OBSERVATIONS ON THE MECHANISM OF FIBRINOLYSIS IN A TWO PHASE SYSTEM

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For some years there have been differences of opinion regarding the relative roles of plasmin and activators of plasminogen in the dissolution of a preformed clot of fibrin. Convincing evidence is available to support the concept that lysis by activation of plasminogen in the gel phase overshadows any action of extrinsic plasmin (Alkjaersig et al., 1959); other studies have been interpreted to justify a participation of plasmin from the fluid phase (Ambrus et al., 1962). The availability of plasminogen-deficient fibrinogen and thrombin, and the development of a convenient analytical technique for kinetic studies of the lysis of fibrin in a reproducible gel-fluid phase arrangement, have made it possible to obtain information which may contribute to an integration of the above views.

Materials and methods. Plasminogen-deficient human fibrinogen and bovine thrombin were obtained as previously described (Maxwell et al., 1962.) Human plasminogen was prepared by chromatography of serum euglobulin on DEAE-cellulose (Wallen and Bergstrom, 1959) to give material with 10-20X the specific activity of the euglobulin. Human urokinase was obtained from Leo Pharmaceutical Products, Copenhagen, and had an activity of 5,300 units/mg. (Ploug and Kjeldgaard, 1957). Crystalline soybean trypsin inhibitor was obtained from Worthington Biochemical Corporation. Fibrinolytic measurements, based on the inherent fluorescence of products in the fluid phase, were carried out as described (Maxwell and Lewandowsk 1962), except that a 1 ml. gel-1 ml. fluid phase system in vials of about 25 mm. diameter was employed. Since the present experiments frequently involved

extensive lysis of the gel phase, fluorescence values had to be corrected for the resulting dilutions of the fluid phase. This correction may be estimated at any degree of lysis from a knowledge of the fluorescence of the totally lysed system. In terms of the arbitrary units reported, this figure was 240.

Results and discussion. Figure 1 is representative of numerous experiments in systems free of inhibitors. The activation of plasminogen in the fluid phase was rapid, and there was minimal lag in the initiation of lysis; similar results were obtained by preincubation of the plasminogen and urokinase. In contrast, lysis by activation of plasminogen in the gel was characterized by a striking lag, but then the rate rapidly overtook and far surpassed that produced by plasmin in the fluid phase. The duration of the lag, as well as the eventual rates in either situation, may be varied at will by manipulations of plasminogen and urokinase concentrations.

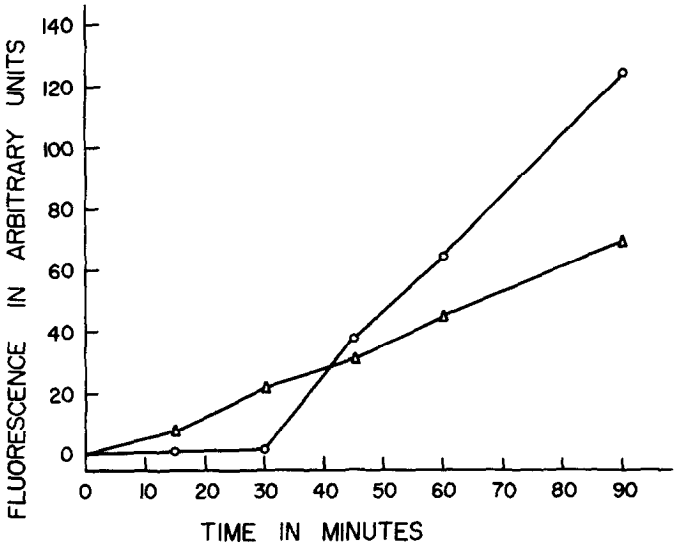

Fig. 1. Fibrinolysis by activation of 10 μg. plasminogen in fluid phase (Δ) or gel phase (0). 100 Units of urokinase in fluid phase.

Addition of an inhibitor to the system resulted in greater divergencies, with additional complications related to the phase location of the inhibitor. Soybean trypsin inhibitor was chosen for initial study because it reacted

immediately with plasmin and had no effect on the activation of plasminogen by urokinase, as established in incidental experiments. As shown in Figure 2, if the inhibitor was in the fluid phase, the lag period for lysis by activation of gel phase plasminogen was markedly prolonged, thus placing fluid phase activation in a more favorable light. However, if both the plasminogen and the inhibitor were in the gel phase, there was no question of the superiority of gel phase activation. By variations of urokinase levels, or of concentrations and phase distributions of plasminogen and inhibitor, the contrasts shown in this figure may be further exaggerated or relatively minimized.

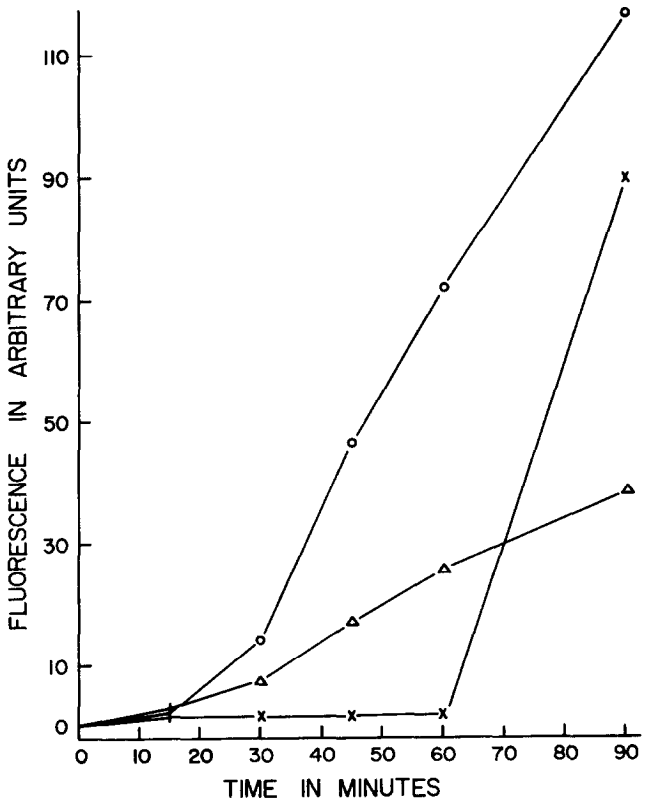

Fig. 2. Effect of 0.25 μg. soybean trypsin inhibitor and phase distributions with 6 μg. plasminogen. O, plasminogen and inhibitor in gel phase; Δ, plasminogen and inhibitor in fluid phase; X, plasminogen in gel phase, inhibitor in fluid phase. 300 Units or urokinase in fluid phase.

Most of the evidence obtained so far is compatible with the concept that lysis is limited to the surface, or perhaps to a very small depth of the gels.

Gross and microscopic examination of lysing gels showed no evidence of discontinuities or separations from the sides of the containers. Since the added plasminogen is homogeneously distributed in the gel, a small fraction of that amount present in the fluid phase must be involved in eventually producing much greater lysis. Observations on autodigestion of fluid phase plasmin, or decrease in rate of its action by dilution or accumulation of end products, indicated that these factors were of minor importance and could not account for the discrepancy. The intimate association of plasminogen with fibrin when in the gel, the possibility that urokinase may be specifically adsorbed on or diffuse beyond the surface, and relative concentrations or rates of reactions in the actual lysing area, are other possibilities for speculation. The unique kinetic and mechanical features of this system, together with the opportunity for controlled variations of the parameters, offer many challenges for further investigation.

From a medical point of view, this system can be no more than a model of unknown degree of artificiality, and may bear only a remote resemblance to the infinitely greater complexities surrounding the lysis of a thrombus in a blood vessel. Nevertheless, the results may afford a unifying perspective with regard to more physiological investigations which, depending on unknown variations in surface area, contact time, and concentrations or phase distributions of plasminogen, activators, and inhibitors, could lead to apparently inconsistent conclusions.

Summary. The relative efficiency of lysis of a preformed clot of fibrin by activation of plasminogen in the gel, as compared to attack by plasmin in the fluid contacting the gel, was shown to be a function of contact time, as well as of concentrations and phase distributions of plasminogen, activator, and inhibitor.

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