On the origin of the fibrin ferment / by L.C. Wooldridge ; communicated by M. Foster.

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"On the Origin of the Fibrin Ferment." By L. C. WOOLDRIDGE, M.B., D.Sc., George Henry Lewes Student. Communicated by Professor M. FOSTER, Sec. R.S. Received February 26, 1884.

The "fibrin ferment" which makes its appearance in shed blood is generally, I believe, supposed to arise from the cellular elements of blood, either from ordinary white corpuscles or from some special kind of corpuscles, the cells so concerned discharging the ferment into the blood or setting it free by their actual disintegration. Without wishing to deny that this may be one source of fibrin ferment, I am able, I think, to bring forward evidence that ferment may make its appearance in blood-plasma perfectly free from cellular, and indeed from all formed elements, in which case it must arise from some constituents of the plasma itself, and not from cells of any kind.

It will be most convenient, perhaps, if I state the facts which I have to bring forward in connexion with two series of experiments.

I. A measured quantity of blood was received directly from the carotid of a dog into a vessel containing an equal bulk of a 10 per cent. solution of common salt, great care being taken that the complete admixture of the blood and salt solution was effected as rapidly as possible. By the help of the centrifugal machine plasma was separated from this "salted blood," and this plasma was again subjected to the action of the machine until all traces of formed elements were removed. As is well known, a portion of such a plasma diluted with five times its bulk of water coagulates rapidly, whereas the undiluted plasma remains liquid for an almost indefinite time.

According to commonly received opinions, such a "salted plasma" contains all the fibrin factors, including the ferment, the latter having already passed out of the cells into the plasma; and the reason given of the absence of coagulation in such a salted plasma and its occurrence upon dilution is, that the presence of the salts presents a hindrance to the action of the fibrin ferment, and that this obstructive influence of the salt is removed by the dilution of the mass.

No one, however, as far as I know, has taken the trouble to ascertain whether fibrin ferment is present in such salted plasma. And, as a matter of fact, it is not; whereas it does make its appearance as soon as dilution with water has taken place, as the following experiment shows:—

A portion of the undiluted salted plasma was treated with absolute alcohol in large excess, and the precipitate after being allowed to remain under the alcohol for three or four weeks was dried at a low temperature and extracted with water—that is to say, the plasma was treated in the way usually adopted for obtaining a solution of ferment fairly free from proteids, &c. A portion of the diluted plasma, or rather of the serum resulting from the coagulation of the diluted plasma, was treated in an exactly similar manner.

The aqueous extract of the diluted plasma brought about coagulation in specimens of magnesium sulphate plasma (such as is usually employed for testing the presence of fibrin ferment) in from ten to fifteen minutes. The aqueous extract of the undiluted plasma brought about no coagulation in specimens of the same magnesium sulphate plasma, even after the lapse of eighteen hours.

The conditions of each experiment were made as exactly alike as possible; and the conclusion seemed inevitable that ferment is present in the diluted and coagulated plasma, but absent from the undiluted plasma.

This conclusion is, moreover, supported by the following experiments:—To a portion of the undiluted plasma above mentioned a small quantity of fibrin ferment was added, in the form of the dried precipitate thrown down by alcohol, *i.e.*, a mixture of coagulated proteids and ferment. Coagulation took place. I have no record of

the exact time elapsing between the addition of ferment and the appearance of the clot, but it was certainly not longer than three or four hours.

II. Of the so-called peptone-plasma (*i.e.*, plasma of the blood of a dog after the injection of peptone into the veins, such blood, as is well known, coagulating with great difficulty), freed from all cellular elements by the centrifugal machine, two portions were taken.

To the one (A) a quantity of *lecithin* was added, the lecithin being rubbed up with the plasma so as to be diffused through it; the other (B) was left untouched.

Through both a stream of carbonic acid was passed, with the result that while A clotted in about ten minutes, B after the lapse of half an hour showed no disposition whatever to coagulate. Both portions were then treated with excess of alcohol for the extraction of fibrin ferment in the usual way. The aqueous extract of A proved to be exceedingly rich in ferment, producing coagulation in magnesium sulphate plasma in about ten minutes. The similarly prepared aqueous solution of B produced no coagulation at all.

Now I have elsewhere,* in discussing the action of lecithin in promoting coagulation, shown that the coagulation which is brought about by the addition of lecithin is not due to the lecithin or to any of its products of decomposition acting after the manner of a ferment, or to its carrying a fibrin ferment with it. In this case, therefore, as in the previous case of "salted" plasma, the ferment appears to be absent *before* coagulation, but to be present *after* coagulation.

I may here call attention to an observation made by Rauschenbach.† This observer found that the addition of yeast to plasma, prevented from coagulating by exposure to cold, brought about coagulation, and at the same time gave rise to the appearance of a large quantity of fibrin ferment. Nevertheless, he completely failed to extract any fibrin ferment from the yeast itself. Now yeast is very rich in lecithin, and it seems highly probable that the coagulation caused by yeast was due to the lecithin contained in it, and hence the appearance of the fibrin ferment after the addition of yeast, and consequent coagulation, is quite parallel to the result of the experiment with lecithin and peptone-plasma recorded above. In both cases the ferment appears to have arisen out of the plasma itself.

It is possible to obtain a coagulation in peptone-plasma without the addition of lecithin. For this purpose large dilution is necessary, followed by the passage of a stream of carbonic acid gas. But in such a case, however, coagulation is not only long in making its appearance, but the fibrin is formed, so to speak, in successive crops. Thus a feeble coagulation first appears, and if the clot so formed be removed, a

* "Journ. of Physiol.," vol. iv, p. 226.

† "Blutplasma u. Protoplasma," Inaug. Diss., Dorpat.

succeeding coagulation is observed some time later, to be followed in turn by a third, and so on. When lecithin, on the other hand, is added, without previous dilution, the clotting is speedy and complete.

If the serum thus resulting from the coagulation of peptone-plasma brought about by large dilution and treatment with carbonic acid, be examined for fibrin ferment in the usual way, it will be found to contain ferment, though much less than could be obtained from a corresponding quantity of the same plasma coagulated rapidly by the addition of lecithin. The relative amount of ferment appearing under different circumstances is illustrated by the following experiment:—

Of three equal portions of the same peptone-plasma, one portion was simply treated with a stream of carbonic acid gas, without any dilution, and did not coagulate; a second was treated with a stream of the same gas after large dilution, and coagulated slowly; to a third lecithin was added, and a stream of carbonic acid passed through it, with the result of producing a rapid and complete coagulation.

All three portions were treated in the same way for the extraction of the fibrin ferment, and the activity of the three aqueous extracts then prepared was tested under exactly the same conditions, with the help of magnesium sulphate plasma.

The first produced no coagulation after the lapse of twenty hours.

The second produced coagulation in four hours.

The third produced coagulation in five minutes.

The amount of ferment seems to be in proportion to the energy of coagulation and the presence of ferment after simple dilution, and the action of carbonic acid gas shows that the ferment appearing after coagulation by the help of lecithin does not come from the lecithin itself.

Thus there is a remarkable coincidence between the occurrence of coagulation itself and the appearance of the fibrin ferment, and that in plasma freed most carefully from all cellular elements.

I believe, therefore, that I am justified in concluding that though fibrin ferment does not pre-exist in normal plasma, it may make its appearance in that plasma in the absence of all cellular elements, and must therefore come from some constituent or constituents of the plasma itself.

I am still engaged in investigations directed to find out what that constituent is, or what those constituents are.

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