ON THE INFLUENCE OF CALCIUM SALTS UPON THE HEAT-COAGULATION OF FIBRINOGEN AND OTHER PROTEIDS

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(Received February 3rd, 1906)

Ringer and Sainsbury¹ found that soluble calcium salts (chloride and nitrate) favour the heat-coagulation of serum proteids, causing a notable lowering of the coagulation temperature, etc., an action shared by salts of the allied elements, strontium and barium, and, to some extent, by magnesium sulphate. They found a certain amount of antagonism between calcium chloride on the one hand, and sodium and potassium chlorides on the other.

Ringer² also found that calcium salts have a remarkable influence in promoting the heat coagulation of alkali-albumin, while they have no such influence on egg-albumin.

As is well known, the heat-coagulation point of fibrinogen is remarkably definite and constant throughout the vertebrate series,³ and it can be studied in its natural state in plasma, etc., with or without the possibly disturbing influence of acidification of the fluid. In view of the great importance of calcium in solution in the coagulation of blood and fibrinogen solutions, and the curious resemblances between the behaviour of fibrinogen in ferment-coagulation and in heat-coagulation—the splitting of the fibrinogen molecule in each case described by Hammarsten, etc.⁴—it seemed very possible that some relation might obtain between calcium salts and heat-coagulation. This has been found to be the case, though perhaps not in the direction that might have been expected.

1. *Journal of Physiol.*, Vol. XII, p. 170.

2. *Journal of Physiol.*, Vol. XII, p. 378.

3. See table in Halliburton's *Text-Book of Chemical Physiology*, p. 248.

4. *Arch. f. d. ges. Physiologie*, Vol. XIX, p. 563.

A large number of plasmas of different kinds were examined, without and with acidulation ; also hydrocele fluid, etc. Halliburton's heat-coagulation method was employed. The rate of heating was made to vary widely, from a rise of 1°C . per minute to three times that rate, and the results were essentially the same in all cases.

Some experiments were performed to try the effect of keeping the proteid at a temperature some degrees below coagulation point for prolonged periods, as there has been some difference of opinion in regard to the influence of the rate of heating upon the coagulation temperature of proteids.

Oxalate and citrate plasmas kept for twenty hours in a warm chamber at 40°C . were found to coagulate at the same point as before, and sodium sulphate plasma kept 24—48 hours at 40°C . showed no appreciable difference.

NOTE.—Horse's blood and ox blood show remarkable differences in regard to the rate of subsidence of the corpuscles in decalcified blood and in sodium sulphate blood respectively. In horse's blood the corpuscles sink rapidly in decalcified blood, and a large amount of clear plasma is soon obtained ; in the salted blood the change is much slower, and much less clear plasma is got. In ox-blood the converse holds good. Measurement of the depth of the layer of clear plasma in each case gave the following relative values :—

Horse's blood after standing 1 hour :	Oxalate blood,	65
" " "	Citrate "	44
" " "	Sod. sulph. "	0
" " 2 hours :	Oxalate blood,	69
" " "	Citrate "	61
" " "	Sod. sulph. "	10

Ox blood, after standing 1—6 hours : no plasma in decalcified or salted samples. After standing 24 hours, the depths of plasma in oxalate, citrate, and sod. sulph. blood were in the relation of 2, 4, and 10 ; after standing 72 hours the relations were as 7, 10, and 22.

Plasma from horse's and ox blood decalcified by addition of 12—15 per cent. sodium oxalate was found, when heated without acidulation, to give heat coagulation of fibrinogen at 49.5° — 50.5°C . with great constancy. After this coagulum had been filtered off there was no evidence of the presence of any fibrinogen remaining in the plasma ; no further coagulation at 56° — 60° , no ppt. on half-saturation with NaCl ,

no attempt at coagulation on adding calcium salts, etc. No notable difference was seen in the behaviour of plasma faintly acidulated with dilute acetic acid before decalcification. Sodium sulphate plasma from the same blood heated alongside of the oxalate plasma showed the usual coagulation at 56°C . Potassium and ammonium oxalates gave results similar to sodium oxalate.

In the foregoing experiments the blood was decalcified by mixing with the oxalate when it was shed. In other cases salted plasma was first obtained, and then it was decalcified by adding oxalate. Essentially similar results were got by both methods.

20 c.c. of sodium sulphate plasma¹ (horse) were mixed with 3 c.c. of 1 per cent. sod. oxalate solution and filtered after some time; then heated alongside of a similar portion of the same plasma, pure or mixed with an equivalent amount of sod. chloride solution instead of sod. oxalate. In the former coagulation occurred at 50.5° , having become opalescent below 50° ; the latter coagulated at 56° , opalescent at 55° .

Sodium sulphate plasma faintly acidified with 2 per cent. acetic acid gave similar results, excepting that the oxalated portion sometimes showed a slightly higher coagulation temperature (51.5° or 52°) than usual; whether the acidification caused a little lime to remain in solution I cannot say. The non-oxalated sample coagulated at 56° .

Is the definite lowering of the heat-coagulation point due to the removal of the lime salts from solution in the plasma or to the presence of a certain excess of soluble oxalate? If the latter were the case, the presence of larger amounts of oxalate would probably increase the effect. This was tried by adding double and treble amounts of oxalate to the blood when shed and in other experiments by adding similar amounts to the ordinary oxalate plasma. All the samples were then heated in a water-bath along with one of the ordinary oxalate plasma (0.15 per cent.) and no appreciable difference was present in the time and manner of coagulation in the different tubes; the coagulation point was at 49.5° — 50.5°C ., while sodium sulphate plasma from the same blood coagulated as usual at 56° .

1. Obtained from blood mixed with half its volume of saturated sod. sulphate solution.

RESTORATION OF SOLUBLE CALCIUM SALT

To test the effect of this, calcium chloride was carefully added to oxalate plasma till any excess of soluble oxalate present was used up and calcium was present in solution. But as such additions would readily lead to ferment coagulation the oxalate plasma was first mixed with sodium sulphate in sufficient amount to prevent spontaneous coagulation. It was found that restoration of lime in solution caused the coagulation point of fibrinogen to rise to the normal level. The addition of the sodium sulphate did not seem to have any disturbing effect, as shown by control experiments in which sodium sulphate was added without the calcium salt; coagulation occurred as usual about 50°C .

ADDITION OF MORE CALCIUM SALT TO PLASMA

The effect of adding more calcium salt to plasma was tried with sodium sulphate and sodium chloride plasmas, the latter obtained from blood mixed with an equal volume of 15 per cent or 20 per cent NaCl solution. There was some evidence of a slight raising of the coagulation above the usual point—at least with certain amounts of calcium salt. Thus when two drops¹ of 10 per cent CaCl_2 solution were added to 10 c.c. sod. sulph. plasma, coagulation occurred at 57.5°C .; after the addition of 10 drops, at 59° . Sodium chloride plasma (containing 10 per cent NaCl) after the addition of 2 and of 10 drops showed somewhat similar results; the coagulum does not separate so readily with the higher proportion of CaCl_2 and it is much less coarsely flocculent than usual. This plasma without addition of CaCl_2 coagulated about 56° .

When relatively large quantities of CaCl_2 were used (*e.g.*, an equal volume or half-volume of 10 per cent. CaCl_2 solution, there appeared to be a lowering of the coagulation point—to 52° - 53° , etc.

Sodium fluoride plasma (from blood mixed with half its volume of 1 per cent. sod. fluoride solution) gave results similar to the preceding. Thus a sample from ox-blood gave coagulation at 49°C ., while

1. 14 drops=1 c.c.

sodium sulphate plasma from the same blood did not coagulate till the temperature rose to 56° (opalescent at 54° — 55°).

Sodium and potassium citrates gave similar results ; coagulation about 50° . The blood was mixed with half its volume or an equal volume of 1 per cent. citrate solution. Doubling or trebling the amount of citrate did not make any appreciable difference.

A combination of decalcifying agents was tried in some experiments ; citrate and fluoride were added to oxalate plasma. Coagulation occurred as before about 50° C.

In some experiments soap was used as a decalcifying agent, but the heat-coagulation was not satisfactorily determined ; there seemed to be a marked tendency for the fibrinogen to become changed into alkali-albumen.

PRECIPITATION OF FIBRINOGEN BY NaCl IN DECALCIFIED PLASMAS

In oxalate plasma it was found that fibrinogen becomes precipitated by a percentage of NaCl much below the usual. Even 5 per cent. NaCl is sufficient to cause precipitation. In plasma mixed with an equal volume of 10 per cent. NaCl or correspondingly smaller amounts of stronger solutions a very considerable amount of stringy or membranous precipitate, with the ordinary microscopical characters of fibrinogen, separates out, though a good deal still remains in solution. Higher percentages of NaCl (7 per cent. and 10 per cent.) give more abundant precipitates. Citrate and fluoride plasmas were tested with similar results ; in all a very large number of plasmas from both horse's and ox blood were examined. Smaller percentages of NaCl fail to cause the precipitation ; less than about 5 per cent. is usually ineffective.

Such results present a marked contrast to what is seen in ordinary sodium chloride plasmas, apart from decalcification. Such plasmas containing 7 per cent. to 10 per cent. NaCl retain fibrinogen in solution for long periods, and are capable of ferment-coagulation after weeks or months.

Sodium chloride plasmas were decalcified by the addition of $\frac{1}{8}$ vol. 1 per cent. sod. oxalate solution, $\frac{1}{2}$ — $\frac{1}{4}$ vol. 1 per cent. sod. fluoride, a similar amount 1 per cent. sod. citrate, or sod. citrate crystals. With oxalate and fluoride a precipitate speedily appears, and separates out in an hour or two. With the citrate the separation is much slower, and there is often no visible change for hours. This fact was taken advantage of to compare the heat-coagulation of the plasma before and after the addition of citrate; a lowering from 56°C . to 51° — 52° was observed.

In the case of all the decalcifying agents a well-marked precipitate was found next day usually in the form of a very coherent shred or membrane lining the bottom of the test-tube. Stained microscopical preparations showed the usual characters of fibrinogen mixed with numerous particles of calcium oxalate, etc.

LEECH EXTRACT PLASMA

When leech extract or 'Hirudin' was used instead of oxalate, etc., to prevent coagulation, there was no lowering of the coagulation point of fibrinogen. Addition of 'Hirudin' to sodium sulphate or sodium chloride plasma—in amounts sufficient to prevent coagulation after dilution—showed no lowering when the plasma was heated either in the diluted or the undiluted state.

HYDROCELE FLUID

Decalcification caused heat-coagulation to occur at about 50° – 51°C ., as compared with 56° in the non-decalcified fluid. Some samples of strongly alkaline hydrocele fluid, poor in fibrinogen, were examined. When heated in the pure state these became opalescent at 56° – 60° , but no visible separation of floccules took place. Similar samples treated with $\frac{1}{10}$ vol. 1% sod. oxalate solution (without acidulation) coagulated at about 51° , numerous stringy flakes separating out. Similar results were obtained after faint acidulation. Addition to the non-oxalated fluid of amounts of sodium chloride equivalent to the

1. Supplied by Sachse & Co., Leipzig.

sodium oxalate used made no difference in coagulation point as compared with the pure fluid.

SERUM-PROTEIDS

Samples of serum were treated with sodium oxalate and allowed to stand for some hours, then heated along with samples of the same serum, pure or mixed with equivalent amounts of sodium chloride. No definite difference could be recognized in the behaviour of the various samples. They all showed opalescence at about the same, and coagulation at the same temperatures.

MUSCLE-PROTEIDS

Saline extracts of muscle were decalcified by oxalate, etc., and tested in the same way as plasma.

·75 per cent. NaCl extract of ox muscle ; 20 c.c. mixed with 4 c.c. sodium oxalate solution (1 per cent.) ; another portion mixed with an equivalent amount of NaCl solution. Abundant coagulation occurred in both tubes at 47° ; the coagulated flakes were much coarser, and perhaps separated more readily in the non-oxalated tube. Filtered and again heated. Coagulation in both at 56° , with the same apparent differences in detail.

5 per cent. MgSO_4 extract of ox muscle treated as above. Coagulation in both between 47° and 50° ; floccules in the oxalated one were not so coarse and did not separate so readily in the oxalated sample. Second coagulation at 55° — 60° in both.

5 per cent. Na_2SO_4 extract of the same muscle gave similar results.

The relations of lime in solution towards the heat-coagulation of fibrinogen seem to be quite different from what has been so far observed in the case of other proteids and in notable contrast to the nature of the effects studied by Ringer and Sainsbury on serum proteids and by Ringer on alkali-albumin. In the latter instances, soluble calcium salt was found to have a remarkable influence in promoting the heat-coagulation of the proteids, lowering the coagulation

point, etc. With fibrinogen on the other hand the influence of lime is in the opposite direction; the removal (or virtual removal) of soluble lime favours coagulation, causing a very decided lowering of the heat-coagulation point.

The foregoing inquiry was carried out on Professor MacWilliam's suggestion.

SUMMARY

1. Decalcification of blood, plasma, hydrocele fluid, etc., by oxalate, citrate, or fluoride, causes a lowering of the heat-coagulation point of fibrinogen from 56° to about 50° . Doubling or trebling the amount of decalcifying agent added makes no appreciable difference.

2. Restoration of the lime is followed by a rise of the coagulation point to about the normal level.

3. Addition of excess of soluble lime salt elevates the coagulation point somewhat.

4. Decalcification changes the fibrinogen in another way; it is precipitated by an unusually low percentage of NaCl—beginning with 5 per cent.

5. No similar lowering of coagulation point was seen in solutions of muscle-proteids, egg-albumin, and serum-proteids. The effect (of rendering the lime insoluble) on fibrinogen is, as far as is known, a special one—in contrast with what Ringer and Sainsbury found in the case of serum-proteids and alkali-albumin where the addition of soluble lime salts favours heat-coagulation, lowering the coagulation point, etc.,

6. In the case of some other proteids (muscle-proteids, egg-albumin) no special effect upon the temperature of coagulation seems to be produced by the presence or absence in solution (as tested by decalcifying agents such as oxalates, etc.) of small amounts of lime salt that are normally present.

7. In regard to fibrinogen the relation of calcium salts to heat-coagulation is very different from their relation to ferment-coagulation.