NOTE ON THE ACTION OF SODIUM CHLORIDE IN DISSOLVING FIBRIN.

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THE question of the solution of fibrin after it has once been formed has been for a long time a subject of controversy. Denis drew a distinction between that which is formed from arterial and that from venous blood, saying that while the former was insoluble in 10% solution of NaCl, that from venous blood was soluble[1]. Further controversy has taken place as to the nature of the proteid thus said to be in solution after the action of the salt, some holding that a transformation takes place during the process whereby the fibrin is changed into a member of the globulin family, and others that the reactions which indicate such a change are dependent on the salt present, and that the fibrin when quite free from the latter manifests its own properties and shows itself to be closely related to the albumins, if not to be an albumin itself. It has been further argued that the changes observable do not take place until putrefaction sets in, and that they are consequently due to the latter cause.

In 1874 Gautier published a paper in which the second of the above views of the nature of the soluble proteid was strongly maintained[2]. He says that the solution in 10% NaCl shows characters which point to fibrin being intermediate between albumin and casein; like the former it coagulates on heating and on the addition of mineral acids, and like the latter is precipitated by $MgSO_4$ and by weak acetic acid. He further says that the reactions which apparently show a difference between it and albumin disappear with the salt. He dialysed it, he says, till it was free from salt, avoiding putrefaction by the presence of HCN, and then evaporated it in vacuo at 45°C., getting a

[1] Gamgee, *Physiological Chemistry*, p. 36.

[2] *Comptes Rend.* 1874, vol. II. p. 227.

neutral solution which coagulated on heating to 61°C., and on addition of mineral acids gave a precipitate with corrosive sublimate, but not with weak acetic acid nor with $CaSO_4$ and other salts.

Wurtz says that on putrefaction in the open air fibrin yields a considerable quantity of albumin.

Hoppe Seyler[1] supports the view that fibrin is changed into a globulin body, having the characters of paraglobulin. The reactions he gives for it are

1. It is precipitated by saturation with solid NaCl.
2. It is precipitated on dilution with water.
3. It does not coagulate under 60°C.
4. It does not form fibrin again when mixed with defibrinated blood.

He also argues that the changes are due to putrefaction.

The questions involved in this controversy are mainly three. 1st, Is fibrin dissolved by neutral salt solutions? 2nd, Is putrefaction necessary for the process? 3rd, Can fibrin as such be recovered from the solution or is it replaced by other bodies. Putting this into slightly different forms it becomes, Is fibrin *dissolved* or is it not rather *decomposed* by solutions of neutral salts? A fourth point closely connected with this third one, if not included in it is, What bodies replace the fibrin if the process is one of decomposition?

To answer these questions I carried out at the suggestion of Dr Sheridan Lea a series of experiments, the results of which I subjoin.

1. *Is fibrin dissolved by salt solutions?*

Some quantity of fibrin was collected by whipping a quantity of bullock's and sheep's blood in contact with air. It was always of bright scarlet colour on separation, and hence may be said to be from arterial blood. It was carefully washed in several changes of water, till it was quite free from haemoglobin and till the water gave no proteid reactions. Then it was chopped fine and put into sufficient 10% solution of NaCl to cover it. After standing under this for 24 hours it was removed, and strained through a cloth. The resulting fluid was markedly opalescent, and would not become clear on filtration. On boiling it there was a marked coagulation, and the liquid gave a very

[1] *Phys. Chem.* p. 417.

pronounced Xanthoproteic reaction. Evidently a quantity of some proteid had gone into solution. The fibrin was freed from moisture by squeezing in a press, chopped again, and again covered with the salt solution. This process was repeated every day or every two days, with always the like result, the fibrin becoming less and less with each successive extraction. Gradually disappearing thus it became more and more of a slimy consistency as the quantity lessened, always being quite firm and leathery again after the pressing. Relatively the quantity passing into solution became greater as the end of the experiment approached, till after the 29th extraction it might all have been said to have been dissolved, for nothing that could be pressed was left. It was only then a very thick opalescent solution.

I made a series of experiments to determine roughly what was the rate of the solution but the results are only approximate, as I could not be sure that I extracted the moisture by pressing the fibrin to the same extent after each weighing.

Beginning with 33 gms. of pressed fibrin, after the first extraction there was a loss of 2·06 gms.; after the second a loss of 1·9 gms., and after each of three subsequent ones an average loss of 1·7 gms. The process therefore seemed to be gradual and regular. The time needed to complete the disappearance was 32—35 days.

Other strengths of solution of NaCl also caused the solution of the fibrin; 5—8 % worked equally well, and nearly as rapidly as the 10 %. A solution containing as little as ·6 % of NaCl worked a little more slowly.

A saturated solution of calcium sulphate behaved similarly.

2. *Is putrefaction necessary for the solution?*

The experiments recorded above were carried out in winter at a temperature a little above freezing point, and no antiseptics were used. It was found that with a salt solution so strong as 10 % there was no danger of putrefaction at the low temperature. No bacteria appeared in any such solution during the course of the experiment. On the other hand it was needful to use antiseptics with the weak salt solution and with the solution of $CaSO_4$.

In the experiments already quoted the results were arrived at in perfect freedom from all putrefaction; as were those which will be detailed in connection with the third question suggested.

3. *Is the fibrin dissolved or decomposed by the salt solution?*

According to Gautier, the fibrin passes into simple solution, and after dialysis can be obtained pure, in which condition it gives the reactions of an albumin. This seems *a priori* improbable, as fibrin has an antecedent which is undoubtedly globulin in nature, viz. fibrinogen. According to Hoppe Seyler on the other hand it has been transformed into a globulin in dissolving. The point is not difficult of determination, for albumins differ conspicuously from globulins in being soluble in distilled water, while the latter require some neutral salt in the solution before they will dissolve. Some of the extract of fibrin in 10 % NaCl was submitted to long-continued dialysis, so long as a trace of salt could be discovered in the dialysate by the aid of $AgNO_3$. The process was a long one, for the last traces of salt were extremely hard to get rid of.

As the dialysis progressed, more and more solid matter was precipitated in the dialyser and the coagulum formed on boiling became less and less. After about 10 days' dialysing, the dialysate, which had been changed every day, sometimes twice a day, gave no reaction with $AgNO_3$, and then no proteid could be detected in the filtrate of the contents of the dialyser. This result is in direct opposition to Gautier's statement that after getting rid of the salt he had a body in solution which he separated out and found to be an albumin. I cannot after several experiments confirm his results, for my solutions always deposited their proteid contents *pari passu* with the abstraction of the salt. As long as the filtrate from the dialyser gave even an opalescence on boiling, I found that a trace of salt could be seen on addition of $AgNO_3$.

The next experiments made bore upon a further difference between albumins and globulins, the latter being precipitated completely from their solutions by $MgSO_4$. I found that the solution was freed from proteid matter by this process just as by dialysis. Saturation with NaCl was not so effectual, a good deal of precipitate falling, but some proteid was left behind, for there was always a coagulum on boiling the filtrate from the excess of salt.

The body, whatever it is, evidently then belongs to the globulins and not to the albumins. The question still remains is it fibrin in solution or has it properties which fibrin does not possess?

All the precipitate obtained by dialysis was soluble in a solution of 10 % NaCl just as the fibrin itself was originally. It dissolved however very readily instead of with the extreme slowness that marked the original

process of solution. On treating this precipitate with 1 % NaCl instead of 10 % a difference was evident. A considerable quantity, probably about four-fifths, dissolved very quickly, but the rest remained in suspension and settled to the bottom of the beaker. Addition of further quantity of the 1 % salt solution did not take it up. It was therefore filtered off and put into fresh 1 % salt solution and left standing in this for 18 hours. It was then filtered again and the filtrate was found to contain no proteid matter whatever. This residue was consequently insoluble in 1 % salt solution. It readily dissolved in 10 %.

Evidently therefore the fibrin was by the action of 10 % salt solution decomposed with the formation of two other bodies, both globulins in nature.

A confirmatory experiment was made in a different way. Some of the extract of fibrin in 10 % salt solution was taken and diluted till the solution was only 1 % strong. On standing a little while a flaky precipitate slowly separated out.

The solutions of the two globulins were then examined separately. The first one, which was soluble in both 1 % and 10 % NaCl, gave the following reactions:

It coagulated on heating at 56° C.

It was precipitated in presence of K_4FeCy_6 by a single drop of acetic acid.

It was readily converted into syntonin and into alkali-albumin.

It was not precipitated by weak acid.

The second one, which was soluble only in 10 % NaCl, was marked by the following reactions:

It coagulated on heating at 59°—60°C.

It was precipitated by weak HCl (a few drops of ·4 %).

It was precipitated by K_4FeCy_6 and acetic acid only when the solution became fairly acid.

It was readily converted into alkali albumin but not into syntonin. The acid added for the latter purpose precipitated it, and in suspension it was not acted on.

In some of its reactions the former body recalls the behaviour of fibrinogen.

The reactions which Hoppe Seyler gives of the body he found produced by putrefactive changes suggest that he suspected his body might be fibrinogen also. Neither of the two however will yield a clot on being treated with fibrin ferment, either alone or with defibrinated blood. Nor does either agree with fibrinogen in its behaviour with

sodium chloride, the latter being precipitated by 13—16%, and afterwards, on adding more salt, being redissolved, coming down finally on saturation. Addition of salt (NaCl) to 16% to the extract or solution of fibrin gave no precipitate. On gradually increasing the amount, the precipitate began to appear and more and more came down between about 23% and saturation. Hoppe Seyler says his body had the characteristics rather of paraglobulin. The latter behaves just as described with salt, but the resemblance fails when the coagulating point is taken into account, paraglobulin coagulating at about 75°C. In the extract of the fibrin nothing remained in solution at a temperature above 60°—65°C.

The outcome of the experiments then may be thus stated:—

Fibrin on being acted on by solutions of neutral salts of 5—10% strength is decomposed with formation of two fibro-globulins, which differ from each other as to their coagulating points, their solubility in 1% salt solution, and their behaviour with acids. Neither body corresponds to either fibrinogen or paraglobulin, and they cannot be made to re-form fibrin. The change is brought about quite apart from putrefactive influences.