride; after five hours' shaking there was still abundance of globulin in solution; after five hours' more there was still a trace; after five hours' more there was still a trace; after three hours' more, still a trace; in fact this trace seemed to be unprecipitable by sodium chloride however long the two were shaken together.

I then took fresh horse serum and had it shaken with excess of common salt for twenty-four hours. I also examined some horse serum which had been standing for months[2] in contact with excess of common salt. In both cases saturation with magnesium sulphate produced no precipitate. I therefore at first concluded that I had obtained complete

[1] Hammarsten, *Erster Abschnitt*, pp. 424—5.

[2] Sodium chloride apparently keeps serum from putrefying for an indefinite length of time. Magnesium sulphate has not such marked antiseptic properties; after three or four weeks, bacteria, then moulds, begin to grow in the liquid, accompanying which is the odour of putrefaction.

precipitation of the serum globulin by means of sodium chloride, but on submitting both these liquids to dialysis, a small amount of precipitation occurred in about twenty-four hours. I therefore concluded that not only does saturation with sodium chloride hold a small amount of globulin in solution, but it also prevents magnesium sulphate from precipitating that same small amount. It is however a very small amount indeed; a mere trace in many cases. After separating all the globulin possible by means of sodium chloride, subsequent saturation with sodium sulphate will precipitate all the remaining proteids in an unchanged form.

9. *Potassium Acetate.* This is a very soluble salt, and readily deliquesces in the air. In order to saturate serum with it, it is necessary to use a vast amount. When added to saturation it completely precipitates all proteids therefrom; it similarly precipitates all proteids from plasma, and this either before or after saturation with magnesium sulphate.

10. *Potassium Phosphate.* This also when added to saturation completely precipitates all proteids from serum. When added to serum after saturation with magnesium sulphate it also causes an abundant precipitate of magnesium phosphate, so that the whole sets into a solid mass. When added however to a solution of the pure serum albumins it completely precipitates all of them.

In the case of both acetate and phosphate of potassium the precipitated proteids are soluble in water.

11. *Calcium Chloride.* When this salt is added to saturation to serum a very abundant precipitate is produced. The precipitate is dense, flocculent, leathery, and perfectly insoluble[1] in water. The filtrate contains no coagulable albumin, but contains a small quantity of proteid, as is shown by the fact that the xanthoproteic reaction can be still obtained.

After saturation with magnesium sulphate the precipitate consists of proteid *plus* calcium sulphate. In fact most of the calcium chloride seems to be concerned in precipitating the sulphate, so that the filtrate is never obtained perfectly free from coagulable proteid.

12. *Salts having no effect in precipitating the proteids of serum.* These are sodium phosphate, potassium chloride, potassium chlorate, potassium nitrate, potassium sulphate, ammonium chloride and barium

[1] In this case the anhydrous salt was first used; so that there was considerable evolution of heat on solution; but the insoluble nature of the precipitate is not due to this, as it is quite as insoluble when produced by the crystalline salt.

chloride. They neither precipitate the proteids in simple serum nor in that liquid previously saturated with magnesium sulphate. Some of these, e.g. sodium phosphate and barium chloride, form a precipitate with magnesium sulphate; this must of course be carefully distinguished from the precipitation of a proteid.

Commercial barium chloride contains as a rule a large quantity of hydrochloric acid, and on adding this to serum the effect of hydrochloric acid on proteids is produced; namely, first a precipitation of the proteid, and then, on adding more, a re-solution of the proteid in the form of acid-albumin. The pure salt must therefore be obtained and used.

As was before stated when speaking of sodium nitrate, potassium nitrate has also some action in lowering the temperatures of heat-coagulation of proteids.

In sheep's serum, after double saturation with magnesium sulphate and potassium nitrate, the temperatures at which the serum albumins were found to coagulate were as follows:

69° C. opalescence,
74° C. abundant flocculent precipitate,
81°–2° C. flocculent precipitate.

The general conclusions that I have arrived at in the action of salts upon the proteids of serum of plasma are then as follows:—

1. That serum globulin can be completely precipitated by means of other salts than magnesium sulphate; these are sodium nitrate, sodium acetate, and sodium carbonate.

2. That magnesium sulphate completely precipitates fibrinogen from its solutions.

3. That certain salts, not previously known to do so, completely precipitate all the proteids of serum in an uncoagulated condition. These are potassium acetate and potassium phosphate.

4. That one salt, viz. calcium chloride, is to be added to the list of those which precipitate proteids in an insoluble form.

5. That double saturation with certain salts will completely precipitate all the forms of serum albumin which can be separated by fractional heat-coagulation. The chief pairs of salts that act so are: magnesium sulphate and sodium sulphate, magnesium sulphate and sodium nitrate, magnesium sulphate and ammonic alum, magnesium sulphate and potassium iodide, and lastly sodium chloride and sodium sulphate.

In the case of the first pair of salts this action is undoubtedly due to the formation of double sulphate of magnesium and sodium. In the case of the others it is probable that similar double salts are formed.

Addenda.

1. *Concerning the existence of Serum Casein.* Hammarsten has clearly shown that what was formerly regarded as serum casein is really serum globulin.

If however an alkali albumin or casein does exist in the serum it would be precipitated by means of magnesium sulphate, with the serum globulin; since both the casein of milk[1] and the alkali albumin prepared artificially from egg albumin[2] are completely precipitable by this method.

If the precipitate produced in serum by magnesium sulphate be dissolved, and the solution rendered slightly acid and boiled, all the proteid is precipitated: none remains in solution. It can also be precipitated at a temperature of 75°C. Under such circumstances, if a serum casein were present it would remain in solution after all the globulin was precipitated; but there is no proteid remaining in solution.

If a serum casein did exist it ought to be precipitated by neutralising the serum, but as a rule no such neutralisation precipitate does appear, unless the serum be much diluted first. The precipitate produced by dilution and addition of acid is really globulin; it does not redissolve in acid so quickly as alkali albumin; moreover it can be collected, and then is found to possess all the characters of serum globulin.

K. A. H. Mörner[3] has shown that sulphate of soda added to saturation to a solution of alkali albumin will precipitate it slightly. Sodium sulphate added to saturation to serum, as we have already seen, produces no precipitate; here we have additional proof that the blood contains no alkali albumin. This however must not yet be taken as an absolute proof since Soyka[4] has asserted that sodium sulphate has no such effect on alkali albumin.

At present then the facts seem to be in favour of the non-existence of serum casein.

[1] Foster's *Physiology*, 4th edition, p. 709. [2] Hoppe-Seyler, *Handbuch*, p. 244.

[3] K. A. H. Mörner. "Studien über das Alkali Albuminat und das Syntonin." *Pflüger's Archiv*, Bd. 17, p. 541—2.

[4] Soyka. "Untersuchungen über das Verhältniss des Acid-Albumins zum Alkali Albuminat." *Pflüger's Archiv*, Bd. XII.

2. *Concerning the precipitation of the Colouring-matter of the Serum.* During my work on the action of salts on the proteids of serum I have come across no special precipitant of the colouring-matter. This material exists in various degrees in different animals; in some, like the rabbit and monkey, it is nearly entirely absent; in others, like the sheep and ox, it is abundant, giving the serum a dark amber colour. When the proteids are finally precipitated, whether by heat-coagulation or by the action of salts, the colouring-matter is precipitated also.

During heat-coagulation about 65°–70°C. the colour changes to brown, and after the first precipitate has occurred the filtrate is of a lighter hue than the original serum: the next precipitate carries more down, and finally a colourless filtrate is obtained. In a serum richly coloured, the occurrence of a colourless filtrate is often an indication of the complete precipitation of the proteids. In a light-coloured serum, all the colouring-matter may have been carried down by the first precipitate. Certain salts which cause no precipitation of proteids often cause the change of the colouring-matter to brown which has just been mentioned as occurring at a heat of 65°–70°C. Barium chloride, potassium chlorate, potassium sulphate are instances of this.

By magnesium sulphate and the other salts precipitating serum globulin much of the colouring-matter is removed; if the serum globulin be redissolved by water the colouring-matter is dissolved also; and the solution is coloured and opalescent. The whole of the colouring-matter can be removed with the proteids by such salts as potassium carbonate, or potassium phosphate. After the removal of part of the colouring-matter with the serum globulin, the serum albumins can be precipitated by subsequent saturation with one of the salts already mentioned; this carries down the rest of the colouring-matter. The serum albumins can be redissolved by the addition of water; this solution is coloured, but unlike that of globulin is clear, not opalescent.

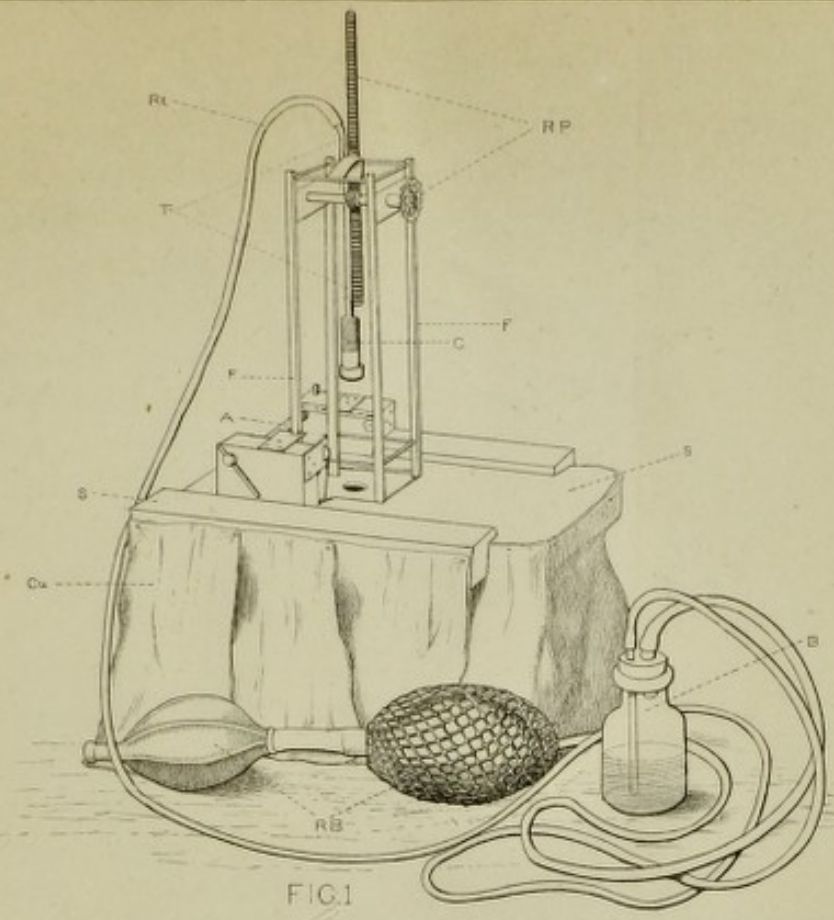

FIG.1

FIG.2.

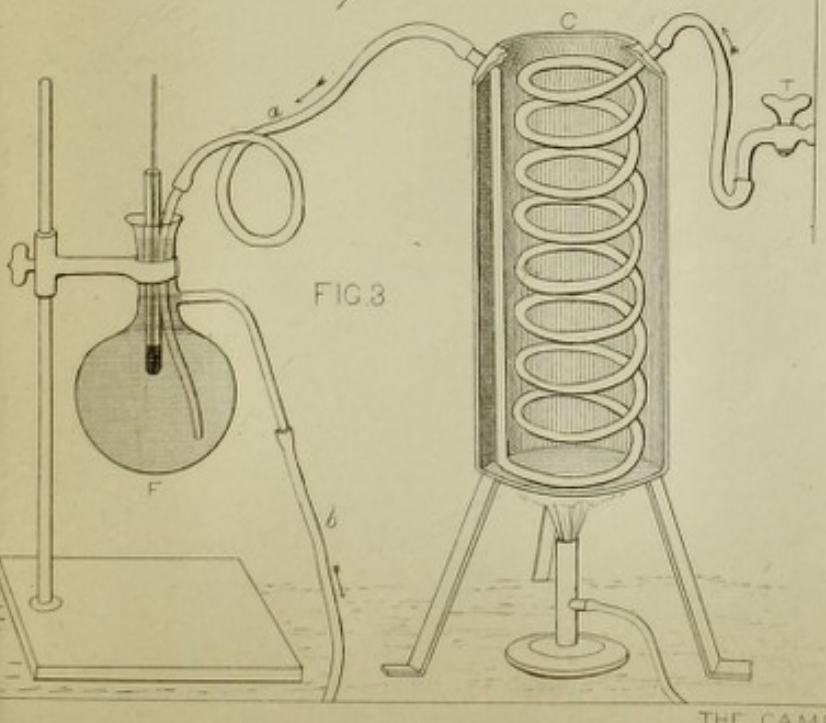

FIG.3

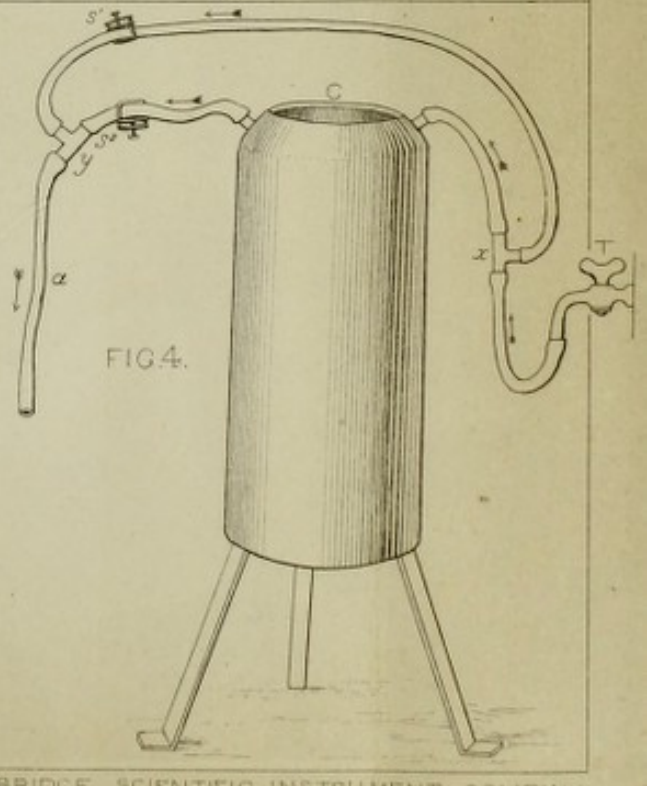

FIG.4.