34.

Sonderdruck Reprint Tirage à part

Supplementum 1 ad Vol. VII

THROMBOSIS ET DIATHESIS HAEMORRHAGICA

PROGRESS IN COAGULATION

Transactions of the Conference Held under the Auspices
of the International Committee on Blood Clotting Factors

Wiesbaden, Germany, September 3—6, 1961

I. S. Wright
Chairman

F. Koller
Editor

E. Beck
Associate Editor

FRIEDRICH-KARL SCHATTAUER-VERLAG · STUTTGART

Natural Inhibitor of Tissue Thromboplastin

P. F. Hjort

My presentation concerns only the natural inhibitor of tissue thromboplastin in serum.

The basic observation is simple and was done more than 50 years ago (7): when tissue thromboplastin is added to normal serum, there is a rapid initial enhancement of the clotting activity, which is followed by a progressive loss, not only of the newly formed activity, but of most of the thromboplastic activity as well. This observation has been confirmed many times with in vivo and in vitro assays of the activity [see Hjort (5) for references].

Tissue thromboplastin does not exist as such in serum, but quickly forms successive intermediates. The first of these we have called "convertin" and described as a complex of thromboplastin, calcium and factor VII (5). Straub and Duckert (13) called it "extrinsic reaction product" and showed that factor X is also involved, probably in such a way that a reaction between thromboplastin, factor X and calcium is catalysed by factor VII. Since this reaction is rapid, it is possible that the inhibitor is directed against this or later intermediates rather than against thromboplastin.

When thromboplastin is added to adsorbed serum, there is no initial activation, and, in my hands, also no progressive inactivation. Therefore, adsorption must either remove the inhibitor, or the initial activation is necessary before the thromboplastin can be inactivated. If an eluate is prepared from serum and incubated with thromboplastin and calcium, the intermediate product is formed, and this product itself is stable, and the eluate does *not* contain the inhibitor. However, if the product is added to adsorbed serum, it is inactivated. The simplest interpretation of these observations is that the inhibitor is present in adsorbed serum, but is directed against the intermediate product and not against tissue thromboplastin.

Unfortunately, there is a remarkable disagreement in the literature on this point. Three investigators (3, 4, 11) find the same thing as I do, namely that adsorbed serum has no effect on thromboplastin. On the other hand there are also three investigators (1, 2, 6), who find that adsorbed serum does inactivate thromboplastin. There are only two variables in the incubation mixture: thromboplastin and serum. I have prepared the thromboplastin according to the description of the other investigators, but my adsorbed serum is still inactive. However,

if the serum is adsorbed with decreasing amounts of $BaSO_4$, it is found that large amounts (about 100 mg/ml) are necessary to remove the effect. Therefore, I believe that these discrepancies may be explained by incomplete adsorption.

To test this explanation, I incubated thromboplastin with unabsorbed serum from a patient with congenital deficiency of factor VII. Thromboplastin was nearly stable in this serum. — Serum from patients on anticoagulant treatment has inhibitory effect, but such sera regularly contain some factor VII.

Calcium is necessary for the inactivation. When citrated serum is added to the intermediate product ("convertin"), a slow inactivation follows, probably because citrate breaks up the intermediate (5). This inactivation is much smaller than that in non-citrated serum. This observation indirectly suggests that calcium is necessary, and further evidence is obtained by adding decalcifying agents at the end of the incubation. The inactivation is then rapidly reversed. Therefore, the inhibitor does not destroy the intermediate; it is only blocked by a mechanism which can be reversed by removing calcium. It is seen that only the thromboplastin activity is reestablished, but not that of the intermediate. This is so because decalcification also breaks up the intermediate. The inactivated thromboplastin can also be recovered by acid (8) and alkali (9).

There is a quantitative relationship between the intermediate and the inhibitor. When decreasing amounts of intermediate are incubated with the same amount of serum, a family of curves is obtained. At the end of the incubation there is a direct relation between amounts of intermediate inactivated and the amounts originally present. When decreasing amounts of serum are incubated with the same amount of intermediate, a family of curves is again obtained, and there is a direct relationship, within a certain range, between the amount of serum and the amount of the intermediate which is inactivated.

In centrifugation experiments it is possible to show that the inhibitor in serum can be exhausted: the serum looses its activity when it is exposed to large amounts of thromboplastin. Similar results were obtained by Lanchantin and Ware (6) and by Deutsch and Fuchs (2). When the incubation mixture is centrifuged at high speed, the inactivated thromboplastin is sedimented. When the sediment is treated with decalcifying agents, thromboplastic activity is regained and after a new centrifugation the inhibitor can be demonstrated in the supernatant. This technique was used to isolate the inhibitor by Lanchantin and Ware (6) and by McClaughry (10). These experiments confirm that the inhibitor forms a complex with the intermediate.

The effect of the inhibitor is slower at low temperature.

The serum inhibitor is stable in serum; it is heat labile and is destroyed at 60° C. It is resistant to acids and alkali between pH 4 and 11; it is not dialysable, it is not adsorbed by $BaSO_4$ or by glass powder, and it is precipitated

in the 33—50% fraction by ammonium sulphate. It is probably an alpha-globulin [Scott et al. (12)].

These observations can all be interpreted on the basis of the following hypotheses. When thromboplastin is added to serum, an intermediate product is formed, and this product is stable when it is allowed to form in a mixture of purified reagents. When the intermediate product is added to serum it is inactivated, probably due to complex formation with an inhibitor in the presence of calcium. The formation of this complex is reversible. When thromboplastin is added to serum, both of these processes take place simultaneously, and the activity curve is the resultant of these two processes.

References

(1) Berry, C. G.: The degeneration of brain thromboplastin in the presence of normal serum. J. clin. Path. **10:** 342—345 (1957).
(2) Deutsch, E., H. Fuchs: Natürlich vorkommende Koagulationsinhibitoren. Acta haemat. **20:** 97—114 (1958).
(3) Egli, H., K. Kesseler, R. Klesper: Über die Inaktivierung von Blutthrombokinase. Acta haemat. **17:** 338—354 (1957).
(4) Fiala, S., K. Roth: Platelets and the thromboplastic system of blood coagulation. Arch. int. Physiol. **61:** 205—231 (1953).
(5) Hjort, P. F.: Intermediate reactions in the coagulation of blood with tissue thromboplastin. Scand. J. clin. Lab. Invest. **9** (Suppl. 27): 183 pp. (1957).
(6) Lanchantin, G. F., A. G. Ware: Identification of a thromboplastin inhibitor in serum and plasma. J. clin. Invest. **32:** 381—389 (1953).
(7) Loeb, L.: Weitere Untersuchungen über Blutgerinnung. Beitr. chem. Physiol. Path. **5:** 534—557 (1904).
(8) Mann, F. D., M. Hurn: Inactivation of thromboplastin by serum. Fed. Proc. **8:** 105 (1949).
(9) Marx, R., H. Bayerle: Von der Antithrombokinaseaktivität des Blutes. Biochem. Z. **319:** 9—17 (1948).
(10) McClaughry, R. I.: The specificity of antithromboplastic activity. J. Mich. med. Soc. **49:** 685, 750 (1950).
(11) Schimpf, K., W. Mühlhäusler, R. Teupel: La place de l'antithrombokinase tissulaire dans la coagulation sanguine. Hemostase **1:** 63—68 (1961).
(12) Scott, T. G., C. Symons, R. L. Markham: Antithromboplastin in human serum and plasma. Nature **186:** 248—249 (1960).
(13) Straub, W., F. Duckert: The formation of the extrinsic prothrombin activator. Thromb. Diath. haem. **5:** 402—424 (1961).