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On the Regulation and Control of Fibrinolysis*

Edward Kowalski Memorial Lecture

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Introduction

Mammalian blood contains an enzymatic system capable of dissolving blood clots, which is called the fibrinolytic enzyme system. Observations on the liquefaction of clotted blood and the enhancement of fibrinolysis in vitro and in vivo date from the 18th and 19th centuries. Whereas clotted whole blood normally does not lyse spontaneously, blood obtained from patients or animals after sudden death or major surgical operations may either be incoagulable or dissolve spontaneously (1–4). The fibrinolytic potential of normal blood may be increased in vitro by treatment of serum with chloroform (5) or by addition of culture fluid from certain strains of hemolytic streptococci (6).

Most of the components of the fibrinolytic enzyme system (Table 1) have been identified between 1930 and 1950. The highlights of this evolution will be briefly summarized. A more extensive review has been given by Astrup (7) and by Fearnley (8). In 1941 Milstone (9) showed that lysis of fibrin by the streptococcal substance described by Tillet and Garner in 1933 (6) depended upon a "lytic factor" present in human serum, and Kaplan (10) and Christensen (11) found that this lytic factor was an enzyme precursor which was converted to an active enzyme by the bacterial fluid. The precursor was called *plasminogen*,, the enzyme *plasmin* and the streptococcal factor *streptokinase* (12). In 1947 Astrup and Permin (13) showed that animal tissues contain an agent which can activate plasminogen. This factor was originally called fibrinokinase but at present the name *tissue activator of plasminogen* is generally accepted. It was also demonstrated in 1947 that blood contains substances which inhibit the fibrinolytic process. Inhibitors of plasmin are called *antiplasmins* and inhibitors of plasminogen activators *antiactivators*. Macfarlane and Pilling (14) found fibrinolytic activity in urine and Williams (15) demonstrated that this was due to the presence of a plasminogen activator which is now called *urokinase*. The origin of fibrinolytic activity in the blood has been much debated. The fibrinolytic agent present in the blood after exercise, venous occlusion, stress or in postmortem blood was shown to be a plasminogen activator (16), which is now called *blood plasminogen activator* or *vascular plasminogen activator* since it appears to originate from the vascular endothelium. Evidence is accumulating that the plasminogen activators from the vascular wall and from tissues are similar or identical, but that they are different from urokinase (17–19). The physiological importance of this plasminogen activator has been inferred from the association between low blood fibrinolytic activity and thrombotic or atherosclerotic disease (20–26). Alternatively, plasminogen activator activity may be induced in blood by contact activation of Hageman factor (27). This humoral plasminogen activation requires the presence of other proteins, one of which has been termed *Hageman factor cofactor* (28). A pathway for plasminogen activation requiring Hageman factor, *high molecular weight kininogen, prekallikrein* and contact activation has been identified (29–31). Even more complicated pathways of humoral plasminogen activation have been proposed (32). The physiological role of these pathways in the removal of fibrin remains speculative, although patients with recurrent deep vein thrombosis have been described in whom an acquired antibody to factor XII was the only detectable defect (33). The main pathways for fibrinolytic activation are summarized in figure 1.

Several inhibitors of plasminogen activators occur in human plasma. Cl-inactivator appears to be the main inhibitor of plasminogen activation through the intrinsic pathway (34). Hedner has described an inhibitor of urokinase-induced fibrinolysis (35). Later work by the same group (36) however indicated

Table 1 Survey of components of the fibrinolytic system

Plasminogen	proenzyme form of the fibrinolytic enzyme
Plasmin	active fibrinolytic enzyme
Tissue activator of plasminogen	enzymes present in tissues which convert plasminogen to plasmin; may be identical or similar to blood plasminogen activator and to vascular plasminogen activator
Vascular plasminogen activator	plasminogen activator present in endothelial cells
Blood plasminogen activator	plasminogen activator present in blood, most likely identical to vascular plasminogen activator
Streptokinase	streptococcal protein which activates the fibrinolytic system in human plasma
Urokinase	plasminogen activator isolated from urine or kidney cell cultures; different from tissue activator
Hageman factor Hageman factor cofactor High molecular weight kininogen Prekallikrein	plasma proteins involved in intrinsic plasminogen activation; Hageman factor cofactor and prekallikrein may be identical
Antiactivators	general designation for inhibitors of plasminogen activation
Antiactivator in blood	inhibitor of vascular plasminogen activator in blood; its existence is doubtful
Antiplasmins	general designation for plasmin inhibitors
α_2-Antiplasmin	specific fast-reacting plasmin inhibitor in human plasma

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that this inhibitor did not inactivate urokinase in a purified system, but inhibited factor XIIa. Antithrombin III in the presence of heparin (37) inactivates activated Hageman factor. α_2-Macroglobulin inhibits kallikrein and may thereby play a role in the regulation of intrinsic fibrinolysis (38).

The presence in plasma of *inhibitors of vascular plasminogen activator* forming a complex which dissociates in the presence of fibrin has been postulated already in the 1950's (39) and also in more recent studies (40) but the evidence for the existence of such a specific inhibitor can at best only be regarded as preliminary (41).

Work by three independent groups (42–44) has revealed the existence of a specific, fast-reacting plasmin inhibitor in human blood, for which the designation *α_2-antiplasmin* was suggested by the Task Force on Nomenclature and Standards of Inhibitors of Coagulation and Fibrinolysis (45). In retrospect, it appears that a fourth group probably had identified the same inhibitor (46). This inhibitor appears to be the main physiological inactivator of plasmin while α_2-macroglobulin reacts slower with plasmin and serves as a second line inhibitor (42, 44, 47). The other plasma proteinase inhibitors do not seem to play a role in the inactivation of plasmin formed in blood (42, 44, 47–49).

The fibrinolytic system plays a role, not only in the removal of fibrin from the vascular bed but also in several other biological phenomena such as tissue repair (50), malignant transformation (51), macrophage function (51), ovulation (52) and embryo implantation (52). In the present review we will however only deal with the role of the fibrinolytic system in thrombolysis.

Main Components of the Fibrinolytic System

Plasminogen

Physicochemical and turnover properties. Human plasminogen is a single chain glycoprotein with a molecular weight of about 90,000, containing about 2 percent carbohydrate. The complete primary sequence of the molecule, represented in fig. 2, has been elucidated mainly by Magnusson's group (53, 54) and by Wallén and Wiman (55) although small contributions have been made by several other groups. The plasminogen molecule consists of 790 (54) or 791 (55) amino acids, it contains 24 disulfide bridges and five homologous triple loop structures or "kringles"(54). In the following discussion, the numbering system proposed by Magnusson's group will be used (54), since it most probably is the correct one.

Native plasminogen has NH_2-terminal glutamic acid ("Glu-plasminogen") but is easily converted by limited plasmic digestion to modified forms with NH_2-terminal lysine, valine or methionine (56, 57), which are commonly designated "Lys-plasminogen". This conversion occurs by hydrolysis of the Arg 67-Met 68, Lys 76-Lys 77 or Lys 77-Val 78 peptide bonds.

The method of choice for the purification of human plasminogen is affinity chromatography on insolubilized lysine, a method introduced by Deutsch and Mertz (58). The Glu-plasminogen and Lys-plasminogen forms can be separated by chromatography on DEAE-Sephadex (57). The hydrodynamic properties of both types have been summarized and discussed elsewhere (55).

The Glu-plasminogen occurring in human blood displays at least two types of microheterogeneity. Affinity chromatography on lysine-Sepharose using gradient elution with 6-aminohexanoic acid separates plasminogen in two fractions, called type I and type II in the order of their elution from lysine-Sepharose (59). The first form appears more easily activatable than the second form and has a larger Stokes radius as evidenced by gel filtration (60). Differences in carbohydrate and amino acid composition have been invoked to explain this heterogeneity (60, 61). Each of these two fractions can be separated in about six forms with different isoelectric points by isoelectric focusing (56, 62) or chromatography on DEAE-Sephadex (60). These forms show differences in sialic acid content. All twelve molecular forms appear to be present in single donor plasmas (60).

The turnover of both Glu-plasmingen and Lys-plasminogen labelled with radioiodine was studied in normal humans (63, 64). Whereas Glu-plasminogen had a plasma half-life of 2.24 ± 0.29 days and a fractional catabolic rate of 0.55 ± 0.10 of the plasma pool per day, Lys-plasminogen disappeared with a half-life of about 0.8 days. The two forms of Glu-plasminogen with different affinity for lysine-Sepharose have similar turnover characteristics in man (63, 64). The turnover of plasminogen was found to be increased during reptilase (Defibrase®) treatment, in patients with cirrhosis and in several clinical conditions associated with intravascular coagulation (63, 64).

Activation to plasmin. Lys-plasminogen forms are converted to plasmin by cleavage of a single Arg-Val bond (65) corresponding to the Arg 560-Val 561 bond. The two-chain plasmin molecule is composed of a heavy chain or A-chain, originating from the NH_2-terminal part of plasminogen and a light chain or B-chain constituting the C-terminal part (66). The B-chain was found to contain an active site similar to that of trypsin, composed of a histidine residue sensitive to tosyl-lysine chloromethyl ketone and a serine residue reactive towards diisopropyl-phosphofluoridate (66). It appears that the active site of plasmin is composed of His 602, Asp 645 and Ser 740 (54).

Activation of Glu-plasminogen to plasmin by urokinase in purified systems occurs about 20 times slower than activation of Lys-plasminogen (67–69), but in either case Lys-plasmin is formed. Rickly and Otavski (70) demonstrated the release of an amino-terminal peptide from human plasminogen by urokinase. Wiman and Wallén (71) initially proposed that activation of Glu-plasminogen occurs in two steps: release of the NH_2-terminal "preactivation" peptide mainly by cleavage at Arg 67-Met 68 yielding Lys-plasminogen, followed by cleavage of the Arg 560-Val 561 bond to generate plasmin. However, when activation is carried out in excess plasmin inhibitor (72, 73), inhibited Glu-plasmin is obtained. In view of the great sensitivity of the Arg 67-Met 68 bond to plasmin it was nevertheless suggested that the major pathway for activation of plasminogen still is via Lys-plasminogen generated by plasmic cleavage of Glu-plasminogen (68, 73). Activation of Glu-plasminogen in the presence of the physiological plasmin inhibitor α_2-antiplasmin however generates inhibited Glu-plasmin (74). Recently Thorsen and Müllertz (75) studied the activation of plasminogen by urokinase in plasma, and found that formation of fibrin resulted in an increased rate of activation associated with the appearance of a component migrating as Lys-plasminogen on polyacrylamide gel electrophoresis in acid-urea. The exact mechanism of activation of plasminogen in vivo thus remains unsettled.

The kinetics of the activation of human plasminogen by urokinase or by streptokinase-plasminogen complex have been studied by several authors and most extensively by Christensen (76) and by Wohl et al. (77). The activation obeys Michaelis-Menten kinetics with K_M values of 1 to 40 μM and k_{cat} values of 0.26 to 26 s^{-1}. Activation of Lys-plasminogen by streptokinase (77) or urokinase (76) appears to be 3 to 10 times faster than that of Glu-plasminogen. The presence of fibrin not only appears to accelerate the activation of plasminogen by tissue activator (17, 78) but also – to a smaller extent – by urokinase (75, 78). The kinetic data obtained in purified systems can therefore not as such be extrapolated to the in vivo situation encountered during thrombolysis.

Lysine-binding sites. The plasminogen molecule contains structures, called lysine-binding sites which interact specifically with certain amino acids such as lysine, 6-amino-hexanoic acid and trans-4-aminomethylcyclohexane-1-carboxylic acid. Plasminogen contains one binding-site with high affinity for 6-aminohexanoic acid ($K_d \approx 9$ μM), and about four with low affinity ($K_d \approx 5$ mM) (79). These lysine-binding sites are located in the plasmin A-chain (80). One site is located in the fourth triple loop structure or kringle (residues 354–439) and at least one in the first three triple loop structures (residues 79–337/353) as shown by their affinities to Lys-Sepharose, whereas the Low-M_r-plasminogen (residues 442–790) has no affinity for Lys-Sepharose (53).

Plasminogen can specifically bind to fibrin through its lysine-binding sites. It has been found in purified systems (81) and in plasma (82) that Lys-plasminogen has a higher affinity for fibrin than the intact Glu-plasminogen. The differences in binding of Glu-plasminogen and Lys-plasminogen to fibrin might be due to an intramolecular interaction of one of the lysine-binding sites with a specific site in the NH_2-terminal region of Glu-plasminogen (83) which is no longer present in Lys-plasminogen. The presence of 6-aminohexanoic acid abolished the adsorption of plasminogen to fibrin both in the purified (81) and in the plasma system (82). These findings indirectly indicate the importance of the lysine-binding sites in plasminogen for the interaction with fibrin. More direct evidence has been obtained by affinity chromatography of plasminogen and plasminogen derivatives on fibrin-Sepharose (84). Thus, it is concluded that one of the functions of the lysine-binding sites in plasminogen is to mediate its interaction with fibrin.

As will be discussed below, the lysine-binding sites of plasmin(ogen) also mediate its interaction with α_2-antiplasmin.

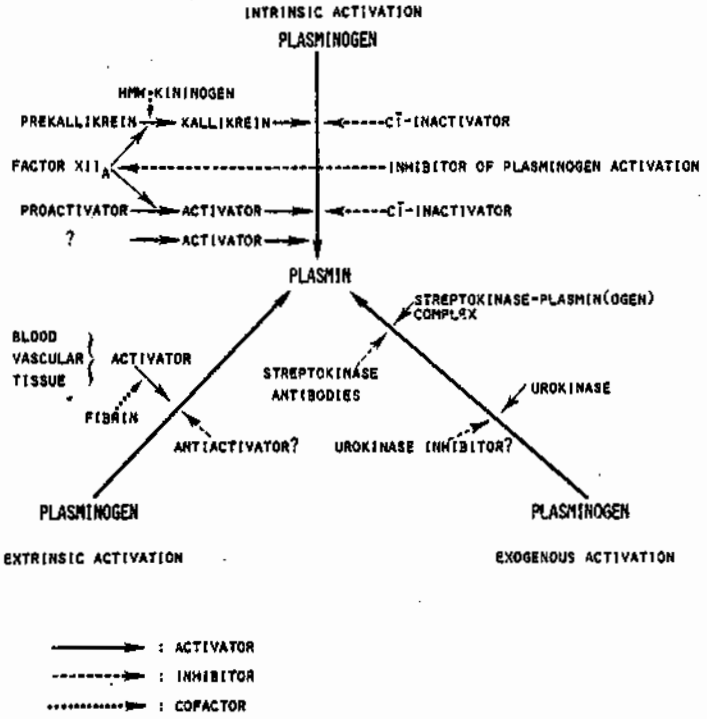

Fig. 1 Schematic representation of activation pathways of plasminogen (→) and site of action of inhibitors (– – →)

Plasminogen Activators

As illustrated in figure 1, plasminogen activation may occur by three different pathways: an intrinsic or humoral pathway in which all components involved are present in precursor form in the blood, an extrinsic pathway in which the activator originates from tissues or from the vessel wall and is released into the blood by certain stimuli or trauma, and an exogenous pathway in which the activating substances streptokinase or urokinase may be infused for therapeutic purposes. All plasminogen activators studied so far exert their action through hydrolysis of the Arg 560-Val 561 bond in plasminogen.

Intrinsic fibrinolysis. The present knowledge on the pathways of intrinsic plasminogen activation has recently been reviewed (85, 86). Intrinsic activation of plasminogen may occur by one or more pathways involving factor XII (Hageman factor), prekallikrein (Fletcher factor), high molecular weight kininogen (Fitzgerald factor) and possibly other components. The exact mechanism of this activation as well as its biological role remain however unknown.

Tissue plasminogen activator, vascular plasminogen activator and blood plasminogen activator. Plasminogen activators are present in many organs, tissues and secretions (87); their extraction from tissues usually requires harsh conditions such as 1 M KSCN or 0.3 M acetate at acid pH. Highly purified activator preparations were obtained from pig heart (88–90), hog ovaries (91) and human uterus (19). These activators appear to be serine proteases with a M_r of approximately 60,000 composed of two disulfide-linked polypeptide chains. Immunological cross-reactivity between these three types of activator but not with urokinase has been reported (19, 88–90). Plasminogen activator has also been partially purified from postmortem vascular perfusates (92, 93) or from postexercise blood (94).

Althouh a strict comparison of highly purified materials has not yet been made, it is very likely that the plasminogen activator found in blood represents released vascular plasminogen activator and that these activators are similar or identical to the tissue activator, but different from urokinase.

An important property of the blood-vascular-tissue type of plasminogen activator is its high affinity for fibrin (16, 39), which has been used for its isolation (17). Tissue activator is a relatively poor plasminogen activator in pure systems but fibrin strikingly stimulates the activation (17, 78).

Plasminogen activator, released in the plasma by nicotinic acid injection in normal subjects has a half-life in vivo of approximately 15 minutes (95, 96).

The physiological importance of extrinsic plasminogen activators has been inferred from the association between low blood fibrinolytic activity and thrombotic or atherosclerotic disease (20–26).

Exogenous plasminogen activators: urokinase and streptokinase. Urokinase is a trypsin-like protease isolated from human urine or cultured human embryonic kidney cells. It may occur in two molecular forms designated S_1 (M_r 31,600) and S_2 (M_r 54,000). The former probably is a proteolytic degradation product of the latter. Urokinase differs from the vascular or tissue plasminogen activator both in its antigenic characteristics (19, 84–87) and in its enzyme specificity, particularly with respect to the activation of fibrin-associated plasminogen (17, 78). The concentration of urokinase in urine is approximately 6 CTA units per ml. The half-life of urokinase in the blood following intravenous injection is approximately 10 min (97). Urokinase has extensively and successfully been used for thrombolytic therapy but its exact place in the management of the several clinical forms of thrombosis remains to be further established (98–100).

Streptokinase is a non-enzyme protein with a M_r 47,000, produced by Lancefield group C strains of β-hemolytic streptococci, which activates the fibrinolytic system indirectly (101). Streptokinase forms a 1 : 1 stoichiometric complex with plasmino-

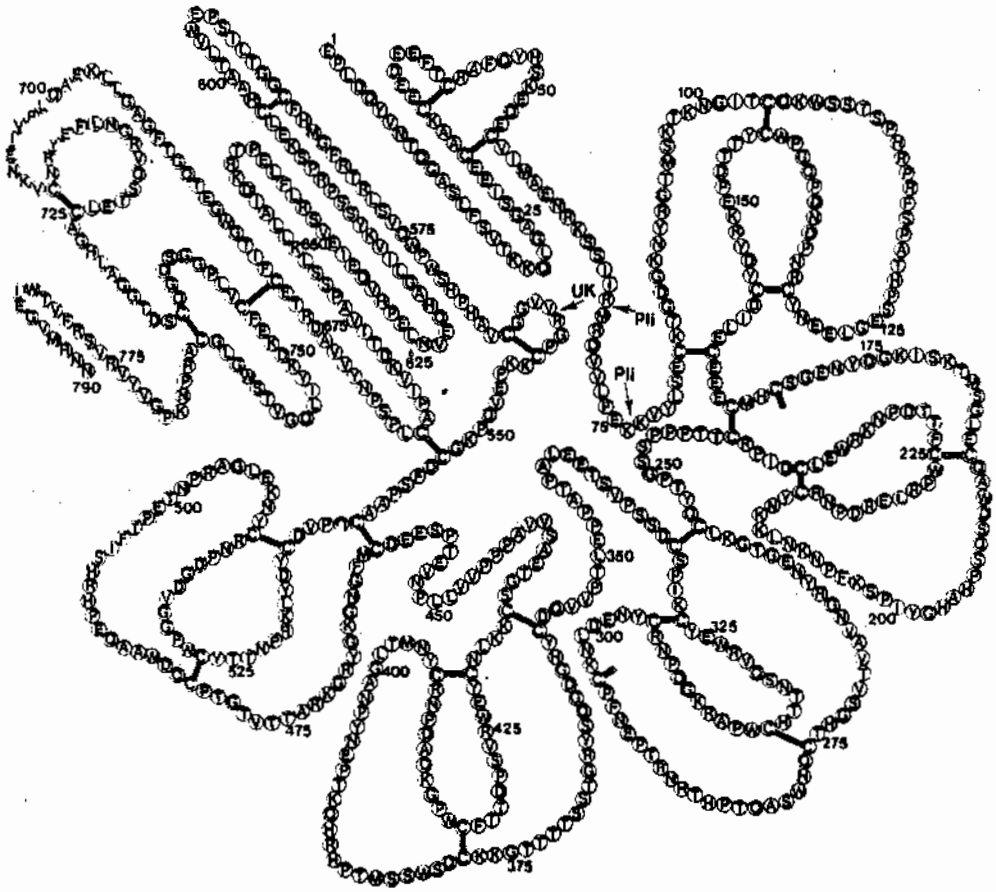

Fig. 2 Primary structure of human plasminogen. Adapted from ref. 54 and ref. 55

gen or plasmin and thereby converts the inactive proenzyme as well as the enzyme into an efficient plasminogen activator. The properties and mechanism of action of streptokinase have been reviewed in detail (102). We have recently studied the kinetics of the streptokinase-human plasmin reaction (103): the complex has a dissociation constant of 5×10^{-11} M and is formed with a rate constant of approximately 3×10^{7} $M^{-1}s^{-1}$, which indicates that the complex is strong and that it is extremely rapidly formed.

Streptokinase is at present the most widely used thrombolytic agent because it is easier to obtain and far less expensive than urokinase; it is however antigenic in humans and may produce pyrogenic or toxic side effects. Although streptokinase is used for thrombolysis since twenty years its optimal dose regimen and exact place in the treatment of thromboembolic disease are still debated (100).

Inhibitors of Plasminogen Activators

Our knowledge on the inhibition of plasminogen activators is still very preliminary in spite of extensive research efforts. The slow progress in this field is partly due to the fact that clot lysis assays for the detection of activator inhibitors are also very sensitive to the presence of plasmin inhibitors which renders the unequivocal identification of the level at which inhibitors act often difficult.

Inhibitors of intrinsic plasminogen activation. Several inhibitors of intrinsic plasminogen activation occur in human plasma, C_1-inactivator (34), an inhibitor of factor XIIa-induced fibrinolysis (35, 36), heparin-antithrombin III complex (37) and α_2-macroglobulin (38). Since the physiological role of the intrinsic fibrinolytic pathway is not established, the role of these inhibitors in the regulation and control of fibrinolysis thus remains entirely speculative.

Inhibitors of extrinsic plasminogen activation. The presence in plasma of inhibitors of extrinsic plasminogen activators forming a complex which dissociates in the presence of fibrin has been postulated already in the 1950s (39) and also in more recent studies (40). The formation of a reversible activator-activator inhibitor complex which dissociates in the presence of fibrin has been invoked to explain the rapid lysis of fibrin in plasma and the resistance of fibrinogen to degradation by plasmin (39). The enhancing effect of fibrin on the plasminogen activation may however be explained by adsorption of activator and plasminogen to its surface facilitating activation (17, 78, 104). The evidence for the existence of a specific inhibitor of extrinsic plasminogen activators in plasma, forming a reversible complex can thus at best be regarded as preliminary (41).

There is good evidence that a significant amount of extrinsic plasminogen activator released in the blood is cleared in vivo by mechanisms other than neutralization by plasmatic inhibitors. Indeed, whereas plasminogen activator, released in the plasma by nicotinic acid injection in normal subjects has a half-life in vivo of approximately 15 min (95, 96), its half-life in plasma in vitro is approximately 75 min (95), as measured with clot lysis assays.

Inhibitors of exogenous plasminogen activation. Human plasma contains antibodies directed against streptokinase, which most probably result from previous infections with beta-hemolytic streptococci. The amount of streptokinase antibodies varies over a wide range amongst individuals. Verstraete et al. (105) found that 352,000 units of streptokinase were required to neutralize the circulating antibodies in 95 percent of a healthy population, but that the individual requirements ranged between 25,000 and 3,000,000 units. Since streptokinase reacts with antibodies and is thereby rendered biochemically inert, sufficient streptokinase

must be infused to neutralize the antibodies before fibrinolytic activation is obtained (106). A few days after streptokinase injection the antistreptokinase titer rises rapidly to 50 to 100 times the preinfusion value and remains high for 4 to 6 months, during which renewed treatment is impracticable. Administration of corticosteroids commonly is used as adjuvant to streptokinase to prevent allergic side-reactions. The streptokinase-plasmin(o-gen) activator complex is virtually unreactive towards α_2-antiplasmin (103).

The mechanism of urokinase inhibition in blood is poorly known. Urokinase inhibitor assays based on clot lysis are strongly dependent on the presence of plasmin inhibitors and are therefore in no way specific. Assayed with clot lysis methods, the half-life of urokinase in vivo is 9 to 16 min but in vitro 27 to 61 min (97), suggesting that clearing of the enzyme from the blood plays an important role. α_2-macroglobulin (107), α_1-antitrypsin (108), antithrombin III (109) and α_2-antiplasmin (43) all inhibit urokinase slowly.

Inhibitors of Plasmin

α_2-Antiplasmin, the physiological plasmin inhibitor of plasma. In 1975 we discovered a new plasmin inhibitor in human plasma (110, 42), which was independently identified by two other groups (43, 44) and most probably also by a third group (46).

α_2-Antiplasmin is a single chain glycoprotein with $\underline{M}_r$ 70,000 containing approximately 13 percent carbohydrate (43, 111). The inhibitor is immunochemically different from α_1-antitrypsin, α_2-macroglobulin, c_1-esterase inhibitor, antithrombin III, α_1-antichymotrypsin and inter-α-trypsin inhibitor (43, 44, 47, 110, 111), and from the inhibitor of plasminogen activation described by Hedner (112).

α_2-Antiplasmin forms a very stable 1 : 1 stoichiometric complex with plasmin, which is devoid of protease or esterase activity (43, 44, 111). Complex formation occurs by strong interaction between the light-(B)-chain of plasmin and the inhibitor. The physiological role of α_2-antiplasmin as an inhibitor of proteases other than plasmin seems negligible (113, 114).

The reaction between plasmin and α_2-antiplasmin proceeds in at least two steps: a very fast reversible second order reaction followed by a slower irreversible first order reaction (115, 116) and may be represented by:

$$P + A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} PA \xrightarrow{k_2} PA'$$

The rate constant k_1 at pH 7.5 is $3.8\times10^7\ M^{-1}s^{-1}$ and $1.8\times10^7\ M^{-1}s^{-1}$ for the two plasmin forms which have different affinities for lysine-Sepharose (116). This reaction rate is one of the fastest so far described for protein-protein interactions and is one order of magnitude higher than the reaction rate of trypsin with its inhibitors. The dissociation constant of the reversible step is approximately 2×10^{-10} M and the rate constant of the second step $4\times10^{-3}s^{-1}$ (116). Plasmin which has 6-aminohexanoic acid (116) or lysine (115) bound to its lysine-binding sites or substrate bound to its active site (116) reacts only very slowly with α_2-antiplasmin. These findings indicate that free lysine-binding sites and a free active site in the plasmin molecule are of great importance for the rate of its reaction with α_2-antiplasmin. As discussed further, these interactions are probably of great importance for the regulation of fibrinolysis in vivo.

Plasmin and α_2-antiplasmin form a stoichiometric 1 : 1 complex with a $\underline{M}_r$ of 150,000 which upon reduction is dissociated in two parts: an intact plasmin A-chain ($\underline{M}_r$ 60,000) and a very stable complex between the plasmin B-chain and α_2-antiplasmin ($\underline{M}_r$ 80,000), provided that the complex formation is performed in excess α_2-antiplasmin (111). Recently we have been able to identify and isolate a low molecular weight peptide ($\underline{M}_r$ about 8,000) which is generated concomitantly with complex formation (74). This peptide is not disulfide bonded to the complex since it can be detected prior to reduction.

The complex can be dissociated in 1.5 M NH_4OH regenerating up to 0.2 mol/mol plasmin activity and a modified inhibitor with a lower $\underline{M}_r$ (60,000) than the virgin inhibitor (70,000). The native α_2-antiplasmin has the NH_2-terminal sequence Asn-Gln-Glu-Gln-Val- and the COOH-terminal sequence -Phe-Leu, while the modified inhibitor has the same NH_2-terminal sequence, but the COOH-terminal sequence -Ala-Leu. The 8,000 $\underline{M}_r$-peptide has the NH_2-terminal sequence Met-Ser-Leu-Ser-Gly-Phe- and the COOH-terminal sequence -Phe-Leu, suggesting that it originates from the COOH-terminal part of α_2-antiplasmin. From these results and by analogy with trypsin-inhibitor complexes it was suggested that the stable complex between plasmin and α_2-antiplasmin is formed by a plasmic attack at a specific leucyl-methionyl peptide bond in the COOH-terminal portion of the

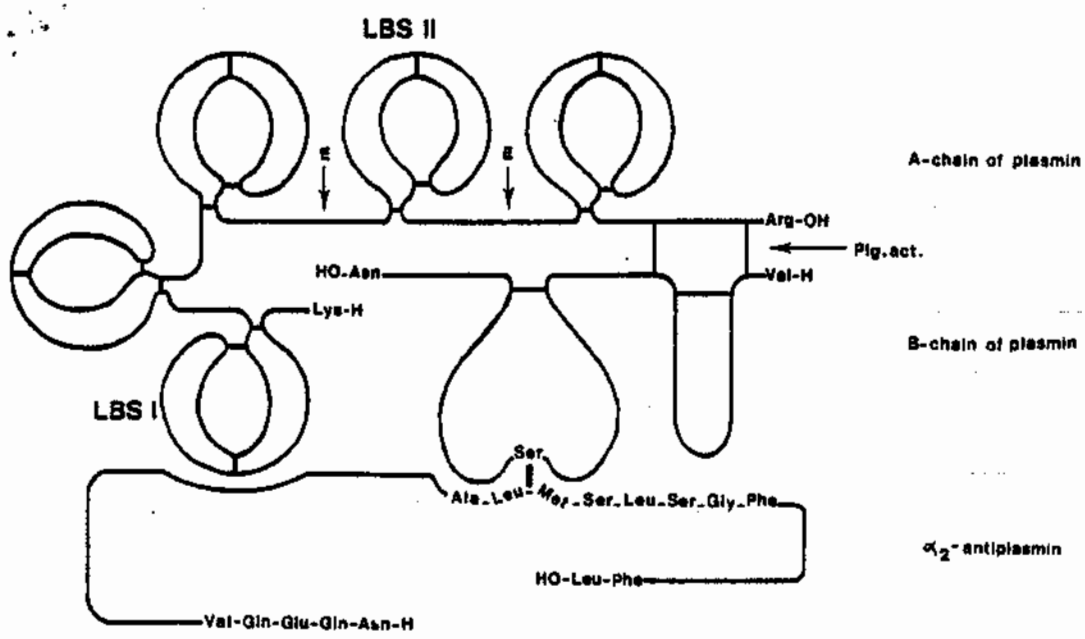

Fig. 3 Schematic representation of the interaction between plasmin and α_2-antiplasmin. Adapted from ref. 74

inhibitor. A strong, probably covalent bond is formed between the active site seryl residue in plasmin and the carbonyl group of this specific leucyl residue in the inhibitor. It remains unsettled whether this bond is of tetrahedral intermediate type or an ester bond (74). The interaction between plasmin and α_2-antiplasmin is schematically represented in fig. 3.

The turnover of ^{125}I-labeled α_2-antiplasmin was studied in control subjects and in patients during thrombolytic therapy (117). In the control group α_2-antiplasmin had a plasma half-life of 2.64 ± 0.32 days and a fractional catabolic rate of 0.53 ± 0.09 of the plasma pool per day. During thrombolytic therapy the half-life shortened to approximately 0.5 days as a result of formation of plasmin-α_2-antiplasmin complex. The long half-life of the plasmin-α_2-antiplasmin complex was confirmed by studying the turnover of the purified complex both before and during thrombolytic therapy in patients with thrombotic disease.

The normal level of α_2-antiplasmin is between 80 and 120 percent (mean ± 2 SD) of the value obtained for pooled normal plasma (118–120). The concentration of α_2-antiplasmin in pooled normal plasma is approximately 1 μM (43, 44, 111). The concentration may decrease to below 30 percent in severe cases of liver disease or intravascular coagulation (118–120), but is normal in patients with cardiovascular, renal or malignant disease. The inhibitor is temporarily exhausted during thrombolytic therapy with streptokinase (119, 121), when measured enzymatically. Residual antigen may however be found immunologically repre-senting complexed and/or degraded inhibitor (121). α_2-Antiplasmin is a weak acute Phase reactant (119). It is possible that some of the α_2-antiplasmin in plasma is inactive (44).

α_2-Macroglobulin. α_2-Macroglobulin represents the slower reacting plasmin inhibitor of plasma, and its role seems to be to inactivate plasmin formed in excess of the inhibitory capacity of α_2-antiplasmin (42, 44). Indeed, when the plasma plasminogen (concentration approximately 1.5 μM) is activated, the formed plasmin is initially primarily bound to α_2-antiplasmin (concentration approximately 1 μM) until after its saturation, excess plasmin is neutralized by α_2-macroglobulin. A number of reviews on the physiology and biochemistry of α_2-macroglobulin have recently appeared (122–124).

Regulation and Control of Fibrinolysis

It has been suggested (7) that the fibrinolytic system is in a dynamic equilibrium with the coagulation system to maintain an intact patent vascular bed. The view that the fibrinolytic system is continuously active in vivo is supported by the finding of plasminogen activator activity in normal blood (16, 125) and a reduced level in patients suffering from venous thrombosis (22) or ischemic heart disease (21, 23). Further evidence for the role of the fibrinolytic system in vivo stems from the finding that pharmacological inhibition of fibrinolysis reduces blood loss after prostatectomy (126) or in patients with primary menorrhagia (127), that high levels of fibrinolytic inhibitors are associated with thrombotic disease (128, 129), that a congenital deficiency of α_2-antiplasmin results in a bleeding tendency (130), and that a partial plasminogen deficiency may be associated with recurrent thrombosis (131). However, subjects with a partial or even a complete congenital deficiency of functional plasminogen without thrombosis have been described (131). This suggests that alternative pathways (leucocyte proteases or phagocytosis?) may also be of significance for the resolution of fibrin.

Turnover studies with labeled components of the coagulation or fibrinolytic system in healthy human subjects have however revealed that consumption of these components by continuous low grade intravascular coagulation and/or fibrinolysis is either non-existent or very small (132, 63). We thus have to consider that the coagulation and fibrinolytic systems are not continuously active but designed to be activated in situ when needed for local hemostasis.

The regulation and control of fibrinolysis appears to occur at several levels: release of plasminogen activator from the vascular wall, fibrin-associated activation of plasminogen and inhibition of formed plasmin by α_2-antiplasmin.

Release of plasminogen activator. The mechanisms controlling the release of plasminogen activator from the endothelial cells have been reviewed recently (133). Parenterally administered adrenaline elicits a release of plasminogen activator. This may be due not only to interaction with peripheral adrenergic receptor sites, presumably at the endothelial cell level (134, 135), but possibly also in part to the central release of vasopressin-like substances which stimulate plasminogen activator release (136). Vasopressin however does not appear to induce local release of plasminogen activator when infused into a brachial artery (134). Furthermore, there is no strict correlation between the release of catecholamines and plasminogen activator following physical exercise (137) or electroshock (138) which suggests additional control mechanisms for plasminogen activator release.

On the basis of this and other evidence Cash has speculated that plasminogen activator release may be under neurohumoral control (133). Higher neurogenic centers or specific peripheral afferent organs might stimulate the release of a plasminogen activator releasing hormone (PARH) with a structure similar to vasopressin from the neurohypophyseal region. This PARH would constitute the major pathway for the release of plasminogen activator from the endothelial cells, whereas the catecholamine pathway would only be involved in severely stressful situations (133).

The observation that venous thrombosis is frequently associated with a decreased fibrinolytic activity has stimulated the interest in drugs which increase the synthesis and release of plasminogen activator in the vessel wall. Many vasoactive drugs such as adrenaline, nicotinic acid, histamine and vasopressin enhance the fibrinolytic activity of blood but their effect is of very short duration (139). The biguanidines and certain anabolic steroids such as ethylestrenol (139) or stanazolol (140) have been reported to produce long-term stimulation of endogenous fibrinolysis by increasing both the synthesis and release of plasminogen activator in the vessel wall (139). A combination therapy with phenformin and ethylestrenol was shown to significantly reduce the occurrence of thrombotic episodes in patients with idiopathic recurrent venous thrombosis (141).

Molecular mechanism of physiological fibrinolysis. The proteolytic enzyme plasmin has a broad specificity, which is not very different from that of trypsin. However, in vivo the main target of plasmin is fibrin. Several hypotheses have been put forward to explain this specificity. Müllertz (39) and Astrup (7) delineated some of the main features of the mechanism of fibrinolysis: activator is present in low concentrations bound to inhibitor in circulating blood, but is adsorbed to and accumulates on the fibrin surface. The effect of the inhibitors against activation and against plasmin is reduced by the adsorption of activator and plasmin to fibrin. The activation of plasminogen proceeds on the fibrin surface, fibrin is lysed and activator and plasmin are released and bound by their respective inhibitors. Alkjaersig, Fletcher and Sherry (142) have suggested that plasminogen is adsorbed to polymerizing fibrin and converted to active enzyme by activators which diffuse into the thrombus. Plasmin would then exert its action in an environment relatively free of inhibitors. Ambrus and Markus (143) have proposed that plasmin-inhibitor complexes, formed in the circulation, dissociate in the presence of fibrin, because plasmin has a greater affinity for fibrin than for its

inhibitors. Chesterman, Allington and Sharp (144) suggested that the activators bind selectively to fibrin and transform plasminogen which diffuses into the thrombus, to plasmin. During the past few years specific interactions at the molecular level have been demonstrated between the different components of the fibrinolytic system which enable us to formulate a molecular model for the regulation of fibrinolysis in vivo.

As discussed above, plasminogen can specifically bind to fibrin through its lysine-binding sites (81–83). When plasma is clotted with thrombin and the clot washed, about 4 percent of the plasminogen remains specifically adsorbed to the fibrin network and is dissociated by 6-aminohexanoic acid (82). Thus, it is concluded that one of the functions of the lysine-binding sites in plasminogen is to mediate its interaction with fibrin.

α_2-Antiplasmin not only forms a stable complex with plasmin, but also interacts weakly with the proenzyme plasminogen and this weaker interaction can be used to efficiently purify α_2-antiplasmin by affinity chromatography on plasminogen-Sepharose (43, 111). Elution can be performed by a low concentration of 6-aminohexanoic acid (5 mM) indicating a specific interaction which is mediated by the lysine-binding sites in plasminogen. The kinetic analysis of the reaction between plasmin and α_2-antiplasmin further demonstrates that the interaction between the lysine-binding sites in plasmin and a corresponding site in α_2-antiplasmin is of great importance for the extreme rapidity of this reaction (115–116).

Fibrinogen can also inhibit the reaction between plasmin and α_2-antiplasmin through a lysine-binding site-mediated interaction. The dissociation constant for this interaction was estimated to be between 10^{-6} and 10^{-7} M (145). Whether the interaction between fibrinogen and plasmin(ogen) is mainly mediated by the same lysine-binding site which is responsible for the plasmin(ogen)-α_2-antiplasmin interaction or by another lysine-binding site is at present unclear.

From the known concentrations of α_2-antiplasmin (about 1 μM) and plasminogen (1.5–2.0 μM) in plasma and the dissociation constant determined for the interaction between these two proteins (4×10^{-6} M) it is concluded that about 30% of the α_2-antiplasmin in the circulation is complexed with plasminogen. The half-life of free plasmin in the circulation can thus be calculated to be about 100 ms, assuming that the concentration of free α_2-antiplasmin is about 0.5 μM and that the concentration of free plasmin under most physiological conditions is far lower (pseudo first order conditions) (146). Thus α_2-antiplasmin rapidly binds plasmin in plasma, and thereby protects fibrinogen, but as soon as plasmin is produced in excess and plasmin-α_2-macroglobulin complex formed, fibrinogen is rapidly degraded (44, 47, 147). In vivo, however, plasmin molecules that are actively degrading fibrin are (presumably) inactivated at a much slower rate by α_2-antiplasmin than free plasmin since they have both their active site and lysine-binding sites protected from the inhibitor. The half-life of plasmin bound to fibrin is therefore expected to be at least two orders of magnitude longer (more than 10 s) than for free plasmin (about 100 ms).

The tissue activator of plasminogen binds strongly to fibrin, thereby efficiently activating the fibrin-bound plasminogen (17, 18, 78). In purified systems tissue activator is a poor plasminogen activator but in the presence of fibrin its efficiency is strikingly stimulated (17, 78). Thus, one way of regulating fibrinolysis is at the level of plasminogen activation localized at the fibrin surface. The fibrinolytic process seems, however, also to be regulated at the level of plasmin inactivation and we propose that plasmin molecules bound to fibrin through their lysine-binding sites and involved in fibrin degradation are protected from rapid inactivation by α_2-antiplasmin (104). Since the fibrin-bound plasmin has an estimated half-life of about 10 s, effective clot dissolution in

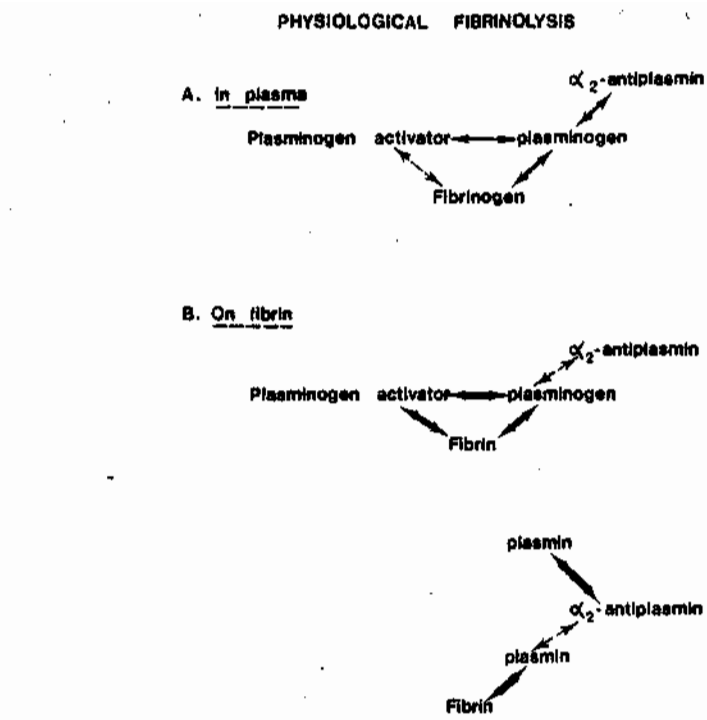

Fig. 4 Schematic representation of the interactions between fibrinogen), plasmin(ogen), α_2-antiplasmin and plasminogen activator. The size of the arrows is roughly proportional to the affinity between the different components

vivo would also seem to require a continuous replacement at the fibrin surface of inactivated plasmin molecules (complexed with α_2-antiplasmin) by plasminogen molecules. These molecular interactions are schematically represented in fig. 4.

In Vivo Findings Supporting the Molecular Model for Fibrinolysis. Several lines of evidence indicate that this model for the regulation of fibrinolysis, which was constructed mainly on the basis of molecular interactions which were demonstrated in purified systems, is also operative in vivo.

Certain amino acids such as 6-aminohexanoic acid or tranexamic acid bind to the lysine-binding sites of plasminogen. In purified systems, the complex between plasminogen and 6-aminohexanoic acid is much more easily activated by urokinase than native plasminogen, most likely as a result of conformational changes in the plasminogen molecule following complex formation (17, 148). In vivo, however, 6-aminohexanoic acid and tranexamic acid are potent inhibitors of fibrinolysis which have successfully been used to reduce bleeding after prostatectomy (126) and in patients with primary menorrhagia (127). The apparent paradox of an enchanced plasminogen activation by these amino acids in purified systems and their potent antifibrinolytic action in vivo can be explained by the fact that they dissociate plasminogen from the fibrin surface and thereby prevent activation of fibrin-bound plasminogen.

Repeated exhaustive physical exercise in healthy subjects results in the release of large amounts of plasminogen activator into the blood but leads only to minimal plasminogen to plasmin conversion (149). In vivo fibrin formation following infusion of reptilase (150, 151) does not induce an increased level of plasminogen activator in the blood but nevertheless leads to a very marked plasminogen activation. This apparent paradox can also easily be explained on the basis of the above molecular model. In the absence of fibrin, plasminogen activator has a poor efficiency; even high concentrations of activator as released by strenuous physical exercise will not lead to significant plasmin

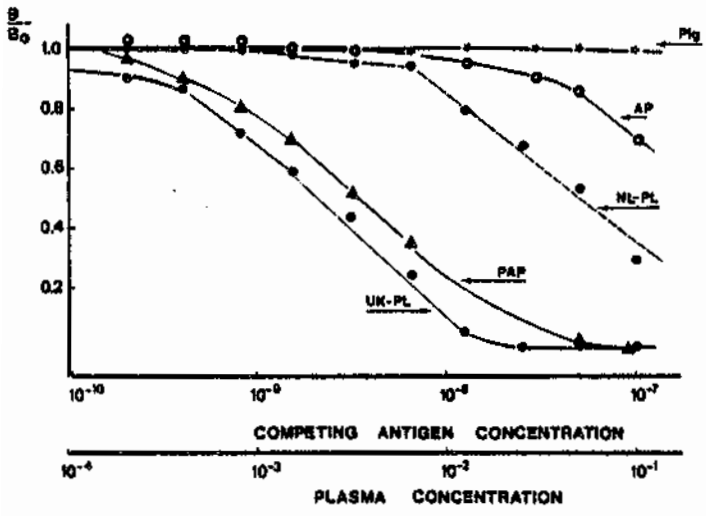

Fig. 5 Competitive inhibition radioimmunoassays using ^{125}I-labeled plasmin-α_2-antiplasmin complex as ligand and plasmin-α_2-antiplasmin complex (PAP), α_2-antiplasmin (AP), plasminogen (Plg), normal plasma (NL-PL) and urokinase-activated plasma (UK-PL) as competing agents

$$\frac{B}{B_0} : \frac{\text{percent ligand bound in the presence of competitor}}{\text{percent ligand bound in the absence of competitor}}$$

Abscissa: competing antigen concentration in M, plasma concentration in vol/vol

formation. In the presence of fibrin however plasminogen activator present in blood or released from the vascular wall will efficiently activate fibrin-bound plasminogen (17, 78).

Plasminogen activator is continuously present in the blood of healthy individuals; yet, only very little – if any – plasminogen activation occurs (152). Intravascular coagulation on the other hand is nearly always associated with activation of the fibrinolytic system (153) ("secondary fibrinolysis"), even in the absence of an increased level of circulating plasminogen activator. These observations can again be explained by the catalytic role of fibrin which triggers and regulates fibrinolysis.

Infusion of plasmin in vivo in amounts equivalent to approximately 50 percent of the circulating α_2-antiplasmin will not lead to rapid systemic fibrinogen breakdown whereas infusion of amounts in excess of the neutralizing capacity of α_2-antiplasmin induces rapid systemic fibrinogen breakdown (147). Infused plasmin will indeed react extremely rapidly with α_2-antiplasmin and not induce systemic degradation of fibrinogen before the inhibitor is exhausted and excess plasmin only slowly inhibited by α_2-macroglobulin.

Koie et al. (130) described a patient with a deficiency of α_2-antiplasmin, who presented with a hemorrhagic diathesis. Plasma-fibrinogen and fibrinogen degradation products in serum were however normal in this patient. These findings can also be interpreted in the framework of the molecular model for fibrinolysis. The bleeding tendency of the patient would be due to premature lysis of hemostatic plugs because due to the absence of α_2-antiplasmin, plasmin molecules generated on the fibrin surface of hemostatic plugs would persist much longer than their estimated normal half-life of the order of magnitude of 10 seconds. The absence of systemic fibrinogen breakdown would then be explained, not by the fact that the other plasma protease inhibitors compensate for the deficient α_2-antiplasmin, but by the lack of systemic plasminogen activation in the absence of fibrin (41).

Biochemical background of thrombolytic therapy. On the basis of the arguments developed in the previous sections efficient thrombolysis requires adsorption of plasminogen activator and plasminogen to the fibrin surface and in loco generation of plasmin, out of reach of the fast-acting α_2-antiplasmin in the blood. Alternatively the fibrinolytic system might be activated to such an extent that the circulating α_2-antiplasmin (concentration 1 μM) is exhausted. This approach is feasible since the concentration of circulating plasminogen is approximately 1.5 μM.

In the former case, systemic fibrinogen breakdown would be minimal since circulating plasmin would be very rapidly neutralized; in the latter case excess plasmin would cause excessive systemic fibrinogenolysis.

Both approaches for therapeutic fibrinolysis have been applied on an empirical basis. Urokinase therapy usually results in mild fibrinolytic activation in which plasminogen is not fully activated and α_2-antiplasmin not depleted. Systemic fibrinogen depletion does not usually occur (97). Under these circumstances the thrombolytically active substance is presumed to be plasmin generated on the fibrin surface and out of reach of α_2-antiplasmin. Thrombolytic schemes with high loading doses of streptokinase result in extensive fibrinolytic activation leading to nearly complete plasminogen to plasmin conversion and α_2-antiplasmin depletion, with marked fibrinogenolysis (105). Under these circumstances, plasmin formed in excess over the neutralizing capacity of α_2-antiplasmin might contribute to clot dissolution. As judged from the clinical results, however, both therapeutic regimens seem to be comparably efficient.

Improved thrombolysis might be expected with the use of tissue activators having a better affinity for fibrin which would ensure more selective activation of plasminogen on the fibrin surface; such activators are however not available at present. Alternatively it might be advantageous to increase the adsorption of plasminogen, thereby increasing the potential amount of in loco formed plasmin. This approach is clinically feasible with the infusion of Lys-plasminogen which has a higher affinity for fibrin than Glu-plasminogen. Kakkar has obtained an increased thrombolytic effect with such therapy in venous thrombosis (154) and Brochier et al. in pulmonary embolism (155). Infusion of plasmin in doses which do not exceed the inhibitory capacity of α_2-antiplasmin is not expected to produce a significant thrombolytic effect, since the enzyme will be very rapidly neutralized by its circulating inhibitor. However, infusion of plasmin or plasminogen before streptokinase (or urokinase) will increase the amount of plasmin which can be formed in excess of the neutralizing capacity of the α_2-antiplasmin and this might contribute to improved thrombolytic effect (156). Finally it could be helpful to reduce the plasma fibrinogen level by infusion of snake venoms such as Defibrase or Arvin, prior to the start of a thrombolytic therapy. This might not only reduce the level of anticoagulant fibrin(ogen) degradation products in the circulating blood (157) but also reduce the concentration of the substrate fibrinogen which competes with fibrin for plasmin formed in excess of α_2-antiplasmin.

Some unresolved questions concerning the regulation and control of fibrinolysis. From the above review it appears that during the past few years significant progress has been made in our understanding of the mechanisms which regulate fibrinolysis, both at the level of the release of plasminogen activator and at the level of the molecular interactions which direct and confine the action of plasmin to fibrin.

There is very significant evidence that deficient fibrinolysis may contribute to the development of thrombotic and possibly atherosclerotic disease (20–26) and some studies indicate that long-term pharmacological stimulation of the synthesis and release of vascular plasminogen activator might prevent thrombosis (141). The mechanisms responsible for the control of plasminogen activator synthesis and the physiological triggers for

its release in thrombotic states in man remain however largely unknown.

The molecular interactions between plasminogen activator, plasminogen, fibrin and α_2-antiplasmin have been sufficiently well delineated to provide a molecular model which can explain the directed action of the fibrinolytic system towards fibrin. However, some important problems remain unresolved such as the cause of the resistance of more than 3 to 5 days old thrombi to lysis, the quantitative differences in the interaction of plasminogen with fibrin as compared to fibrinogen and the sensitivity of fibrin-adsorbed plasminogen to plasminogen activators. In a plasma clot system activated with urokinase, clot dissolution only seems to occur after depletion of α_2-antiplasmin (44, 47, 75). Yet during thrombolytic therapy with urokinase, α_2-antiplasmin usually is not exhausted. How and whether this paradox may be explained by a continuous replacement of plasminogen and activator at the surface of a thrombus also remains to be established. Another puzzle is why tissue plasminogen activator has such a low specific activity in the absence of fibrin and such a high specific activity in its presence. It is possible that the activator is reversibly bound to an inhibitor or to other plasma proteins and released by fibrin or it may be that the activator is modified by fibrin. It is also possible that the catalytic role of fibrin is exclusively due to concentration of fibrinolytic components at its surface.

Plasmin-α_2-Antiplasmin complex, an Indicator of In Vivo Activation of the Fibrinolytic System

Activation of the fibrinolytic system results in the formation of plasmin, which has a short lifespan in the blood as it is rapidly bound to and neutralized by α_2-antiplasmin. The plasmin-α_2-antiplasmin complex however (as well as the thrombin-antithrombin III complex) contains new antigenic structures which render it immunochemically distinct from the precursor molecules (158, 159).

For the study of the occurrence in vivo and clinical relevance of this complex, we have developed a simple latex agglutination test for its rapid quantitation in plasma (160). In this method, polystyrene (latex) particles are coated with purified gamma globulins from antisera obtained after prolonged immunization which were absorbed with the precursor proteins. The purified complex was found to cause a clear agglutination of the particles at a concentration of 0.1–0.2 mg per liter. Purified plasminogen and α_2-antiplasmin were 100–500 times less reactive. Activation of fresh human plasma with urokinase caused progressive generation of agglutinating activity up to a plasma dilution of 1/480.

Intravenous injection of streptokinase into patients resulted in an increase of the plasmin-α_2-antiplasmin complex titer to at least 1/240. The titer remained high during the first 3 h after injection and was still raised after 24 h, indicating and confirming that the half-life of this complex in plasma is several hours (117). Seven out of eight patients with diffuse intravascular coagulation of various origin had plasmin-α_2-antiplasmin titers of 1/80 or 1/160 (160). Similar studies were carried out with the thrombin-antithrombin III complex and markedly elevated levels were found in patients with disseminated intravascular coagulation (161).

From these findings it was concluded that primary activation of the fibrinolytic system in vivo, or activation secondary to in vivo coagulation is associated with the appearance of circulating plasmin-α_2-antiplasmin complexes, which can be directly assayed in plasma on the basis of their neoantigenic expression. The measurement of plasmin-α_2-antiplasmin complex may therefore represent an index of ongoing fibrinolytic activation in vivo.

Later it was shown that the discriminatory potential of the hyperimmune antisera used in these earlier studies was not due to the presence of antibodies which are entirely specific for the complex but to antibodies which react faster with the complex than with its precursors (mainly the inhibitor) (162). The development of applicable latex reagents therefore required careful titration of the antibodies to obtain a maximal discrimination between complex and precursors (at least 250 times), and application of this technology on a routine base proved to be tedious.

Recently we have applied different immunization and absorption procedures and thereby obtained antisera which in equilibrium competitive inhibition radioimmunoassays discriminate the plasmin-α_2-antiplasmin (and the thrombin-antithrombin III) complex from their precursors by at least a factor 100. Fig. 5 shows competitive inhibition radioimmunoassays using iodine-labeled plasmin-α_2-antiplasmin complex as ligand and the purified complex and its precursors as competitors. It is hoped that with these more discriminating antisera it will be possible to develop more specific and more sensitive assays for the determination of plasmin-α_2-antiplasmin as well as thrombin-antithrombin III complexes in human blood.

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References

1 Morgagni J B. In: "The seats and causes of diseases", 1769; vol: 3, book 4, London, p. 173. Cited by Fearnley G R (ref 8).
2 Hunter J. In: "A treatise on blood, inflammation and gunshot wounds" 1794; J. Richardson for G. Nicol, London, p. 26. Cited by Fearnley G R (ref 8).
3 Morawitz P. Beitr Chem Physiol Pathol 8, 1. Cited by Fearnley G R (ref 8).
4 Macfarlane R G. Fibrinolysis following operation. Lancet I 1937; 10–12. Cited by Fearnley G R (ref 8).
5 Denys J, de Marbaix H. La Cellule 1889; 5: 197. Cited by Fearnley G R (ref 8).
6 Tillett W S, Garner R L. Fibrinolytic activity of hemolytic streptococci. J Exper Med 1933; 58: 485–502.
7 Astrup T. Fibrinolysis in the organism. Blood 1956; 11: 781–806.
8 Fearnley G R. Fibrinolysis. Adv Drug Res 1973; 7: 107–163.
9 Milstone H. Factor in normal blood which participates in streptococcal fibrinolysis. J Immunol 1941; 42: 109–116.
10 Kaplan M H. Nature and role of the lytic factor in hemolytic streptococcal fibrinolysis. Proc Soc Exper Biol Med 1944; 57: 40–43.
11 Christensen L R. Streptococcal fibrinolysis: proteolytic reaction due to serum enzyme activated by streptococcal fibrinolysin. J Gen Physiol 1945; 28: 363–383.
12 Christensen L R, Macleod C M. Proteolytic enzyme of serum: characterization, activation, and reaction with inhibitors. J Gen Physiol 1945; 28: 559–583.
13 Astrup T, Permin P M. Fibrinolysis in animal organism. Nature 1947; 159: 681–682.
14 Macfarlane R G, Pilling J. Fibrinolytic activity of normal urine. Nature 1947; 159: 779.
15 Williams J R B. The fibrinolytic activity of urine. Brit J Exper Pathol 1951; 32: 530–537.

16 Müllertz S. Plasminogen activator in spontaneously active human blood. Proc Soc Exper Biol Med 1953; 82: 291–295.
17 Wallén P. Activation of plasminogen with urokinase and tissue activator. In: "Thrombosis and urokinase" (Paoletti R, Sherry S eds). Academic Press, London 1977; p 91–102.
18 Thorsen S. Human urokinase and porcine tissue plasminogen activator. Thesis, University of Copenhagen, Laegeforeningens Forlag, Copenhagen 1977.
19 Rijken D C, Wijngaards G, Zaal-de Jong M, Welbergen J. Purification and partial characterization of plasminogen activator from human uterine tissue. Biochim Biophys Acta 1979; 580: 140–153.
20 Cash J D. A new approach to the studies of the fibrinolytic enzyme system in man. Amer Heart J 1968; 75: 424–428.
21 Chakrabarti R, Fearnley G R, Hocking E D, Delitheos A, Clarke G M. Fibrinolytic activity related to age in survivors of myocardial infarction. Lancet 1966; I: 573–574.
22 Pandolfi M, Isacson S, Nilsson I M. Low fibrinolytic activity in the walls of veins of patients with thrombosis. Acta Med Scand 1969; 186: 1–5.
23 Rosing D R, Redwood D R, Brakman P, Astrup T. Impairment of the diurnal fibrinolytic response in man. Effects of aging, type IV hyperlipoproteinemia, and coronary artery disease. Circ Res 1973; 32: 752–758.
24 Peabody R A, Tsapogas M J, Wu K T. Altered endogenous fibrinolysis and biochemical factors in atherosclerosis. Arch Surg 1974; 109: 309–313.
25 Pilgeram L O. Abnormalities in clotting and thrombolysis as a risk factor for stroke. Thromb Diath haemorrh 1974; 31: 245–264.
26 Clayton J K, Anderson J A, McNicol G P. Preoperative prediction of postoperative deep vein thrombosis. Br Med J 1976; 2: 910–912.
27 Niewiarowski S, Prou-Wartelle O. Role of the contact factor (Hageman factor) in fibrinolysis. Thromb Diath haemorrh 1959; 3: 593–603.
28 Ogston D, Ogston C M, Ratnoff O D, Forbes C D. Studies on a complex mechanism for the activation of plasminogen by kaolin and by chloroform: the participation of Hageman factor and additional cofactors. J Clin Invest 1969; 48: 1786–1801.
29 Griffin J H. New hypothesis for the molecular mechanism of surface-dependent activation of Hageman factor (factor XII). Thromb Haemost 1977; 38: 50 (Abstr.).
30 Griffin J H. Role of surface in surface-dependent activation of Hageman factor (blood coagulation factor XII). Proc Natl Acad Sci 1978; 75: 1998–2002.
31 Kaplan A P, Castellino F J, Collen D, Wiman B, Taylor F B. Molecular mechanisms of fibrinolysis in man. Thromb Haemost 1978; 39: 263–283.
32 Kluft C. An inventory of plasminogen activators in human plasma. Thromb Haemost 1978; 38: 134.
33 Hedner U, Nilsson I M. Acquired anticoagulants against factors XI and XII in patients with severe thrombotic disease. 16th Internat Congr of Haematology Kyoto, Japan 1976; (Abstract no 8–88) p 341.
34 Kluft C. Elimination of inhibition in euglobulin fibrinolysis by use of flufenamate: involvement of C1-inactivator. Haemostasis 1977; 6: 351–369.
35 Hedner U. Studies on an inhibitor of plasminogen activation in human serum. Thromb Diath haemorrh 1973; 30: 414–424.
36 Hedner U, Martinsson G. Inhibition of activated Hageman factor (factor XIIa) by an inhibitor of plasminogen activation (PA-inhibitor). Thromb Res 1978; 12: 1015–1023.
37 Stead N, Kaplan A P, Rosenberg R D. Inhibition of activated factor XII by antithrombin-heparin cofactor. J Biol Chem 1976; 251: 6481–6488.
38 McConnell D J. Inhibitors of kallikrein in human plasma. J Clin Invest 1972; 51: 1611–1623.
39 Müllertz S. Mechanism of activation and effect of plasmin in blood. Ph D Thesis, Eijnar Munksgaard, Copenhagen 1956.
40 Gurewich V, Hyde E, Lipinski B. The resistance of fibrinogen and soluble fibrin monomer in blood to degradation by a potent plasminogen activator from cadaver limbs. Blood 1975; 46: 555–565.
41 Collen D. α_2-Antiplasmin inhibitor deficiency. Lancet 1979; I: 1039–1040.
42 Collen D. Identification and some properties of a new fast-reacting plasmin inhibitor in human plasma. Eur J Biochem 1976; 69: 209–216.
43 Moroi M, Aoki N. Isolation and characterization of alpha 2-plasmin inhibitor from human plasma. A novel proteinase inhibitor which inhibits activator-induced clot lysis. J Biol Chem 1976; 251: 5956–5965.
44 Müllertz S, Clemmensen I. The primary inhibitor of plasmin in human plasma. Biochem J 1976; 159: 545–553.
45 Hedner U, Abildgaard U. Report on the joint Meeting of the task forces on nomenclature and standards of inhibitors of coagulation and fibrinolysis. Thromb Haemostas 1978; 39: 524–525.
46 Bagge L, Björk I, Saldeen T, Wallin R. Purification and characterization of an inhibitor of plasminogen activation from posttraumatic patients. Forensic Sci 1976; 7: 83–86.
47 Müllertz S. Different molecular forms of plasminogen and plasmin produced by urokinase in human plasma and their relation to protease inhibitors and lysis of fibrinogen and fibrin. Biochem J 1974; 143: 273–283.
48 Nilehn J-E, Ganrot P O. Plasmin, plasmin inhibitors and degradation products of fibrinogen in human serum during and after intravenous infusion of streptokinase. Scand J Clin Lab Invest 1967; 20: 113–121.
49 Collen D, Wiman B. Fast-acting plasmin inhibitor in human plasma. Blood 1978; 51: 563–569.
50 Astrup T. Fibrinolysis – An overview. In: "Progress in chemical fibrinolysis and thrombolysis, vol 3" (Davidson J F, Rowan R M, Samama M M, Desnoyers P C eds). Raven Press, New York 1978; p 1–57.
51 Reich E. Plasminogen activator: secretion by neoplastic cells and macrophages. In: "Proteases and biological control" (Reich E, Rifkin D B, Shaw E. eds). Cold Spring Harbor Laboratory 1975; p 333–341.
52 Strickland S. Studies on the role of plasminogen activator in ovulation and early embryogenesis. In: "Regulatory proteolytic enzymes and their inhibitors" (Magnusson S, Ottesen M, Foltman B, Danø K, Neurath H, eds). Pergamon Press, Oxford 1978; p. 181–185.
53 Sottrup-Jensen L, Claeys H, Zajdel M, Petersen T E, Magnusson S. The primary structure of human plasminogen: isolation of two lysine-binding fragments and one "mini"-plasminogen (M. W. 38,000) by elastase-catalyzed-specific limited proteolysis. In: "Progress in chemical fibrinolysis and thrombolysis, vol 3" (Davidson J F, Rowan R M, Samama M M, Desnoyers P C, eds). Raven Press, New York 1978, p 191–209.
54 Sottrup-Jensen L, Petersen T E, Magnusson S. In: "Atlas of protein sequence and structure", 1978; Vol 5: suppl 3, p 91.
55 Wiman B. Biochemistry of the plasminogen to plasmin conversion. In: "Fibrinolysis. Current fundamental and clinical aspects" (Gaffney P J, Balkuv-Ulutin S, eds). Academic Press, London 1978; p 47–60.
56 Wallén P, Wiman B. Characterization of human plasminogen. I. On the relationship between different molecular forms of plasminogen demonstrated in plasma and found in purified preparations. Biochim Biophys Acta 1970; 221: 20–30.
57 Wallén P, Wiman B. Characterization of human plasminogen. II. Separation and partial characterization of different molecular forms of human plasminogen. Biochim Biophys Acta 1972; 257: 122–134.
58 Deutsch D G, Mertz E T. Plasminogen: purification from human plasma by affinity chromatography. Science 1970; 170: 1095–1096.
59 Brockway W J, Castellino F J. Measurement of the binding of antifibrinolytic amino acids to various plasminogens. Arch Biochem Biophys 1972; 151: 194–199.
60 Collen D, De Maeyer L. Molecular biology of human plasminogen. I. Physicochemical properties and microheterogeneity. Thromb Diath haemorrh 1975; 34: 396–402.
61 Castellino F J, Siefring G E Jr, Sodetz I M, Bretthauer R K. Amino terminal amino acid sequences and carbohydrate of the two major forms of rabbit plasminogen. Biochem Biophys Res Commun 1973; 53: 845–851.
62 Summaria L, Arzadon L, Bernabe P, Robbins K C. Studies on the isolation of the multiple molecular forms of human plasminogen and

plasmin by isoelectric focusing methods. J Biol Chem 1972; 247: 4691–4702.

63 Collen D. Plasminogen and prothrombin metabolism in man. Thesis, University of Leuven, Belgium 1974.

64 Collen D, Verstraete M. Molecular biology of human plasminogen. II. Metabolism in physiological and some pathological conditions in man. Thromb Diath haemorrh 1975; 34: 403–408.

65 Robbins K C, Summaria L, Hsieh B, Shah R J. The peptide chains of human plasmin. Mechanism of activation of human plasminogen to plasmin. J Biol Chem 1967; 242: 2333–2342.

66 Groskopf W R, Summaria L, Robbins K C. Studies on the active center of human plasmin. Partial amino acid sequence of a peptide containing the active center serine residue. J Biol Chem 1969; 244: 3590–3597.

67 Claeys H, Vermylen J. Physico-chemical and proenzyme properties of NH_2-terminal glutamic acid and NH_2-terminal lysine human plasminogen. Influence of 6-aminohexanoic acid. Biochim Biophys Acta 1974; 342: 351–359.

68 Wallén P, Wiman B. On the generation of intermediate plasminogen and its significance for activation. In: "Proteases and biological control" (Reich E, Rifkin D B, Shaw E, eds). Cold Spring Harbor Laboratory, p 291–303.

69 Thorsen S, Kok P, Astrup T. Reversible and irreversible alterations of human plasminogen indicated by changes in susceptibility to plasminogen activators and in response to epsilon-aminocaproic acid. Thromb Diath haemorrh 1974; 32: 325–340.

70 Rickli E E, Otavsky W I. Release of an N-terminal peptide from human plasminogen during activation with urokinase. Biochim Biophys Acta 1973; 295: 381–384.

71 Wiman B, Wallén P. Activation of human plasminogen by an insoluble derivative of urokinase. Structural changes of plasminogen in the course of activation to plasmin and demonstration of a possible intermediate compound. Eur J Biochem 1973; 36: 25–31.

72 Summaria L, Arzadon L, Bernabe P, Robbins K C. The activation of plasminogen to plasmin by urokinase in the presence of the plasmin inhibitor trasylol. The preparation of plasmin with the same NH_2-terminal heavy (A) chain sequence as the parent zymogen. J Biol Chem 1975; 250: 3988–3995.

73 Violand B N, Castellino F J. Mechanism of the urokinase-catalyzed activation of human plasminogen. J Biol Chem 1976; 251: 3906–3912.

74 Wiman B, Collen D. On the mechanism of the reaction between human α_2-antiplasmin and plasmin. J Biol Chem 1979; (in press).

75 Thorsen S, Müllertz S. Reaction sequences and the mechanism of plasminogen activation in urokinase-activated plasma. Effects of fibrin and 6-aminohexanoic acid. In: "Progress in chemical fibrinolysis and thrombolysis, 1979; Vol: 4" (in press).

76 Christensen U. Kinetic studies of the urokinase-catalysed conversion of NH_2-terminal glutamic acid plasminogen to plasmin. Biochim Biophys Acta 1977; 481: 638–647.

77 Wohl R C, Summaria L, Arzadon L, Robbins K C. Steady state kinetics of activation of human and bovine plasminogens by streptokinase and its equimolar complexes with various activated forms of human plasminogen. J Biol Chem 1978; 253: 1402–1407.

78 Camiolo S M, Thorsen S, Astrup T. Fibrinogenolysis and fibrinolysis with tissue plasminogen activator, urokinase, streptokinase-activated human globulin, and plasmin. Proc Soc Exper Biol Med 1971; 138: 277–280.

79 Markus G, De Pasquale J L, Wissler F C. Quantitative determination of the binding of epsilon-aminocaproic acid to native plasminogen. J Biol Chem 1978; 253: 727–732.

80 Rickli E E, Otavsky W I. A new method of isolation and some properties of heavy chain of human plasmin. Eur J Biochem 1975; 9: 441–447.

81 Thorsen S. Differences in the binding to fibrin of native plasminogen and plasminogen modified by proteolytic degradation. Influence of omega-amino-carboxylic acids. Biochim Biophys Acta 1975; 393: 55–65.

82 Rákóczi I, Wiman B, Collen D. On the biological significance of the specific interaction between fibrin, plasminogen and antiplasmin. Biochim Biophys Acta 1978; 540: 295–300.

83 Wiman B, Wallén P. Structural relationship between "glutamic acid" and "lysine" forms of human plasminogen and their interaction with the NH_2-terminal activation peptide as studied by affinity chromatography. Eur J Biochem 1975; 50: 489–494.

84 Wiman B, Wallén P. The specific interaction between plasminogen and fibrin. A physiological role of the lysine binding site in plasminogen. Thromb Res 1977; 10: 213–222.

85 Ratnoff O D. The surface-mediated initiation of blood coagulation and related phenomena. In: "Haemostasis: biochemistry, physiology and pathology" (Ogston D, Bennett B, eds). John Wiley & Sons, London 1977; p 25–55.

86 Ogston D, Bennett B. Surface-mediated reactions in the formation of thrombin, plasmin and kallikrein. Br Med Bull 1978; 34: 107–112.

87 Astrup T. Tissue activators of plasminogen. Fed Proc 1966; 25: 42–51.

88 Cole E R, Bachmann F W. Purification and properties of a plasminogen activator from pig heart. J Biol Chem 1977; 252: 3729–3737.

89 Wallén P, Kok P, Rånby M. The tissue activator of plasminogen. In: "Regulatory enzymes and their control" (Magnusson S, Ottesen M, Foltman B, Danø K, Neurath H, eds). Pergamon Press, Oxford 1978; p 127–135.

90 Rickli E E, Zaugg G. Isolation and purification of highly enriched tissue plasminogen activator from pig heart. Thromb Diath haemorrh 1970; 23: 64–76.

91 Kok P, Astrup T. Isolation and purification of a tissue plasminogen activator and its comparison with urokinase. Biochemistry 1969; 8: 79–86.

92 Aoki N, von Kaulla K N. The extraction of vascular plasminogen activator from human cadavers and a description of some of its properties. Am J Clin Pathol 1971; 55: 171–179.

93 Pepper D S, Allen R. Isolation and characterization of human cadaver vascular endothelial activator. In: "Progress in chemical fibrinolysis and thrombolysis, 1978; Vol 3" (Davidson J F, Rowan R M, Samama M M, Desnoyers P C, eds). Raven Press, New York, p 91–98.

94 Radcliffe R, Heinze T. Isolation of plasminogen activator from human plasma by chromatography on lysine-Sepharose. Arch Biochem Biophys 1978; 189: 185–194.

95 Fletcher A P, Biederman O, Moore D, Alkjaersig N, Sherry S. Abnormal plasminogen-plasmin system activity (fibrinolysis) in patients with hepatic cirrhosis: its cause and consequences. J Clin Invest 1964; 43: 681–695.

96 Tytgat G, Collen D, De Vreker R A. Investigations on the fibrinolytic system in liver cirrhosis. Acta Haematol 1968; 40: 265–274.

97 Fletcher A P, Alkjaersig N, Sherry S, Genton E, Hirsh J, Bachmann F. The development of urokinase as a thrombolytic agent. Maintenance of a sustained thrombolytic state in man by its intravenous infusion. J Lab Clin Med. 1965; 65: 713–731.

98 Kakkar V V, Scully M F. Thrombolytic therapy. Br Med Bull 1978; 34: 191–199.

99 Paoletti R, Sherry S (eds). Thrombosis and urokinase. Academic Press, London 1977.

100 Verstraete M. A far stretched program: rapid, safe and predictable thrombolysis in man. In: "Fibrinolysis" (Kline D L, Reddy N N, eds). CRC Press, Cleveland 1979 (in press).

101 Müllertz S, Lassen M. An activator system in blood indispensable for the formation of plasmin by streptokinase. Proc Soc Exper Biol Med 1953; 82: 264–268.

102 Brogden R N, Speight T M, Avery G S. Streptokinase: a review of its clinical pharmacology, mechanism of action and therapeutic uses. Drugs 1973; 5: 357–445.

103 Cederholm-Williams S A, De Cock F, Lijnen H R, Collen D. Kinetics of the reactions between streptokinase, plasmin and α_2-antiplasmin. Eur J Biochem 1979; 100: 125–132.

104 Wiman B, Collen D. Molecular mechanism of physiological fibrinolysis. Nature 1978; 272: 549–550.

105 Verstraete M, Vermylen J, Amery A, Vermylen C. Thrombolytic therapy with streptokinase using a standard dosage scheme. Brit Med J 1966; 5485: 454–456.

106 Johnson A J, McCarty W R. The lysis of artificially induced intravascular clots in man by intravenous infusions of streptokinase. J Clin Invest 1959; 38: 1627–1643.

107 Ogston D, Bennett B, Herbert R J, Douglas A S. The inhibition of urokinase by α_2-macroglobulin. Clin Sci 1973; 44: 73–79.

108 Clemmensen I, Christensen F. Inhibition of urokinase by complex formation with human alpha 1-antitrypsin. Biochim Biophys Acta 1976; 429: 591–599.

109 Clemmensen I. Inhibition of urokinase by complex formation with human antithrombin III in absence and presence of heparin. Thromb Haemost 1978; 39: 616–623.

110 Collen D, De Cock F, Verstraete M. Immunochemical distinction between antiplasmin and α_1-antitrypsin. Thromb Res 1975; 7: 245–249.

111 Wiman B, Collen D. Purification and characterization of human antiplasmin, the fast-acting plasmin inhibitor in plasma. Eur J Biochem 1977; 78: 19–26.

112 Hedner U, Collen D. Immunochemical distinction between the inhibitors of plasminogen activation and antiplasmin in human plasma. Thromb Res 1976; 8: 875–879.

113 Edy J, Collen D. The interaction in human plasma of antiplasmin, the fast-reacting plasmin inhibitor with plasmin, thrombin, trypsin and chymotrypsin. Biochim Biophys Acta 1977; 484: 423–432.

114 Ohlsson K, Collen D. Comparison of the reactions of neutral granulocyte proteases with the major plasma protease inhibitors and with antiplasmin. Scand J Clin Lab Invest 1977; 37: 345–350.

115 Christensen U, Clemmensen I. Kinetic properties of the primary inhibitor of plasmin from human plasma. Biochem J 1977; 163: 389–391.

116 Wiman B, Collen D. On the kinetics of the reaction between human antiplasmin and plasmin. Eur J Biochem 1978; 84: 573–578.

117 Collen D, Wiman B. Turnover of antiplasmin, the fast-acting plasmin inhibitor of plasma. Blood 1979; 53: 313–324.

118 Edy J, Collen D, Verstraete M. Quantitation of the plasma protease inhibitor antiplasmin with the chromogenic substrate S-2251. In: "Progress in chemical fibrinolysis and thrombolysis, Vol 3" (Davidson J F, Rowan R M, Samama M M, Desnoyers P C, eds). Raven Press, New York 1978; p 315–322.

119 Teger-Nilsson A C, Friberger P, Gyzander E. Determination of a new rapid plasmin inhibitor in human blood by means of a plasmin specific tripeptide substrate. Scand J Clin Lab Invest 1977; 37: 403–409.

120 Aoki N. Natural inhibitors of fibrinolysis. Progr. Cardiovasc Dis 1979; 21: 267–286.

121 Verstraete M, Vermylen J, Schetz J. Biochemical changes noted during intermittent administration of streptokinase. Thromb Haemostas 1978; 39: 61–68.

122 Barrett A J, Starkey P M, Munn E A. The unique nature of the interaction of α_2-macroglobulin with proteinases. In: "Proteinase inhibitors" (Fritz H, Tschesche H, Greene L J, Truscheit E, eds). Springer Verlag, Berlin 1974; p 72–77.

123 Laurell C B, Jeppsson J O. Protease inhibitors in plasma. In: "The plasma proteins" second edition, vol 1 (Putnam F W, eds). Academic Press, New York 1975; chapter 5.

124 Harpel P C. Human alpha 2-macroglobulin. Methods Enzymol 1976; 45: 639–652.

125 Sawyer W D, Fletcher A P, Alkjaersig N, Sherry S. Studies on the thrombolytic activity of human plasma. J Clin Invest 1960; 39: 426–434.

126 McNicol G P., Fletcher A P, Alkjaersig N, Sherry S. Impairment of hemostasis in the urinary tract: the role of urokinase. J Lab Clin Med 1961; 58: 34–46.

127 Nilsson L, Rybo G. Treatment of menorrhagia with an antifibrinolytic agent, tranexamic acid (AMCA). A double-blind investigation. Acta Obstet Gynec Scand 1967; 46: 572–580.

128 Nilsson I M, Krook H, Sternby N H, Söderberg E, Soderström N. Severe thrombotic disease in a young man with bone marrow and skeletal changes and with a high content of an inhibitor of the fibrinolytic system. Acta Med Scand 1961; 169: 323–337.

129 Brakman P, Albrechtsen O K, Astrup T. A comparative study of coagulation and fibrinolysis in blood from normal men and women. Brit. J Haemat 1966; 12: 74–85.

130 Koie K, Kamiya T, Ogata K, Takamatsu J. α_2-Plasmin-inhibitor deficiency (Miyasato disease). Lancet 1978; ii: 1334–1336.

131 Aoki N, Moroi M, Sakata Y, Yoshida N. Abnormal plasminogen. A hereditary molecular abnormality found in a patient with recurrent thrombosis. J Clin Invest 1978; 61: 1186–1195.

132 Collen D, Tytgat G N, Claeys H, Piessens R. Metabolism and distribution of fibrinogen. I. Fibrinogen turnover in physiological conditions in humans. Brit J Haemat 1972; 22: 681–700.

133 Cash J D. Control mechanisms of activator release. In: "Progress in chemical fibrinolysis and thrombolysis, Vol 3" (Davidson J F, Rowan R M, Samama M M, Desnoyers P C, eds). Raven Press, New York 1978; p 65–75.

134 Cash J D. Platelet, fibrinolysis and stress. In: "Thrombosis-risk factors and diagnostic approaches" (Brinkhous K M, ed). Schattauer Verlag, Stuttgart 1972; p 93.

135 Markwardt F, Klocking H P. Studies on the release of plasminogen activator. Thromb Res 1976; 8: 217–223.

136 Barker J L, Crayton J W, Nicoll R A. Supraoptic neurosecretory cells: adrenergic and cholinergic sensitivity. Science 1972; 171: 208–210.

137 Hawkey C M, Britton B J, Wood W G, Peele M, Irving M H. Changes in blood catecholamine levels and blood coagulation and fibrinolytic-activity in response to graded exercise in man. Br J Haematol 1975; 29: 377–384.

138 Pina-Cabral J M, Rodrigues C. Blood catecholamine levels, factor VIII and fibrinolysis after therapeutic electroshock. Br J Haematol 1974; 28: 371–380.

139 Nilsson I M. Effect of drugs on activator synthesis and release. In: "Progress in chemical fibrinolysis and thrombolysis, Vol 3" (Davidson J F, Rowan R M, Samama M M, Desnoyers P C, eds). Raven Press, New York 1978; p 77–89.

140 Davidson J F, Lochhead M, McDonald G A, McNicol G P. Fibrinolytic enhancement by stanazolol: a double blind trial. Br J Haematol 1972; 22: 543–559.

141 Nilsson I M. Phenformin and ethylestrenol in recurrent venous thrombosis. In: "Progress in chemical fibrinolysis and thrombolysis, Vol 1" (Davidson J F, Samama M M, Desnoyers P C, eds). Raven Press, New York 1975; p 1–12.

142 Alkjaersig N, Fletcher A P, Sherry S. The mechanism of clot dissolution by plasmin. J Clin Invest 1959; 38: 1086–1095.

143 Ambrus C M, Markus G. Plasmin-antiplasmin complex as a reservoir of fibrinolytic enzyme. Amer J Physiol 1960; 199: 491–494.

144 Chesterman C N, Allington M J, Sharp A A. Relationship of plasminogen activator to fibrin. Nature 1972; 238: 15–17.

145 Wiman B, Lijnen H R, Collen D. On the specific interaction between the lysine-binding sites in plasmin and complementary sites in α_2-antiplasmin and in fibrinogen. Biochim Biophys Acta 1979; 579: 142–159.

146 Wiman B, Collen D. On the role of α_2-antiplasmin in the regulation of fibrinolysis. In: "The physiological inhibitors of coagulation and fibrinolysis" (Collen D, Wiman B, Verstraete M, eds). Elsevier/North Holland 1979; p 177–185.

147 Collen D, Verstraete M. α_2-Antiplasmin consumption and fibrinogen breakdown during thrombolytic therapy. Thromb Res 1979; 14: 631–639.

148 Thorsen S, Müllertz S. Rate of activation and electrophoretic mobility of unmodified and partially degraded plasminogen. Effects of 6-aminohexanoic acid and related compounds. Scand J Clin Lab Invest 1974; 34: 167.

149 Collen D, Semeraro N, Tricot J P, Vermylen J. Turnover of fibrinogen, plasminogen, and prothrombin during exercise in man. J Appl Physiol 1977; 42: 865–873.

150 Collen D, Vermylen J. Metabolism of iodine-labeled plasminogen during streptokinase and reptilase therapy in man. Thromb Res 1973; 2: 239–250.

151 Collen D, Verstraete M. Plasmin-antiplasmin complex formation during defibrase infusion in man. Thromb Res 1977; 11: 417–420.

152 Collen D, Tytgat G, Claeys H, Verstraete M, Wallén P. Metabolism of plasminogen in healthy subjects: effect of tranexamic acid. J Clin Invest 1972; 51: 1310–1318.

153 Collen D, Rouvier J, Chamone D F, Verstraete M. Turnover of radiolabeled plasminogen and prothrombin in cirrhosis of the liver. Eur J Clin Invest 1978; 8: 185–188.

154 Kakkar V V, Sagar S, Lewis M. Treatment of deep-vein thrombosis with intermittent streptokinase and plasminogen infusion. Lancet 1975; ii: 674–676.

155 Brochier M, Planiol T, Griguer P, Raynaud Ph, Fauchier J P, Charbonnier B, Latour F, Pellois A. Intérêt du traitement séquentiel lysyl-plasminogène-urokinase et thérapeutique thrombolytique. Coeur Med Interne 1977; 16: 513–521.

156 Marbet G A, Walter M, Six P, Nyman D, Rüst O, Biland L, Duckert F, Madar G, Da Silva A, Widmer L K, Schmitt H E, Vokal J. Vergleich verschiedener fibrinolytischer Methoden zur Behandlung venöser Thrombosen. In: "Blutgerinnung und Antikoagulation (Neuhaus K, Duckert F, eds). Schattauer Verlag, Stuttgart 1976; p 87.

157 Latallo Z S, Lopaciuk S, Meissner J. A combined treatment with Defibrase and streptokinase. Akt Probl Angiol 1975; 26: 181.

158 Collen D, De Cock F. Emergence in plasma during activation of the coagulation or fibrinolytic systems of neoantigens, associated with the complexes of thrombin or plasmin with their inhibitors. Thromb Res 1974; 5: 777–779.

159 Collen D, De Cock F. A tanned red cell hemagglutination inhibition immunoassay (TRCHII) for the quantitative estimation of thrombin-antithrombin III and plasmin-α_1-antiplasmin complexes in human plasma. Thromb Res 1975; 7: 235–238.

160 Collen D, De Cock F, Cambiaso C L, Masson P. A latex agglutination test for rapid quantitative estimation of the plasmin-antiplasmin complex in human plasma. Eur J Clin Invest 1977; 7: 21–26.

161 Collen D, De Cock F, Verstraete M. Quantitation of thrombin-antithrombin III complexes in human blood. Eur J Clin Invest 1977; 7: 407–411.

162 Plow E F, De Cock F, Collen D. Immunochemical characterization of the plasmin-antiplasmin system. Basis for the specific detection of the plasmin-antiplasmin complex by latex agglutination assay. J Lab Clin Med 1979; 93: 199–209.