Partial purification of an activator inhibitor in serum¹

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The presence of an activator inhibitor in serum has been assumed by several authors (Müllertz, 1953; Nilsson *et al*, 1961; Paraskevas *et al*, 1962; Brakman and Astrup, 1963; Brakman *et al*, 1966; Bennett, 1967; Lauritsen, 1968). Knowledge of the nature of this or these inhibitor(s) of plasminogen activation is, however, still vague, mainly because of the lack of suitable analytical methods. Most of the methods hitherto used do not differentiate between the activator inhibitor and plasmin inhibitor. However, Lauritsen (1968) has described a caseinolytic method in which the effect of antiplasmin is eliminated.

Human normal serum has been used as a starting material for the isolation of the inhibitor of plasminogen activation. During the purification procedure the inhibitor of the plasminogen activation was measured both with the clot lysis method of Paraskevas *et al* (1962) in which urokinase is used as an activator, and with the caseinolytic method of Lauritsen (1968). In addition, we followed the content of α_2 macroglobulin, plasminogen, antiplasmin (a caseinolytic method in which the serum fraction was incubated with plasmin for 120 min), α_2 -antitrypsin, and prothrombin.

As an initial step in the purification process haptoglobin was removed from the serum by specific absorption with haemoglobin, linked to sepharose by using bromcyanide (Fig.).

The next step consisted of ion exchange chromatography on DEAE-Sephadex of the serum deprived of haptoglobin in 0.15 M Tris buffer containing 0.15 M NaCl, pH 9.0. The activator inhibitor fraction was obtained well separated from the albumin as well as from the α_2 macroglobulin. This fraction also contained plasminogen and α_2 antiplasmin, but no α_2 macroglobulin. On gel filtration of this fraction on Sephadex G-200 the activator inhibitor was obtained in two peaks, the first of which was situated in the first protein zone, where the macromolecular proteins are found and the second, just before the third protein peak. Prepara-

Fig. Steps in the partial purification of activator inhibitor from human serum. AI = activator inhibitor.

tive agarose gel electrophoresis (veronal buffer containing 2 mM ca-lactate) of the fraction containing the activator inhibitor activity obtained after ion-exchange chromatography gave a distinct zone in the α_2 region, one zone in the α_1 region, and another in the β_2 region. Activator inhibitor activity could be demonstrated only in the α_2 zone. This zone was eluted and after electrophoresis on agarose it gave only one protein zone in the α_2 region.

An antiserum against this fraction has been raised in rabbits, and gives three precipitation lines against normal serum as tested in agarose gel with the method of Williams as modified by Scheidegger (1955), which combines electrophoresis and immunodiffusion. Antigen-antibody crossed electrophoresis according to Laurell (1965) gave three distinct

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peaks. One of these peaks was identical with ceruloplasmin, another with prothrombin, and the third one with the activator inhibitor. By means of absorption with pure ceruloplasmin and prothrombin an antiserum was obtained, which produced only one precipitation line against normal serum. This line proved to be identical with the activator inhibitor.

The activator inhibitor fraction obtained is thus contaminated with ceruloplasmin and prothrombin, but in such small amounts that no activity is obtained in tests performed during the purification procedure.

The activator inhibitor reacts as an immediate inhibitor of the plasminogen activation, is thermolabile, acid labile, and is inactivated by methylamine to about 40% of the original level. After adsorption on BaSO_4 the activity of the activator inhibitor decreases to about 60% of the original level. The inhibitor reacts as a competitive inhibitor of the activation of plasminogen by urokinase as well as streptokinase. It has no antiplasmin activity, no antithrombin activity and no plasminogen activity.

An increased content of the activator inhibitor, as measured with both of the above mentioned methods, has been found in 15 patients with renal grafts. The increased content of inhibitor has proved to persist for two to three years after the operation and to rise to still higher values during rejection of the graft.

Patients with chronic or acute uraemia have also proved to have increased content of activator inhibitor.

An increased content of the activator inhibitor has also been found in patients with malignant tumours, in foetuses, and in newborns.

After gel filtration on Sephadex G-200 of sera from patients belonging to these groups the second peak of the activator inhibitor, situated just before the albumin peak, was found to be very markedly elevated. Most of the patients also had an abnormally high first peak of the activator inhibitor found in the zone of the macromolecular proteins.

In two patients with an abnormally high proteolytic capacity, as measured by Dr Jacobsen, Oslo, we found a very low concentration of this activator inhibitor. After gel filtration on Sephadex G-200 sera from these patients the patterns obtained were found to lack the second peak of the activator inhibitor. These results indicate that the second peak, as obtained after gel filtration of serum on Sephadex G-200, is the one mainly responsible for the serum activity of the activator inhibitor. It has also been shown that this second peak inactivated the urokinase added to serum, while the first peak is unchanged after the addition of urokinase. Judging from the elution pattern on Sephadex G-200, the active fraction of activator inhibitor has a molecular weight just above that of human serum albumin (69 000) and very close to that of human plasminogen (80 000).

A high level of this activator inhibitor has also been found in a few patients with recurrent venous thrombosis. Serum from one of these patients showed also a very high second peak of the activator inhibitor after gel filtration on Sephadex G-200.