

Further observations on the coagulation of the blood / by L.C. Wooldridge.

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FURTHER OBSERVATIONS ON THE COAGULATION
OF THE BLOOD. BY L. C. WOOLDRIDGE, D.Sc., M.B.,
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(From the Leipzig Laboratory.)

IN a previous communication on the relation of the white blood corpuscles to the coagulation of the blood¹ I pointed out that there are essentially two processes to be considered.

The blood plasma after it has left the vessels exerts an active destructive influence on the white cells whereby the latter are themselves converted into fibrin, but at the same time a certain substance (or substances), which I then, in accordance with the usual doctrine, called fibrin ferment is liberated from the cells.

This substance (or these substances) is able to bring about the coagulation of the fibrinogen in the plasma.

Previous to my communication the active destructive power of the plasma had been entirely overlooked. Writers on the subject always speak of a breaking up (*Zerfaß*) or death (*Absterben*); there is never the slightest hint that the plasma plays an active part in the matter.

Rauschenbach² under the direction of Prof. Alexander Schmidt has repeated and extended my experiments, using cooled plasma instead of peptone plasma. He comes to exactly the same conclusion as I do with regard to the active destructive power of the plasma, except that he does not make the distinction which I do between plasma in the vessels and plasma which has left the vessels. He with, I think, perfect right makes much of this action of the plasma (*Spaltende Wirkung*), but he does not seem to be fully convinced that I had discovered the fact long previous to his communication.

In extending my own researches I first directed my attention to the body or bodies which are separated from the white cells and which

¹ *Proc. Roy. Soc.*, Vol. xxxii., p. 413. 1881.

² *Blut Plasma und Protoplasma*, Inaugural Dissertation, Dorpat.

induce coagulation of the fibrinogen of the plasma; and in doing so I have come upon a fact which is of the very highest importance not only for the question of the coagulation of the blood but for the very much greater question of the nature of the chemical processes in protoplasm which constitute life.

This fact is that lecithin, a body omnipresent in protoplasm, can bring about coagulation.

I describe now the experiments on which this statement is based.

The experiments were performed on dog's blood which had been prevented from coagulating by injection of peptone.

For the satisfactory carrying out of the experiments in question the peptonization must be very complete, the plasma from the blood must be centrifuged until absolutely no further sediment is obtained. Such a plasma is not coagulated by passing through it a stream of carbonic acid, no matter how long or how frequently this may be repeated, nor is it coagulated by adding other acids, e.g. acetic, till a slightly acid reaction is present.

But although a stream of carbonic acid or the addition of another acid does not induce coagulation it does bring about a certain change in the plasma, as will be apparent from the following experiment.

Exp. Peptone plasma uncoagulable with Co_2 . To 1 part plasma, 1 part normal serum is added. After 24 hours a scarcely perceptible clot; no further increase on standing 24 hours longer.

To one part of same plasma, but one through which a stream of Co_2 had been passed, 1 part normal serum is added. In 10 minutes complete coagulation has occurred so that the vessel can be inverted without anything falling out.

We see therefore that a plasma practically incoagulable with serum is rendered easily coagulable after a stream of carbonic acid is passed through it. The plasma in question is totally uncoagulable with fibrin ferment, but it becomes readily coagulable with fibrin ferment after a stream of carbonic acid has been passed through it. It is not necessary to use carbonic acid. Neutralization with acetic acid acts just in the same way.

Plainly therefore in peptone plasma either fibrinogen is not present as such (it is present, for it can be obtained by the salt method), or there is something present which prevents its coagulation. The acidification does away with these obstacles, and when the necessary additions have been made coagulation occurs. To this point I will return later.

I will call to mind that I am speaking of a plasma which is not coagulable with acids.

If to such a plasma lymph cells obtained in the method previously described by me be added coagulation occurs. If sufficient lymph cells be added the coagulable substance in the plasma disappears, that is to say you get a serum. This serum will bring about coagulation in a further portion of plasma.

Now the usual doctrine about cells is that they give out ferment and paraglobulin.

But it is quite evident they must do more than this, for, as we have seen above, normal serum which contains both produces by itself alone a very faint coagulation. But it always does induce a certain amount of coagulation, which is as it ought to be, since normal serum of course contains the products of the disintegration of white cells although to a much less extent than the plasma I have been talking about.

Both the cells and the serum from the coagulation brought about by cells act with very great rapidity and completeness. They must therefore give out something which exerts a similar influence to that exerted by the passage of a stream of carbonic. What this influence is I do not at present know, but I have learnt something as to what is the body or what are bodies which the cells give out.

For I find that the *alcoholic extract* of the cells acts just as well as the cells themselves. Now the alcoholic extract has invariably an acid reaction, and as will be remembered we are now dealing with a plasma not coagulated with acids but rendered coagulable by acids. The acid of the extract plays a part, but it is not sufficient for coagulation; the other substance in the alcohol extract is necessary, and this other substance is lecithin.

The alcoholic extract is prepared as follows :

The lymph cells are extracted with hot alcohol, this is filtered off and allowed to cool. A precipitate occurs on cooling and this is again filtered; the filtrate is evaporated to dryness. The residue is treated with a little cold absolute alcohol; a very large portion is left undissolved. After filtration the clear alcoholic solution is evaporated to dryness. It is then dissolved in cold absolute ether and this is filtered and evaporated. The ethereal solution has an acid reaction. One portion of this extract is used for coagulation experiments. The other is used for analytical purposes.

The coagulation experiments are made in the following manner :

A portion of the extract is rubbed up to a paste or thick emulsion

with a drop or two of dilute sodium carbonate. This neutralizes the acid present. On diffusing this emulsion through a portion of plasma no coagulation results. But when I pass through this plasma a current of Co_2 complete coagulation occurs in from 10 to 20 minutes.

The plasma without this emulsion is totally uncoagulable with Co_2 .

Now as to the chemical nature of this extract which can bring about coagulation, the residue from the ether solution is not crystalline but forms a yellowish waxy mass. In water it is not soluble, but it swells up and if examined under the microscope the formation of the peculiar myelin drops, characteristic of lecithin, is observed with great distinctness. If a portion be incinerated with sodic carbonate and saltpetre it leaves an ash very rich in phosphoric acid. If it be dissolved in a little alcohol and to this be added an alcoholic solution of platinum chloride a voluminous yellowish white precipitate is caused. This precipitate is not distinctly crystalline and is very easily soluble in chloroform. It contains platinum, chlorine and phosphorus. The filtrate from the platinum chloride precipitate when freed from superfluous platinum by a stream of H_2S and evaporated to dryness, leaves a comparatively very small residue which melts on the water bath. This is easily soluble in ether and alcohol, the solutions having an acid reaction. It is not soluble in water, but is soluble in dilute alkalies. If the alkaline solution be acidified and heated oily drops appear on the surface. If the substance be treated with concentrated caustic soda, a jelly like mass is the result. In fact the residue in question consists of fatty acids.

Now if this residue be treated with a little carbonate of soda and the influence of the resulting soap on coagulation be tested in the manner above described, it is found that it is absolutely without any influence.

The alcohol ether extract which brings about coagulation in the manner above described consists then chiefly of lecithin; besides the lecithin there is a small quantity of fatty acids. When the lecithin has been removed, the extract has lost its power of bringing about coagulation.

A peptone plasma which does not coagulate with Co_2 is a plasma in which there are no white cells and no products of the breaking up of white cells; and we have seen that the addition of lecithin, which is abundantly present in the cells, renders the plasma coagulable under the above-mentioned circumstances.

I will only remark, although it is scarcely necessary for me to do so, that the alcohol ether extract certainly does not contain any fibrin

ferment. Heating it to 100° C. in water does not destroy in any way its activity.

The ordinary Schmidt's ferment is free from lecithin.

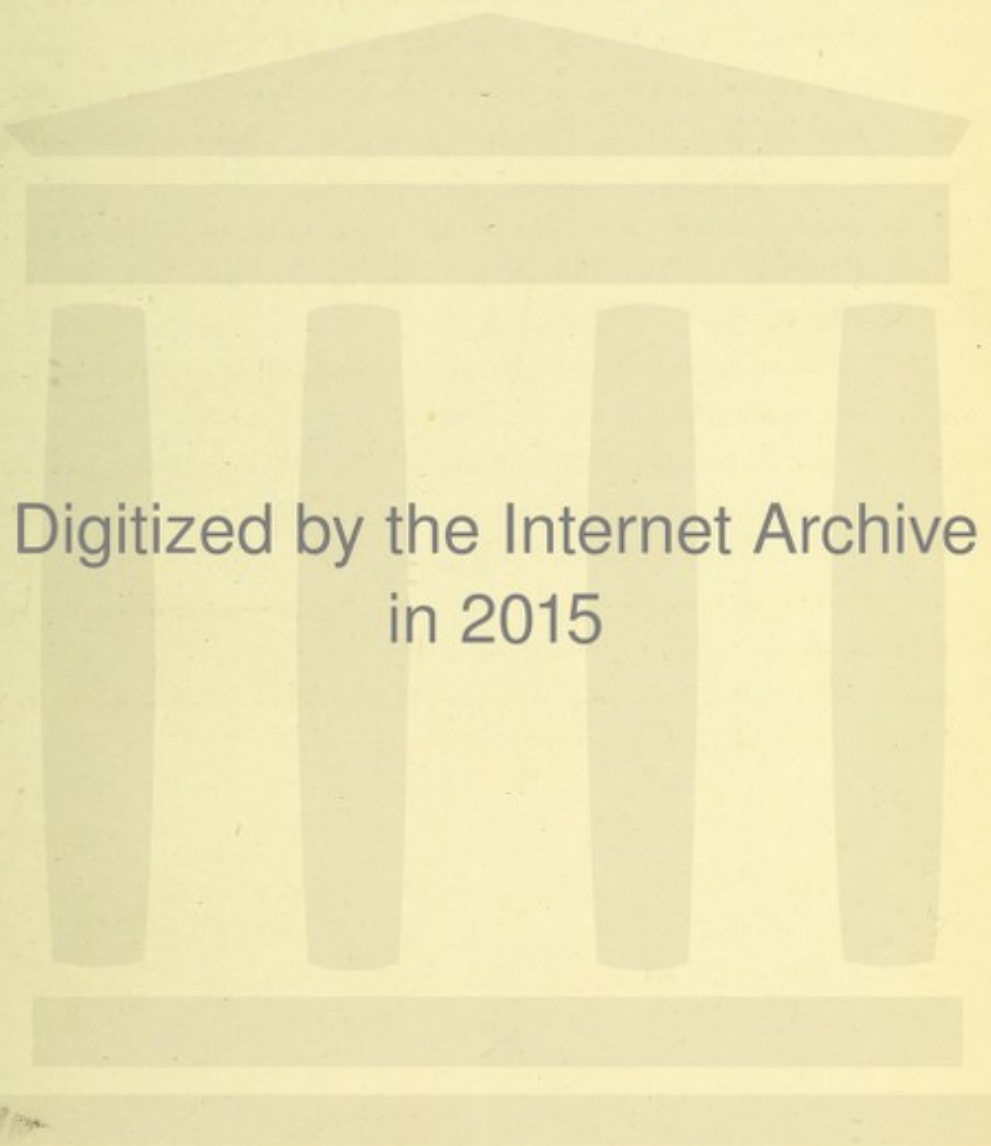
I will call to mind certain well-known facts in coagulation. The transfusion of serum or defibrinated blood is as a general rule not followed by thrombosis. Yet a solution of fibrinogen coagulates at the temperature of the body with great rapidity. This has always been a point of great difficulty in connection with the coagulation doctrine. The peptone plasma behaves, in this respect, just like the plasma in the vessels. The peptone plasma behaves towards ferment just as if it contained no fibrinogen. After a current of Co_2 has been passed through it, it behaves just like a solution of fibrinogen.

It is on evidence of this nature that we talk of zymogen in gland cells. And I think it is almost admissible to talk about a mother substance of fibrinogen in the blood.

The able researches of Hammarsten have shewn that one albuminous body is sufficient for fibrin formation.

But Hammarsten also finds that when a fibrinogen has been dissolved and reprecipitated a great number of times *special* ferments are necessary for fibrin formation; and it is a very suggestive circumstance that these special ferments lose their activity after standing some little time under alcohol. (Removal of lecithin.)

I need hardly add that the above brief and condensed statement is intended only as a preliminary communication.



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