Deterioration of AHG (Factor VIII) and Fibrinolysis

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In one of his articles Brinkhous (1) suggested that deterioration of AHG in plasma or blood might be due to fibrinolysis. In view of the importance of blood- and plasma-transfusions to hemophiliacs we decided to study this possible relationship between AHG-deterioration and fibrinolysis.

Methods

For determining fibrinolytic activity we used several methods i.e.
the fibrin-plate method of Astrup (2), with some modifications;
the profibrinolysin-method by Marbet (3);
Loomis' method (4);
and by estimating fibrinogen concentrations according to Parfentjev et al. (5).
For determining AHG activity we used:
one of the methods of Brinkhous (partial thromboplastin time-determination) (6);
the recalcification time of haemophilic plasma before and after addition of the specimen to be investigated;
the method of Bounameaux (7).

Results

The fibrin-plate method of Astrup can be carried out with untreated fibrin plates in which the proenzymes are kept intact and with fibrin plates in which the proenzymes have been inactivated by heating at 80° C for 30 minutes (8). In the first case fibrinolysis can be provoked by adding a small amount of activator e.g. streptokinase to the plate; in the second case activated fibrinolysin must be added to obtain actual fibrinolysis.

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Our first experiments were carried out with fibrin plates, made from bovine fibrinogen. As no fibrinolysis could be demonstrated with normal platelet-rich or platelet-poor human plasma we turned to using human fibrinogen, because species specificity might play a role here. The results were however not improved.

To make unclear results more visible, methyleneblue was added to the fibrinogen before thrombin was added. In this way blue fibrin-plates were obtained from which the blue color could be washed off from the spots where actual fibrinolysis had occurred. The results could be made still more clear by examing the blue plates over a yellow paper.

The methods of Marbet and Loomis are based on the addition of streptokinase to the plasma to be investigated, prior to the addition of thrombin. In this way profibrinolysin (plasminogen) is measured. We tried to use both methods for measuring spontaneous fibrinolysis by omitting the addition of streptokinase, but in this way no fibrinolysis could be demonstrated in normal human plasma samples.

We then turned to the method of Parfentjev for estimation of fibrinogen by turbidity. With this method we hoped to measure a decrease in fibrinogen after keeping the plasma at different temperatures. But spontaneous fibrinolysis could not be demonstrated with this method either.

Our first experiments on the influence of fibrinolysis on the AGH mainly consisted of incubating human plasma at different temperatures (37° C, 20° C, 4° C and — 25° C) and determining AHG and fibrinolytic activity after certain periods of time.

As could be expected and as has been demonstrated already by Brinkhous et al. (9), AHG-activity decreased, invertly proportional to the incubation temperature. Fibrinolytic activity however, as measured by the above mentioned methods, was always zero.

For this reason we tried to find out what would happen with the AHG if real fibrinolytic activity was provoked. For that purpose varying amounts of Varidase*) were added to different plasma specimens before incubation. As was to be expected fibrinolytic activity could then be demonstrated in the treated plasma, even on pre-heated fibrin plates. AHG-determination however, whether carried out according to Brinkhous or by recalcification of haemophilic plasma, proved impossible because fibrinolysis interfered with them in such a way that no good clotting times could be observed.

Because is was not yet clear whether these failures were caused by streptokinase still present in the mixture (and which might activate plasminogen in the haemophilic substrate plasma) or by fibrinolysin, we first tried to remove the streptokinase from the plasma by shaking with Al $(OH)_3$-gel. This did not improve the results, probably because plasmin was not removed and because other clotting factors were eventually removed by the Al $(OH)_3$.

We then tried to separate the AHG from the plasmin by precipitating the AHG with alcohol in the same way that it is done in the fractionation of plasma

*) The Varidase preparations used only contained 5000 U of streptodornase to 150 000 U of streptokinase and were kindly supplied to us by courtesy of Lederle Lab. Div.

proteins according to Cohn. The very small quantities of precipitate obtained were already proof that most of the fibrinogen had been destroyed by the plasmin.

After centrifuging in the cold the precipitate was redissolved in physiological saline to the original plasma volume, after which the AHG was to be determined. Even then these determinations failed because together with the AHG a small amount of plasmin was precipitated. This could be demonstrated on both heated and non-heated fibrin plates. Qualitatively the presence of AHG in the precipitate could be confirmed however.

We then considered the following: a relatively small amount of streptokinase will cause a considerable plasmin-activity and it may hardly be considered possible to provoke a certain specified fibrinolytic activity in plasma by the addition of a certain amount of streptokinase. Fibrinolytic activity depends more on the time interval elapsed after the addition of streptokinase than on the amount of streptokinase added. For this reason we decided to prepare a specimen of human plasmin ourselves and add specified amounts of it to the plasma to be incubated. The preparation of this (impure) plasmin was accomplished according to Marbet's method and the strength of this preparation was determined with Loomis' method.

Finally small amounts of this preparation were added to human plasma before incubation and the influences on the AHG followed:

a) qualitatively by means of the changes in the T.G.T.
b) quantitatively by means of Brinkhous' method.
c) quantitatively by means of Bounameaux' method.

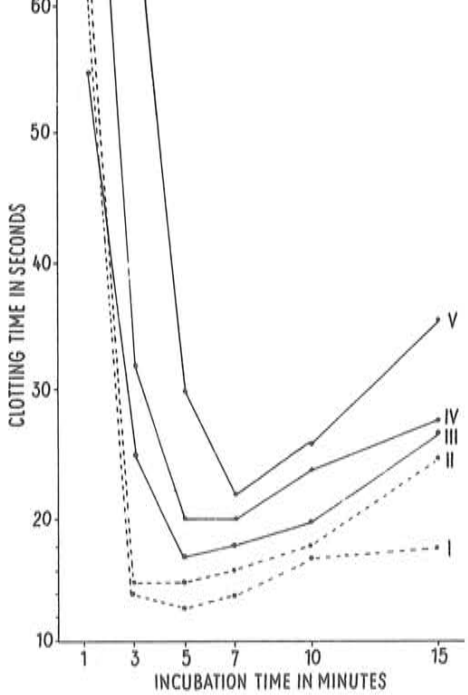
This time the determinations could be performed without difficulty as can be seen from fig. and table below.

Influence of varying amounts of fibrinolysin on AHG- and fibrinogen content of plasma after 24 hours incubation at 37° C.

Fibrinolysin	AHG-content after 24 hours	Fibrinogen content after 24 hours
0. U	70%	90% Parfentjev
0.5 U	1%	—
0.4 U	1%	—
0.25 U	3%	5% Parfentjev
0.125 U	60%	70% Parfentjev
0.0625 U	85%	85% Parfentjev

The solutions of fibrinolysin in the concentrations mentioned in this table, were clearly active in the plate-method of Astrup, except in the lowest concentration (0.0625 U/cc), where this was not convincing.

We investigated with different methods how much fibrinolysis takes a part in the decrease of AHF in stored plasma. There was no conclusive evidence about the role of fibrinolysin in the deterioration of AHF, but the findings were more against than in favour of fibrinolysis.



I. $\frac{N\,N\,S}{N}$

II. $\frac{N'N\,S}{N}$ N' = normal plasma, incubated during 24 hours at room temperature.

III. $\frac{F\,N\,S}{N}$ F = normal plasma + 1/4 Loomis U fibrinolysin per ml, incubated during 24 hours at room temperature.

IV. F'N S F' = normal plasma + 1/2 Loomis U fibrinolysin per ml, incubated during 24 hours at room temperature.

V. $\frac{F''N\,S}{N}$ F'' = normal plasma + 1 Loomis U fibrinolysin per ml, incubated during 24 hours at room temperature.

Summary

The possible relationship between antihemophilic globulin (Factor VIII) and fibrinolysis was investigated. No conclusive evidence about the role of fibrinolysis in the deterioration of AHF was obtained but the findings were more against than in favour of the role of fibrinolysis.

Résumé

Les auteurs ont étudié les relations possibles entre la globuline antihémophilique (AHG, facteur VIII) et la fibrinolyse. L'influence de cette dernière sur la détérioration de l'AHG n'a pas pu être prouvée; l'évidence est même nettement contre une telle éventualité.

Zusammenfassung

Es wurde die Möglichkeit einer Beziehung zwischen Verlust der Aktivität an Faktor VIII und Fibrinolyse bei Lagerung von menschlichem Plasma untersucht. Es konnte kein schlüssiger Beweis für eine Rolle der Fibrinolyse bei der Zerstörung von AHF gefunden werden. Vielmehr sprechen die Ergebnisse mehr gegen als für eine Bedeutung der Fibrinolyse.

References

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