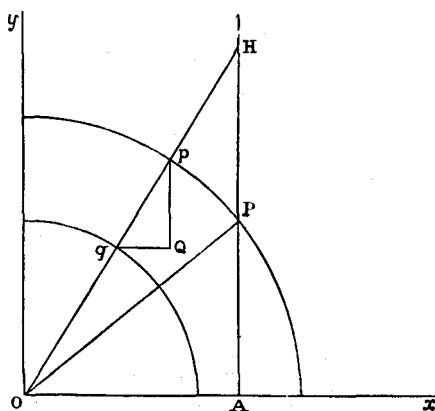


axis of x , and draw AP parallel to y . Make $AOP = \lambda$, and $yOH = h$. Draw the circles whose centre is O , and radii OP and AP respectively. Let OH meet them in p, q . From p and q draw lines parallel to Oy, Ox , respectively. Their point of intersection, Q ,



belongs obviously to the ellipse λ , and to the hyperbola h . A somewhat similar, simple, construction can easily be given for the circle.

On the Coagulation of Egg and Serum Albumen, Vitellin, and Serum Globulin, by Heat. By John Berry Haycraft, M.D., D.Sc., and C. W. Duggan, M.B.

(From the Physiological Laboratory of the University of Edinburgh.)

(Read July 15, 1889.)

A large number of proteid substances, when in solution, are coagulable by heat. As the temperature of such a fluid is raised, faint opalescence at first appears, and then, at a higher temperature, masses (floculi) of albumen separate out, in most cases, suddenly, from the fluid. It is generally held that each coagulable albumen is so affected at a definite temperature peculiar to itself; thus, egg albumen is said to become opalescent at 60° C., and to separate out in floculi at 63° C. Unfortunately, hardly two observers agree as to the exact temperature at which opalescence and coagulation

occur; thus, keeping to the example, egg albumen, Wurtz puts the coagulation point at 73° C., and Henrijean at 60° to 61° C.

It is hardly possible to explain such differences, either on the assumption that any of the above authors had used imperfect apparatus, or, that they had been guilty of inaccurate observation. It is more probable that the conditions, under which the experiments were performed, were not always the same. What are the conditions which are capable of modifying the coagulation point of albumen? It seemed to us a not unimportant point to investigate systematically these conditions; as such investigation is calculated to throw light on the nature of coagulation itself, and may enable one to arrive at the exact specific coagulation points of the more important proteids, heated as they should always be under exactly similar conditions. The conditions modifying coagulation, which we have studied, are,—the rapidity at which the coagulation is allowed to take place, the degree of concentration of the proteid substance itself, the presence of acids and alkalies, and the presence of soluble salts.

The Rapidity at which Coagulation is allowed to take place.

This is an acknowledged factor varying the indicated temperature of coagulation, and at least one author has alluded to it in the case of the particular albumen studied by himself. If a solution of a coagulable proteid be heated quickly, the proteid will be found to coagulate at a higher temperature than if the heat be applied more slowly. Thus we found that egg albumen, diluted with one volume of water, coagulated at 64° C., when slowly heated, the temperature taking forty minutes to reach this point. Another portion of the same solution coagulated at 66° C., when heated rapidly, the experiment taking in this case only one minute. It is not difficult to explain this fact. If a drop of an albuminous fluid is mounted for microscopical examination, and, if it be heated on the stage of the microscope, the process of coagulation can be readily followed out. When opalescent, the fluid will be found to contain numbers of tiny granules. These granules increase in size, and apparently become adherent, and run together to form granular masses or flocculi. This naturally requires time, and if the fluid be heated

rapidly the temperature may materially increase above the point at which, were the fluid kept for a sufficient time, coagulation would occur. Although our experiments convince us of the general truth of this fact, it occasionally happens that an albumen slowly heated coagulates at a very high temperature, and perhaps never forms distinct flocculi, the coagulation being in the form of a thin jelly. Another portion of the same solution quickly heated coagulates in flocculi at a lower temperature. We have found this occur with some specimens of serum albumen. We are inclined to explain this occurrence on the supposition that the slow and continuous heating in these cases causes some chemico-physical change in the albumen itself, whereby its coagulation is affected.

The Influence on the Coagulation Point of the Degree of Concentration of the Albumen itself.

We find, as the result of our experiments, that in all the albuminous solutions we have investigated, the coagulation point is considerably raised by diluting the solution. A very dilute solution may not coagulate even on boiling, and egg white, diluted, but nevertheless forming a comparatively strong solution, cannot be coagulated, as Sir William Roberts long ago pointed out.

In our experiments we invariably proceeded in the same way as regards the rapidity with which the solutions were heated, so as to eliminate any fallacy which might arise on this score. The usual method for determining coagulation points was adopted. The solution was placed in a test-tube containing a thermometer which could be used as a stirrer. The test-tube was immersed in a water-bath consisting of two beakers, one within the other, and each one filled with cold tap water. The water-bath was heated by a Bunsen, the flame of which was kept always at the same height, and so arranged that it took some forty minutes for the fluid in the test-tube to reach the temperature of 80° C. All our experiments were performed in this way, so that uniformity of results was obtained. We are inclined to think, however, that the heating process was unnecessarily slow, not only on account of loss of time, but what is more important, because it permitted changes to take place in the albuminous solution, especially when acids or alkalies were present in the fluid.

The Effect of Dilution on the Coagulation Point of Egg Albumen.

Egg albumen was prepared by cutting up the glairy white of an egg and squeezing it through a linen cloth. When this was diluted with water, the dilute solutions were carefully filtered. The egg albumen was always alkaline in reaction, but we decided not to neutralise it.

In the first experiments the opalescence of the heated solution alone was observed.

- (1) Undiluted egg-white became opalescent at 58° C.
- (2) Egg-white, diluted with one volume of water, became opalescent at 58°·75 C.
- (3) Egg-white, diluted with two volumes of water, became opalescent at 59°·75 C.
- (4) Egg-white, diluted with three volumes of water, became opalescent at 60°·5 C.
- (5) Egg-white, diluted with four volumes of water, became opalescent at 61°·75 C.

In the second experiment the appearance of flocculi was noted as well as the opalescence.

Opalescence appeared in the undiluted egg-white at 59° C., but did not appear so soon in the diluted portions, occurring about 1° C. higher for each dilution.

- (1) The undiluted albumen coagulated with the formation of flocculi at 64° C.
- (2) With one volume of water flocculi formed at 65°·5 C.
- (3) With two volumes of water flocculi formed at 69° C.
- (4) With three volumes of water a few flocculi formed at 80° C., the albumen never completely separating out.
- (5) Greater dilutions showed opalescence, but flocculi did not appear.

The Effect of Dilution on the Coagulation Point of Serum Albumen.

Serum albumen is said by Hoppe-Seyler (iii. p. 232) to become opalescent at 60° C., and to coagulate at 72° C. to 73° C., and Schäfer places it at 70° C. (4, p. 181).

Serum albumen was prepared in the following way:—The serum from bullock's blood was saturated by the hand with magnesium

sulphate, the precipitated globulin filtered off; by this means one obtains a solution of serum albumen in a saturated solution of magnesium sulphate. It would have been useless to dilute this solution with water, for, in that case, both the albumen and the magnesium sulphate would suffer dilution. Dilution was effected by the addition of a saturated solution of magnesium sulphate.

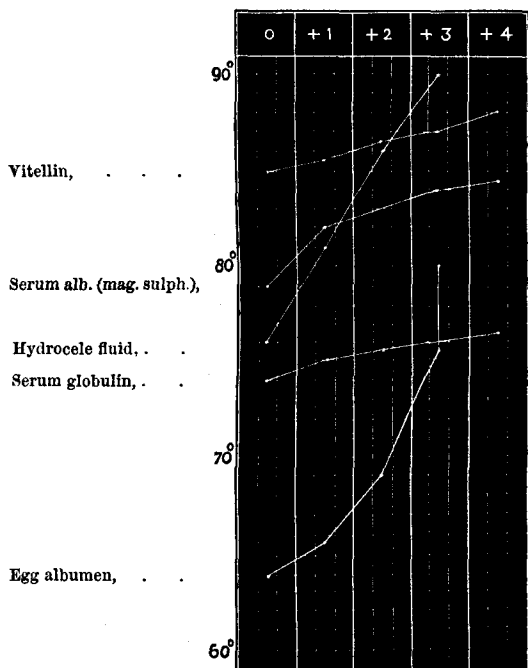


FIG. 1.—Showing the Temperature at which certain Albumens coagulate when diluted with One, Two, Three, and Four Volumes of Fluid.

(a) Undiluted serum albumen, saturated with magnesium sulphate, becomes opalescent at 77° C., and coagulates at 79° C.

(b) The same solution, diluted with one volume of a saturated watery solution of magnesium sulphate, becomes opalescent at 79° C., and coagulates at 82° C.

(c) When diluted with two volumes, opalescence occurs at 79° C., and coagulation at 83° C.

(d) When diluted with three volumes, opalescence begins at 81° C., and coagulation at 84° C.

(e) When diluted with four volumes, opalescence begins at $81^{\circ}5$ C., and coagulation $84^{\circ}75$ C.

(f) When diluted with five volumes, opalescence begins at 82° C., and coagulation at $85^{\circ}25$ C.

The numbers quoted do not give us the correct coagulation points for diluted solutions of serum albumen; they are the coagulation points of diluted solutions plus magnesium sulphate, which raises the coagulation point considerably, as we shall subsequently see. The experiment only serves to show how coagulation varies with dilution of the albumen. In this experiment fine flocculi appeared even in the more dilute solutions, and their presence rendered the determination of the coagulation point quite easy, even in the most dilute solutions.

In order to determine the action of magnesium sulphate, serum albumen was prepared in another way.

Blood serum was diluted with two volumes of water, and a stream of carbon dioxide passed through it. The precipitate of globulin was filtered off. By this method the albumen was obtained mixed with a small quantity of globulin; its presence, however, did not prevent the recognition of the point of opalescence and the coagulation point of the albumen.

(a) The serum albumen became opalescent at 70° C., and coagulated in flocculi at $74^{\circ}25$ C. The coagulation point being raised two or three degrees above the figure given by Hoppe-Seyler on account of its dilution.

(b) This solution of serum albumen, diluted with one volume of water, became opalescent at 74° , the opalescence becoming very dense at 78° C. No flocculi appeared.

On comparing these figures with those given for serum albumens in a saturated solution of magnesium sulphate, it will be seen that the former are uniformly lower, the presence of magnesium sulphate tending to elevate the coagulation point. The effect of dilution is more marked in the case of serum albumen by itself than in that of serum albumen in the saturated magnesium sulphate solution. In the first place, the coagulation becomes very imperfect in the dilute solutions; in the second place, the temperature in the dilute solution is very much raised.

The Effect of Dilution on the Coagulation Point of Vitellin.

The yolks of several eggs were dissolved in 6 per cent. solution of sodium chloride and filtered. The filtrate was poured into a large volume of distilled water, the precipitate of vitellin redissolved in saline solution, reprecipitated in distilled water, and dissolved in 5 per cent. solution of sodium chloride. In this case the vitellin, prepared from six eggs, was dissolved in 300 c.c. of the solution. In order to study the effect of dilution, a 5 per cent. solution of sodium chloride was added in all cases.

(a) The vitellin solution became opalescent when heated to 80° C., and coagulated at 85° C.

(b) When diluted with one volume of 5 per cent. solution of sodium chloride, the vitellin became opalescent at 81° C., and coagulated at 85°·5 C.

(c) When diluted with two volumes, it became opalescent at 82° C., and coagulated at 86°·5 C.

(d) When diluted with three volumes, it became opalescent at 82° C., and coagulated at 87° C.

(e) When diluted with four volumes, it became opalescent at 83° C., and coagulated at 88° C.

The experiment was repeated, giving a result almost precisely the same. It will be noticed that in this proteid the coagulation point does not vary to a very considerable extent with dilution.

The Effect of Dilution on the Coagulation Point of Serum Globulin.

The coagulation point of serum globulin is given by Halliburton as 75° C. (Reference 6, p. 163).

In the first experiment the globulin was precipitated from bullock's blood by magnesium sulphate. The precipitate was, after washing, dissolved in a 5 per cent. solution of magnesium sulphate. It was diluted with a 5 per cent. watery solution of magnesium sulphate. Unfortunately the opalescence was not noted down. The flocculi were well marked.

(a) The solution of serum globulin in a 5 per cent. solution of magnesium sulphate coagulated at 74° C.

(b) The solution, when diluted with an equal volume of 5

per cent. watery solution of magnesium sulphate, coagulated at 75° C.

- (c) When diluted with two volumes, coagulated at 75°·5 C.
- (d) When diluted with three volumes, it coagulated at 75°·5 C.
- (e) When diluted with four volumes, it coagulated at 76°·25 C.
- (f) When diluted with five volumes, it coagulated at 77° C.
- (g) When diluted with six volumes, it coagulated at 77° C.

In another experiment serum globulin was prepared by passing a stream of carbon dioxide through dilute blood serum. The precipitated globulin was dissolved in 5 per cent. solution of sodium chloride. The solution of globulin not being of the same strength (a little weaker), and the salt used for its solution being a different one, the coagulation points do not correspond with those obtained in the previous experiment.

(a) Serum albumen, dissolved in 5 per cent. solution of sodium chloride, became opalescent at 74° C., and coagulated at 79° C.

(b) Serum albumen, dissolved in 5 per cent. solution of sodium chloride and diluted with one volume of a 5 per cent. watery solution of sodium chloride, became opalescent at 77°·5 C., and coagulated at 81°·5 C.

(c) Diluted with two volumes, opalescence commenced at 78°·5 C., and it coagulated at 82°·5 C.

(d) Diluted with three volumes, opalescence commenced at 79° C., and coagulated at 84° C. The albumen at this stage had begun to putrefy, and on repeating the experiments it was found that the coagulation point was raised about two degrees for (a), (b), (c), and that (d) did not coagulate even on boiling.

The Effect of Dilution on the Coagulation Point of Hydrocele Fluid.

Hydrocele fluid contains the same proteids as are found in blood plasma, namely, fibrinogen, serum globulin, and serum albumen. In a case of chronic hydrocele there may be an almost entire absence of proteid matter. The proteid substance when present varies in amount, and the coagulation point varies with it. On diluting hydrocele fluid the coagulation point is raised.

(a) Hydrocele fluid became opalescent at 65° C.; at 72° C. it assumed the consistence of a thin jelly which thickened, and at 76° C. flocculi separated out.

(b) Diluted with one volume of water, it became opalescent at 67° C., and coagulated at 81° C.

(c) Diluted with two volumes of water, it became opalescent at 69° C., and coagulated at 86° C.

(d) (a) Diluted with three volumes of water, it became opalescent at 73° C., and a few flocculi separated out at 90° C.

Another specimen of hydrocele fluid, apparently containing less proteid matter, became opalescent at 70° C., and coagulated with the formation of flocculi at 80°·5 C.

A third specimen became opalescent at 70° C., flocculi forming at 78° C.

General Conclusions regarding Dilution.

In the case of albumens and globulin existing in a natural condition within an animal fluid, such as white of egg, serum, or hydrocele fluid, the point of opalescence is gradually and almost uniformly raised by successive dilutions. The coagulation point, on the other hand, rises rapidly, and the more dilute fluids often refuse to form flocculi, or even to coagulate at all.

When a globulin is dissolved in an artificially prepared saline solution, both the point of opalescence and coagulating point are uniformly raised on diluting the solution. The same appears to apply to serum albumen saturated with magnesium sulphate.

The Action of Salts on the Coagulation Point of Albumen.

It is known that the addition of many neutral salts to an albuminous solution has an important action on the temperature at which it coagulates. Some salts are stated to lower and others to raise the coagulation point. It is impossible to explain at present their action, and we have accordingly commenced a somewhat systematic examination of the question. Our results are far from complete, and will subsequently, we hope, be more fully extended.

We have at present studied the action of two important salts, namely, magnesium sulphate and common salt, on the coagulation points of egg and serum albumen, vitellin, and globulin, and the action of these salts has been studied in all degrees of strength up to complete saturation.

Although we feel that it would be quite out of place to attempt general conclusions, yet we believe one or two inferences may be drawn from the facts that we have gleaned.

Some of the facts we have already obtained are sufficiently striking to justify us in thinking that a more extended investigation, made on similar lines, may throw some light on the mutual relationship existing between the albuminous and saline molecules when in solution together. We are aware of the extreme difficulty of the subject, since so little is known as yet regarding simpler problems, such as the mutual relationships that exist between simple mixtures of inorganic salts.

The Action of various Salts on the Coagulation Point of Egg Albumen.

Varenne (Reference 8) finds that many salts by their addition elevate the temperature of coagulation, such are, common salt and sulphate of magnesium; others, such as sulphate of copper and chloride of barium, lower it; while a third series, such as sulphate of sodium and chlorate of potassium, have no effect.

TABLE I. showing the Action of various Salts on the Coagulation Point of Egg Albumen.

Salt added.	Proportion.	Opalescence.	Coagulation.
	Per cent.	° C.	° C.
<i>Original solution of Albumen,</i>	...	61	65
Lithium chloride, . . .	10	65	70
Sodium chloride, . . .	10	64	66.5
Potassium fluoride, . . .	10	66	71
Potassium chloride, . . .	10	63	68
Potassium bromide, . . .	10	67	77.75
Potassium iodide, . . .	10	67	75
Ammonium chloride, . . .	10	64.5	70
Ammonium nitrate, . . .	10	71	73.5
Ammonium sulphate, . . .	10	67	74
Magnesium chloride, . . .	10	69	75.5
Magnesium nitrate, . . .	10	68	70.5
Magnesium sulphate, . . .	10	65	70
Potassium nitrate, . . .	10	68	76.25
Potassium sulphate, . . .	10	65	68.5

Béchamp (Reference 5, p. 29) finds, on the other hand, that sulphate of magnesium, alum, and the salts of sodium and potassium lower the coagulation point. He came to this conclusion after working with

very dilute solutions of albumen; these did not coagulate at all, until after the addition of the salts mentioned. He added very small quantities of the salts to the albuminous solution, viz., less than one per cent. Had he worked with coagulable solutions of albumen, and had he added larger quantities of salt, his result would have been different. While, as we shall afterwards show, these salts as a rule raise the point of coagulation, it is not at all improbable that dilute uncoagulable solutions of egg albumen may be enabled to coagulate, when they otherwise would not; in fact, our results point to this conclusion. If so, it is only one of the many facts which indicate how little is at present known as to properties of the albuminous molecules and the factors which determine their solubilities.

In the preceding table we have placed some of our own results. In all cases the temperature, at which opalescence and coagulation occur, has been raised, though often, as in the case of common salt, to a very slight extent.

The Precipitation of Egg Albumen by Single and by Double Saturation with Neutral Salts.

By complete saturation of an albuminous fluid with a neutral salt the proteid may be precipitated at the temperature of the laboratory. Thus Hammarsten has shown that globulin may be precipitated from serum by the addition of magnesium sulphate. In this case the globulin is not converted into a coagulated proteid, but can again be dissolved after the magnesium sulphate has been diluted.

The Action of Magnesium Sulphate.—The egg albumen was diluted with one volume of water and freed as much as possible from membrane. A portion of this was saturated with magnesium sulphate and filtered. The saturated solution contained about 100 per cent. of magnesium sulphate. In order to obtain solutions of albumen containing a lower percentage of the salt, the saturated solution was diluted with portions of the original albumen.

The original diluted albumen became opalescent at 65° C., and coagulated, forming flocculi, at 66°·5 C.

(a) The saturated solution became opalescent at 78° C., and coagulated at 80° C.

(b) Egg albumen, containing 50 per cent. of magnesium sulphate, became opalescent at 67°·25 C., and coagulated at 68°·5 C.

(c) Egg albumen, containing 25 per cent. of magnesium sulphate, became opalescent at 65° C., and coagulated at 67° C.

(d) Egg albumen, containing 12·5 per cent. of magnesium sulphate, became opalescent at 63°·25 C., and coagulated at 65° C.

(e) Egg albumen, containing 6·25 per cent. of magnesium sulphate, became opalescent at 63° C., and coagulated at 65° C.

The action of this salt seems a very curious one, for while in large quantity it raises the coagulation point very considerably, small quantities seem to lower it slightly, and no doubt Béchamp

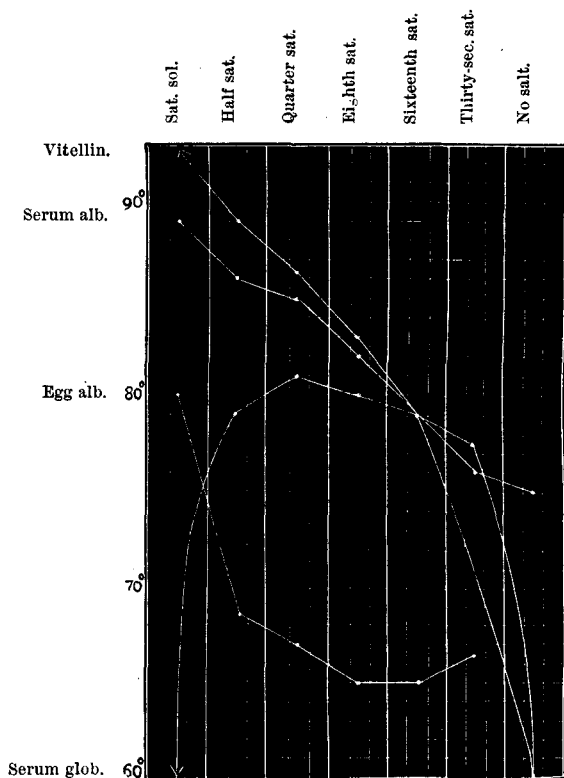


FIG. 2.—Showing the Effect of different Strengths of Magnesium Sulphate on the Coagulation Points of certain Albumens.

is correct when he states that the dilute uncoagulable albumen can readily be coagulated after the addition of the salt. He is hardly,

however, justified in speaking of magnesium sulphate as lowering the coagulation point of albumen by its presence.

It is a point of some interest to discover whether a salt, which, by its addition to an albuminous solution, raises the temperature at which coagulation occurs, will produce the same result on an albuminous solution already saturated with another salt. This we have determined to some extent.

Effect of the Addition of various Salts on the Coagulation Point of Egg Albumen already saturated with Magnesium Sulphate.

Egg albumen was diluted with two volumes of water, and saturated with magnesium sulphate. The solution was filtered, and it was found on heating to become opalescent at 79° C., coagulating at 81·75 C. The salts added, most of which have already been studied in respect to their action on the coagulation point of egg albumen (Table I.), are seen to lower the coagulation point of egg albumen saturated with magnesium sulphate.

TABLE II. *showing the Action of various Salts upon the Coagulation Point of Egg Albumen already saturated with Magnesium Sulphate.*

Salt added.	Proportion.	Opalescence.	Flocculi.
	Per cent.	° C.	° C.
<i>Albumen saturated with magnesium sulphate,</i>	6	79	81·75
Sodium chloride,	6	72	79
Sodium iodide,	6	...	70
Sodium sulphate,	6	79	81·5
Potassium chloride,	6	72	79
Potassium bromide,	6	70	74
Potassium nitrate,	6	70	73·75
Potassium chlorate,	6	71	74·5
Potassium sulphate,	6	74	77
Ammonium chloride,	6	62	73
Ammonium nitrate,	6	63	65

On comparing this table with that on page 370, it will be noted, first, that those salts which on Table I. do not raise the coagulation point of egg albumen to any great extent, NaCl, KCl, K₂SO₄, and Na₂SO₄ (Varenne), do not lower the coagulation point (Table II.) to any great extent. On the other hand, salts like KBr, K₂NO₃, and NH₄NO₃, which raise the coagulation point in Table I., depress

it in Table II. It is possible still more to lower the coagulation point by the addition of larger quantities of the latter salts, until one can precipitate the albumen by double saturation at the temperature of the laboratory. On the other hand, the addition of large quantities of NaCl and Na_2SO_4 exerts very little action.

Effect of Magnesium Sulphate on the Coagulation Point of Serum Albumen.

Although Dr Halliburton has succeeded (Reference 6, p. 192) in precipitating serum albumen by double saturation by means of sulphate of magnesium in conjunction with such salts as sodium sulphate, sodium nitrate, potassium iodide, &c., magnesium sulphate in itself raises the coagulation point of serum albumen.

(a) Serum albumen, containing 100 per cent. magnesium sulphate, became opalescent at 84°C. , and coagulated at 89°C. , a slight opalescence appearing at 40°C. , due to a trace of serum globulin.

(b) Serum albumen, containing 50 per cent. magnesium sulphate, became opalescent at 77°C. , and coagulated at 86°C.

(c) Serum albumen, containing 25 per cent. magnesium sulphate, became opalescent at 76°C. , and coagulated at $84^\circ\cdot75 \text{C.}$

(d) Serum albumen, containing $12\frac{1}{2}$ per cent. magnesium sulphate, became opalescent at 76°C. , and coagulated at 82°C.

(e) Serum albumen, containing $6\frac{1}{4}$ per cent. magnesium sulphate, became opalescent at 74°C. , and coagulated at $78^\circ\cdot25 \text{C.}$

(f) Serum albumen, containing $3\frac{1}{8}$ per cent. magnesium sulphate, became opalescent at 72°C. , and coagulated at 76°C.

(g) Serum albumen, somewhat diluted in this experiment, became opalescent at 68°C. , and coagulated at 75°C. , without the formation of well-marked flocculi.

Sodium Chloride.—Although Hoppe-Seyler states that this salt lowers the coagulation point of serum albumen, we find that this is only the case when present in large quantity. Small quantities appear, if anything, to raise it.

A saturated solution of the same serum albumen as that used for the last experiment coagulated at 72°C. , when saturated with common salt. A solution, containing 20 per cent., became opalescent at 74°C. , and coagulated at $80^\circ\cdot5 \text{C.}$

*The Action of Sodium Chloride on a Solution of Serum Albumen
already saturated with Magnesium Sulphate.*

In this case the coagulation was lowered as sodium chloride was added in greater and greater quantity.

(a) Serum albumen, saturated with magnesium sulphate, became opalescent at 77° C., and coagulated at 79° C.

(b) The same solution, plus 10 per cent. sodium chloride, became opalescent at 72°·5 C., and coagulated at 75° C.

(c) The same solution, plus 20 per cent. sodium chloride, became opalescent at 70° C., and coagulated at 73° C.

A larger quantity of common salt was not added, since 20 per cent. did not dissolve readily.

*The Action of Magnesium Sulphate on the Coagulation
Point of Vitellin.*

Some vitellin was dissolved in a dilute solution of magnesium sulphate. Some of this was saturated with the salt, the precipitate filtered off, and the filtrate tested.

(a) Vitellin, dissolved in a saturated solution of magnesium sulphate (100 per cent.), became opalescent at 88° C. Coagulation did not occur even on boiling, a few flocculi alone appearing.

(b) Vitellin, dissolved in a 50 per cent. solution of magnesium sulphate, became opalescent at 87° C., and coagulated at 89° C. with flocculi.

(c) Vitellin, dissolved in a 25 per cent. solution of magnesium sulphate, became opalescent at 81° C., and coagulated at 86°·5 C.

(d) Vitellin, dissolved in a solution containing 12·5 per cent. magnesium sulphate, became opalescent at 79° C., and coagulated at 82°·5 C.

(e) Vitellin, dissolved in 6·25 per cent. solution of magnesium sulphate, became opalescent at 74° C., and coagulated at 79° C.

(f) Vitellin did not completely dissolve in 3½ per cent. solution of magnesium sulphate. It was not heated.

When further diluted until only about 1 per cent. magnesium sulphate was present, a distinct precipitate separated out in the cold.

This experiment was repeated with a more dilute solution of vitellin. The coagulation points at corresponding strengths of the

magnesium sulphate were all higher. The result was otherwise the same, the saturated solution requiring the greatest temperature for its coagulation.

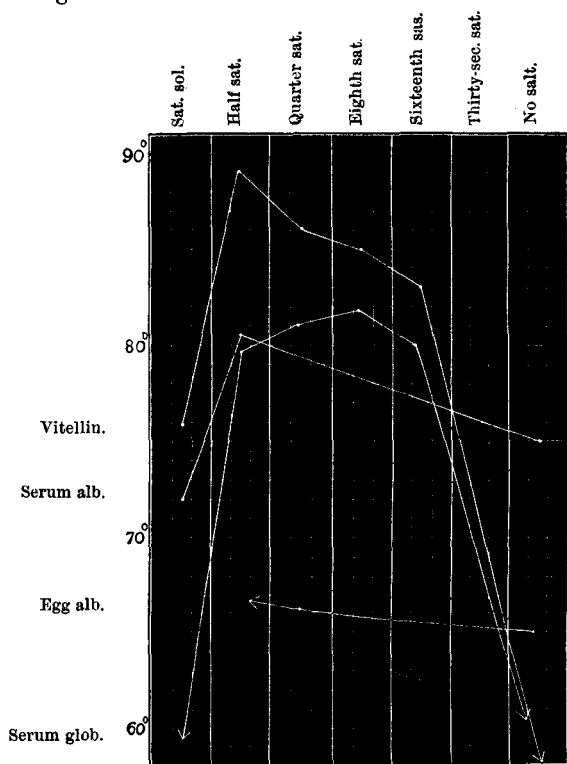


FIG. 3.—Showing the Effect of different Strengths of Sodium Chloride on the Coagulation Points of certain Albumens.

The Action of Sodium Chloride on the Coagulation Point of Vitellin.

Some vitellin was dissolved in 5 per cent. solution of common salt. It was saturated with the salt, and a precipitate of globulin filtered off.

(a) Vitellin, dissolved in saturated solution of common salt, became opalescent at 70° C., and coagulated at 76° C.

(b) Vitellin, dissolved in 20 per cent. solution of common salt, became opalescent at 83° C., and coagulated at 89° C.

(c) Vitellin, dissolved in 10 per cent. solution of common salt, became opalescent at 80° C., and coagulated at 86° C.

(d) Vitellin, dissolved in 5 per cent. solution of common salt, became opalescent at 79° C., and coagulated at 85° C.

(e) Vitellin, dissolved in 2·5 per cent. solution of common salt, became opalescent at 78° C., and coagulated at 83° C.

This experiment was repeated, and showed that common salt raises the coagulation point of vitellin, but that it is lowered just before the point of saturation, and that it continues to be lowered until saturation occurs.

Action on the Coagulation Point of Vitellin of both Common Salt and Magnesium Sulphate dissolved together in the Solution.

If, to vitellin in a saturated solution of common salt, some magnesium sulphate be added, the latter dissolves with difficulty, precipitating the vitellin in flocculi; on heating, other flocculi appear.

If, to vitellin in a saturated solution of magnesium sulphate, common salt be added, the coagulation point is lowered. Thus, on adding 15 per cent. of common salt, coagulation occurs at 88° C., and with a little over 20 per cent. it is lowered to 70° C.

Serum Globulin.—Serum globulin is precipitated by magnesium sulphate in excess, as Hammarsten has shown. The same observer obtained a precipitation by saturating with common salt.

The Action of Magnesium Sulphate on the Coagulation Point of Serum Globulin.

Serum globulin was precipitated from the serum of ox's blood by passing a stream of CO₂ through it. The precipitate after careful washing was dissolved in magnesium sulphate solution.

(a) Serum globulin is precipitated in the cold by saturating the solution with magnesium sulphate.

(b) Serum globulin, dissolved in a solution containing 50 per cent. magnesium sulphate, became opalescent at 74·5 C., and coagulated at 79° C.

(c) Serum globulin, dissolved in a solution containing 25 per cent. magnesium sulphate, became opalescent at 78·5 C., and coagulated at 80·75 C.

(d) Serum globulin, dissolved in a solution containing 12·5 per cent. of magnesium sulphate, became opalescent at 77°·5 C., and coagulated at 80° C.

(e) Serum globulin, dissolved in a solution containing 6·25 per cent. of magnesium sulphate, became opalescent at 76° C., and coagulated at 78°·75 C.

(f) Serum globulin, dissolved in a solution containing 3·125 per cent. magnesium sulphate, became opalescent at 71°·5 C., and coagulated at 77° C.

Effect of Sodium Chloride on the Coagulation Point of Serum Globulin.

(a) Serum globulin, saturated with sodium chloride, is precipitated in the cold.

(b) Serum globulin, containing 20 per cent. sodium chloride, became opalescent at 77° C., and coagulated at 79°·5 C.

(c) Serum globulin, containing 10 per cent. sodium chloride, became opalescent at 79° C., and coagulated at 81° C.

(d) Serum globulin, containing 5 per cent. sodium chloride, became opalescent at 79° C., and coagulated at 81°·75 C.

(e) Serum globulin, containing 2·5 per cent. sodium chloride, became opalescent at 78° C., and coagulated at 80° C.

(f) Serum globulin in much smaller quantity does not dissolve to form a clear solution.

Tentative Conclusions regarding the Action of Salts.

(1) A salt may raise the temperature of coagulation if present in a certain percentage; at another percentage it may lower it. Thus common salt raises the coagulation points of both vitellin and serum globulin when present in moderately small quantity. Large quantities lower the coagulation point.

(2) If a proteid be present in a saturated solution of a salt—such as magnesium sulphate—and, if another salt be then added, which by itself would raise the coagulation point, the coagulation point may in this case be lowered. It appears, too, that salts which are most active in raising the coagulation point are most active in lowering it, when added to a solution already saturated by another salt.

*Statement as to whether it is possible to speak of the Specific
Coagulation Point of an Albumen.*

From what has been already said, it is obvious that it is a difficult and perhaps a valueless task to attempt to determine what may be termed the "specific coagulation point" of an albumen. The coagulation point varies with the rapidity of heating, with the concentration of the fluid, with its reaction, and with the saline substances present. All that one can say is that, under such and such conditions, an albumen coagulates at such a temperature. It is probably hardly possible to obtain even two albumens under such similar conditions that their coagulation points may with advantage be compared. The nearest approach to this would perhaps be to dissolve a certain weight, say both of vitellin and another globulin such as serum globulin, in the same volume of salt solution. The coagulation points may, in this case, with advantage be compared. But what would be the value of the coagulation points so obtained for purposes of comparison with serum or egg albumen dissolved in water? The coagulation points quoted by previous writers cannot be taken in any sense as absolute values for the albumen named, modifying conditions having, as a rule, been totally disregarded. The same may be said of our own results, for the percentage strengths of the albuminous solutions used by us were in no case determined with any care. Although the forms of the curves represented in the charts are not affected by this, their altitudes in the scale of temperatures may be so to some considerable extent.

On so-called Fractional Coagulation.

So far we have been dealing with albumen in its natural condition, or mixed and possibly combined with neutral salts which we had added.

The solutions were alkaline, and, as we found, when dealing with the natural albuminous solution, difficult to coagulate, especially if in a dilute condition. Let us now consider the coagulation point of an albuminous solution to which an acid has been added. On adding an acid to an albumen solution, the coagulation is rendered, as every one knows, more easy, and it occurs at a lower temperature. The very dilute solutions, uncoagulable in the alkaline solution,

are at once coagulated after the addition of a few drops of weak acid. No one has brought this out more clearly than Dr Halliburton in a most suggestive paper (Reference 6), which will presently be quoted in relationship to fractional coagulation. He showed that the coagulation point of serum albumen varies with the amount of acid present, the greater the quantity added, the lower the coagulation point, until finally coagulation could be produced at the temperature of the laboratory. If then the coagulating point depended on the two factors, heat and the amount of acidity, it seemed to him a natural deduction, that, on keeping one of these, the acidity, a constant quantity, it might be possible to separate by fractional coagulation two or more albumens mixed together, and having different coagulation points. He investigated serum albumen, and found that if it be neutralised by the addition of some drops of a 2 per cent. solution of acetic acid, and if, further, it be rendered slightly acid by the addition of one drop of the dilute acetic acid to seventy-five drops of the albuminous solution, then it coagulates at 70° to 71° C., and if this coagulum be filtered off, and the solution again brought to the same degree of acidity, a coagulum occurs the second time at 77° to 78° C. If this coagulum be filtered off and the filtrate acidified as before, a third coagulum may be produced at 82° C. Dr Halliburton considers that the serum albumen, originally regarded as one proteid, in reality consists of three.

MM. Corin and Berard have followed this process of fractional coagulation, applying it to egg-white. This substance, commonly held to consist of albumen and globulin, they believe to consist of three albumens and two globulins.

They neutralise some egg-white, slightly acidify it, and raise its temperature, until opalescence appears; then they keep the temperature constant for a considerable time—an hour or even more. They filter off the coagula, re-acidify to the same degree, raise the temperature until opalescence occurs, and then after more prolonged heating flocculi again appear.

In this way they have succeeded, as already stated, in fractionating five proteids.

Without doubting that it may be possible to fractionate some proteids, nevertheless the results of our own work, and many of

the facts frankly stated by Dr Halliburton, seemed to throw some doubt upon the correctness of his deductions in the case of serum albumen, and this applied with equal force to the experiments conducted by MM. Corin and Berard on egg albumen.

Our previous experiments have shown that, in alkaline solution, the more dilute a solution is, the higher is its coagulation point, and we have found that we could never completely precipitate any albumen at the temperature at which flocculi first appeared. The reason of this is very simple; as soon as a solution begins to coagulate, the remnant, still soluble, is practically a more dilute solution of the same proteid, and must be heated two or three degrees more before it will begin to precipitate. In this case, also, the coagulating proteid will leave another soluble remnant, coagulable at a still higher temperature, and so on. In fact, we may venture to make this general statement—*In order to coagulate completely any proteid it must be heated to that temperature at which its most dilute solutions are coagulable.* We have not made so systematic an investigation upon the effect, on its coagulating point, of diluting acid solutions of albumen, but we have assured ourselves that the more dilute solutions coagulate at a higher temperature. One out of several experiments may be quoted the following :—

Some egg albumen was diluted with two volumes of water and carefully neutralised. It was then brought to the same degree of acidity as is recommended for fractional coagulation, 1 cubic centimetre of a 2 per cent. solution of acetic acid being added to 75 cubic centimetres of the albumen. This solution was found to coagulate at 53° C.

When diluted with one volume of water, acidulated to the same degree, it coagulated at 54° C.

With three volumes of water, it coagulated at 58° C.

With seven volumes of water, it coagulated at 62° C.

With fifteen volumes of water, it coagulated at 68° C.

It is seen, therefore, that dilution has the effect of raising the coagulation point a great many degrees, the more dilute albumen requiring a much higher temperature for its separation. This may be shown in the most striking manner by heating some of the acidulated water to between 60° and 70° C.; and dropping in some acidulated egg albumen it at first dissolves. Now divide the solu-

tion into two portions, A and B, and heat A to 75° C., and keep B at the original temperature. A will coagulate, showing that although in too dilute a solution to coagulate below 70° C., it could nevertheless coagulate, provided its temperature be raised. B will remain clear, but, if more albumen be dropped into it, a point will be reached, at which it will cease to dissolve, and then it will separate out in flocculi.

Here then, without going any further, one has come across an observation which, if it does not explain all the facts described under the head of fractional coagulation, must at any rate account for some of them.

Both Dr Halliburton and MM. Corin and Berard found that after coagulation the filtrate, which they separated from the clot, was less acid than it was before coagulation had occurred, the latter observers finding that, as a rule, it was actually alkaline. Here, again, is a factor which we cannot afford to lose sight of. If the coagulation point is lowered by acidity, as all persons are agreed, one would expect that, while coagulation is proceeding, and while *pari passu* the acidity is decreasing, that the decrease of acidity would at last bring the coagulation—at that temperature—to a standstill. In this case one would expect, that on re-acidifying to the same degree, another crop of coagula might fall at the same temperature as did the first crop.

Dr Halliburton does not mention any such coagulation, although undoubtedly it occurs, and we have found it on repeating his observations, but MM. Corin and Berard evidently find that one is produced, and in consequence they heat the albumen for an hour or more before filtering off the coagulum. After this time, they found that the albumen never gave a second coagulation at the same temperature. We cannot but conclude from this that their experiments clearly indicate that the albuminous solutions with which they worked must have been very materially changed by the temperature, nor is it at all improbable that very material changes may occur in a solution of egg albumen kept in an acid solution at a high temperature for over an hour; in one of their quoted experiments fractionation lasted over six hours.

We may, we think, make this statement, and one fully borne out by our own experiments, that during coagulation in an acid medium

the coagulation point is being continually raised, both in virtue of the albumen becoming more dilute and in virtue of its becoming less acid; these factors bring the coagulation to a standstill, but, after filtering off the coagulum, if the fluid be brought back to its original degree of acidity, and heated to the same temperature, coagula will again form, unless the albumen has undergone some physico-chemical change.

It follows, too, that it is impossible to separate two albumens from one another by heat coagulation, unless, during the process of coagulation, the degree of acidity is kept uniform by the addition of small quantities of fresh acid, and unless the coagulation point of *the most dilute solution* of one of the albumens present be below the coagulation point of the other albumen. We became more convinced of this, when repeating in detail the experiments on fractional coagulation. After keeping an albuminous solution, either egg or serum albumen, at the temperature at which flocculi appear, for five or six minutes, and then filtering off the flocculi, we found that fresh flocculi appeared, when the filtrate had been re-acidified, and again raised to the same temperature. Two or three crops might be thus removed in the case of egg-white. Keeping up the same degree of acidity, and raising the temperature, we were able to get other crops of albumen. We were struck, however, by the fact that, while dealing with the more dilute albumen, the coagulation took place with difficulty, and it was longer delayed. This was particularly the case with egg-albumen. If the fluid filtrate from the coagulated flocculi be divided into two parts, and one portion raised gradually in temperature, opalescence followed by the formation of flocculi will appear. If the other portion be raised in temperature and kept for, say, three minutes at a temperature one or two degrees below the temperature at which opalescence appeared in the first portion, it will become opalescent and perhaps form flocculi. We found, in fact, that it was impossible to get the subsequent coagulation at definite points, as indicated by the previous observers, for the coagulation point depended upon the way in which the operations had been carried out. This was especially the case, when dealing with egg albumen, and we have little doubt that MM. Corin and Berard, working with careful method, invariably raised their temperatures to points which perhaps their first experiments

had suggested. They, no doubt, produced coagula, but, had they tried the experiment, they would have obtained them equally well at a lower temperature provided they had raised the temperature more slowly. It is not difficult to fractionate egg albumen ten or twelve times.

Another point that struck us was the smaller and smaller amount of coagulation produced, as the temperature of the solution was raised and successive crops produced. This was noticed by Dr Halliburton in the case of egg albumen. It is certainly the case with egg albumen. This, of course, in itself renders it highly probable that we are dealing in both cases only with one albumen, the more dilute solutions of which are alone coagulated at the higher temperatures. Even supposing that the γ serum albumen of Dr Halliburton, of which he "in some case only found a trace," and which coagulates at 82° C., is different from α and β serum albumen, found in greater quantity, and coagulating at lower temperatures, yet fractional coagulation could not give us the means of proving this. One cannot compare the coagulating points of a dilute with a strong solution of two albumens, and presuming that γ serum albumen is a dilute solution of an albumen differing from α and β , yet its coagulation point would be lower than 82° C. in a solution of corresponding strength.

It is, of course, possible that serum albumen may consist of more than one albumen, although it is probable, from what we have brought forward, that all the albuminous matter present coagulates at the same degree of concentration—at or about the same temperature. Other methods may enable the physiologist to separate these, if they exist, from one another, and no methods have in the past yielded such valuable results as those in which separation has been obtained by the addition of neutral salts. Dr Halliburton has by this means tried to separate the α , β , and γ serum albumens from one another, and frankly states that he has failed to do so (Reference 6, p. 173).

In conclusion, we may state that the method of fractional coagulation could only be of service when the coagulation points of the albumens present are widely separated from each other. In reality, fractional coagulation has been for a long time in use, and one of the few cases in which, as far as we can see, it is at all

applicable, is the separation of serum globulin from serum albumen. Serum globulin is precipitated at the atmospheric temperature on acidifying by a stream of carbon dioxide, or by the addition of weak acetic acid. This precipitation is not a complete one, however, as Hammarsten has shown. The reason is, that, at the atmospheric temperature, part of the globulin remains in solution.

This paper contains some of the results of a research, towards the expenses of which a grant of money was voted by the Scientific Grants Committee of the British Medical Association.

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**Some New Points in Connection with Muscle
Contraction.** By **Alexander James, M.D.**

(Read July 15, 1889.)

The interval which elapses between tapping a muscle or tendon and the resulting movement of the limb has been estimated by many observers—Burckhardt, Tschirjieu, Waller, Brissaud and François Franck, Eulenberg, De Watteville, &c.—but the precise signification of these so-called reflexes is not yet fully understood. What follows is intended to add to our knowledge of this subject.

The observations were made on a patient in the Royal Infirmary, aged 26, who, as the result of a blow on the left side of the neck, sustained three years previously, presented (1) greatly impaired voluntary motor power in the left arm and left leg; (2) marked jerks on tapping the tendons of the left supinator longus, left