High accumulation of plasminogen and tissue plasminogen activator at the flow surface of mural fibrin in the human arterial system

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Purpose: We assessed the fibrinolytic activity of the organized mural thrombus lining of aneurysms and prosthetic grafts.

Methods: Between May 1995 and April 1998, the full-thickness mural thrombi of aneurysms and the pseudointima lining of vascular grafts were obtained from 12 patients, ranging from 55 to 78 years in age, who underwent elective surgery. These included five aortic arch aneurysms, four abdominal aortic aneurysms, and three patent synthetic vascular grafts. The specimens were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/immunoblot and immunohistochemistry for human plasmin/plasminogen, tissue plasminogen activator (tPA), and fibrin degradation product (D-dimer).

Results: In the SDS-PAGE/immunoblot, 25- and 27-kd bands appeared specifically in experimental fibrin plates after limited digestion by plasmin and were also recognized in the mural thrombi. The presence of bands at 25 and 27 kd, which were most prominent in sections near the flow surface layer, was consistent with the hypothesis that the mural fibrin was digested by the endogenous plasmin. Apparent immunoreactivity was found at the flow surface of the masses at a thickness of 10 to 400 μm, suggesting the presence of a plasminogen and tPA-rich layer, with D-dimer as a consequential product of fibrinolysis.

Conclusion: The hypothesis that fibrin surfaces in the arterial system acquire fibrinolytic activity because of digestion by circulating endogenous plasmin was confirmed; this may contribute to the antithrombogenicity of these flow surfaces. (J Vasc Surg 2000;32:374-82.)

Most aneurysms are commonly associated with a thick mural thrombus, whereas oversized prosthetic grafts deposit a laminated mural thrombus, which shapes its lumen down to a normal-size channel.[1,2]

After being narrowed down to the size of runoff, the caliber remains fairly constant as long as the hemodynamic conditions maintain stability, which suggests sufficient antithrombogenicity of the fibrin flow surface with a physiologic shear rate. Some reports have suggested that the fibrin flow surface may acquire antithrombogenicity[3-5]; however, these reports did not provide any clues concerning the substantial mechanism that explains the increased antithrombogenicity.

In the last three decades, the activation pathways of plasminogen and its inhibitors have been extensively investigated.[6,7] These studies have demonstrated that when fibrin is digested by plasmin, the binding of both plasminogen and tissue plasminogen activator (tPA) to fibrin increases. Thus, a plasmin-digested fibrin enhances the activation rate

of plasminogen[8,9] and potentiates the fibrinolytic process. Sakharov and Rijken[10] recently reported in their experimental model that the generation of surface-associated plasminogen-binding sites during thrombolysis results in a strikingly high plasminogen concentration at the surface of a lysing clot. Despite this background, however, few reports have focused on the fibrinolytic activity on the flow surface of mural fibrin in the human arterial system.[3,4,11]

To identify the mechanisms responsible for the antithrombogenicity suggested by the experimental and clinical findings, we investigated the fibrinolytic activity of the mural thrombus of aneurysms and prosthetic grafts in humans.

METHODS

Between May 1995 and April 1998, the full-thickness mural thrombi of unruptured aortic aneurysms and the pseudointima of synthetic vascular grafts were obtained from 12 unselected patients who underwent elective surgery (11 men and one woman; age range, 55-78 years; mean age, 68.5 ± 6.9 years). These included five distal aortic arch aneurysms, four abdominal aortic aneurysms, and three patent synthetic vascular grafts: two knitted Dacron grafts with well-healed inner capsules (Bionit II, C.R. Bard, Billerica, Mass, and Gelsoft, Vascutek, Ayr, United Kingdom) and an ovine biosynthetic graft (Omniflow, Bio Nova, North Melbourne, Australia), which is a composite vascular prosthesis composed of glutaraldehyde-tanned ovine tissue and polyester mesh tubing. These grafts were removed during revision operations for graft complication at 8, 28, and 36 months after implantation. The indications for revision included distal anastomotic stenosis, graft infection of the aortofemoral bypass graft, and an aneurysm of the femoropopliteal bypass graft.

Specimen preparation. The aortic aneurysms were opened longitudinally after circulatory arrest was established or after crossclamping of the infrarenal aorta, and the mural fibrin masses, which had a thickness of 1 to 4 cm, were obtained en bloc from the lumen of the aorta. The synthetic grafts were crossclamped after 1 mg/kg of heparin was administered, and a disease-free segment with a length of 5 cm was dissected by means of the no-touch technique. These fibrin specimens were immediately rinsed gently, then frozen in normal saline at –80°C for later examinations. The frozen fibrin specimens were defrosted in normal saline at 37°C, cross-sectioned, and divided into two to ten 2 mm-thick layers, according to the thickness of the thrombus. The samples obtained from each layer were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)/ immunoblot, whereas the remaining specimens were also subjected to histologic examination, scanning electron microscopy (SEM), and immunohistochemical analysis.

Preparation of plasmin-treated fibrin plates. Cross-linked fibrin plates were prepared by dissolving 100 mg human fibrinogen (Yoshitomi Pharmacy, Osaka, Japan) in 50 mL phosphate buffered saline (PBS; 50 mmol/L, pH 7.5). This fibrinogen preparation contained approximately 10 U/mg of factor XIII (manufacturer's data). A 0.2-mL aliquot of the solution was placed on a plate and spread uniformly, and 10-µL of 20 U/mL thrombin solution (Mochida Pharmacy, Osaka, Japan) containing 20 mmol/L $CaCl_2$, 2 mmol/L $MgCl_2$, and 5 mmol/L HEPES buffer (pH 7.4; Sigma, Tokyo, Japan) with 150 mmol/L NaCl was added. The mixture was incubated for 24 hours at 37°C, and a cross-linked fibrin plate was then obtained.

To assist in the possible identification of the fibrin digestion products of our human specimens, we exposed the fibrin plates to various proteolytic enzymes, including plasmin, elastase, and a neutrophil extract. For the analysis of plasmin digestion, 1 mL of 0.01 U/mL plasmin solution (Protogen AG, Läufeltingen, Switzerland) was applied to 0.4 mg of the fibrin plate for either 1 or 3 hours, and it was rinsed with normal saline to remove residual plasmin. For elastase digestion analysis, 0.01 U/mL of elastase (Cosmo Bio, Tokyo, Japan) was applied to 0.4 mg of the fibrin plate. Neutrophils were separated by means of isopyknic centrifugation by using a Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden).[12] The extract of neutrophil solution contained cathepsin G, neutrophil elastase, and other proteases. The mixture was incubated for 1 hour at 37°C, and each protease that had been applied to the fibrin plates was then discarded; the remaining fibrin sample was then rinsed with normal saline and analyzed by means of SDS-PAGE. The in vitro data obtained from the plasmin-treated fibrin plate were used to identify the specific bands of the analyzed mural fibrin samples.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis/immunoblot. The fibrin plate samples (0.4 mg) were placed in an incubation well containing 0.5 mL of 10 mmol Tris/HCl/buffer (pH 7.4) with 2% SDS, 9 mol/L urea, and 2% mercaptoethanol (fibrin-dissolving buffer) and were incubated in a shaker for 18 hours at 60°C. Each dissolved
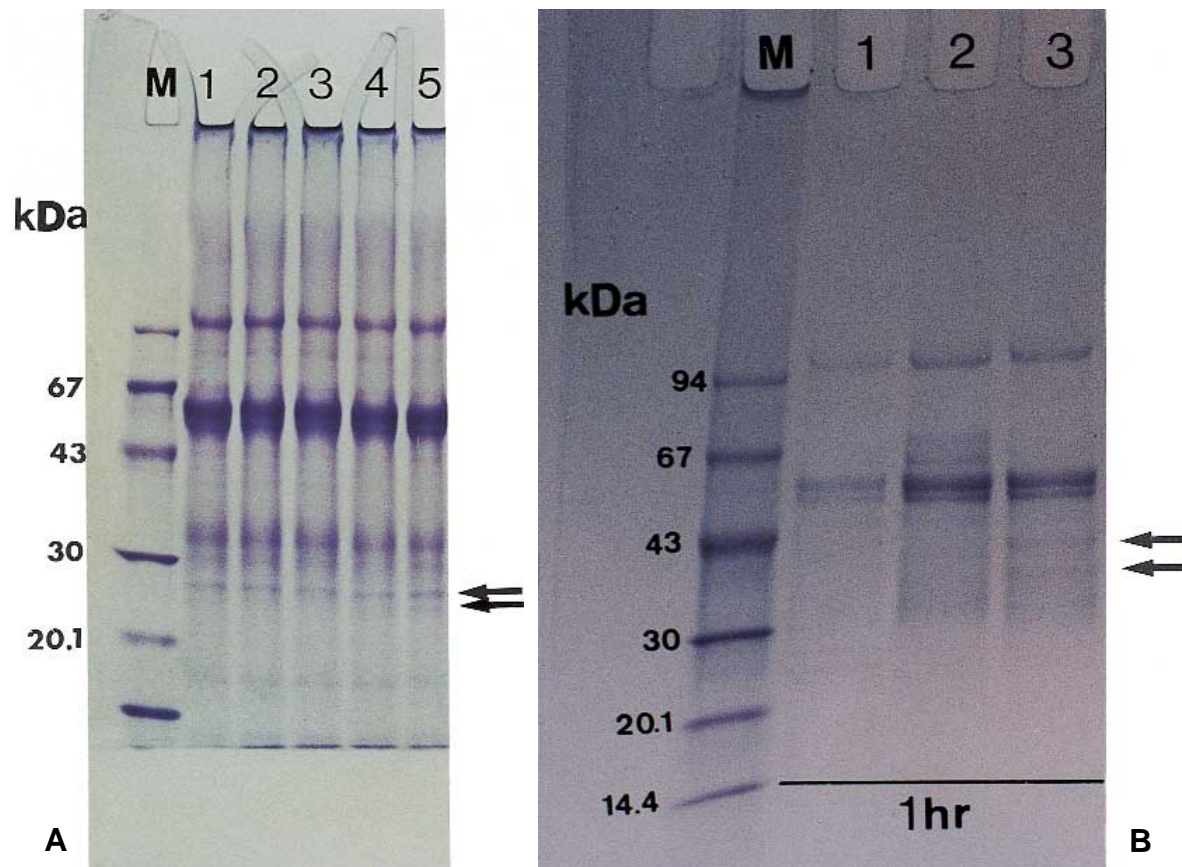
Fig 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the cross-linked fibrin plates after limited digestion with various enzymes. **A,** 25- and 27-kd bands (*arrows*) were recognized specifically after the plasmin digestion. *1,* Untreated fibrin; *2,* plasmin digestion for 5 minutes; *3,* plasmin digestion for 15 minutes; *4,* plasmin digestion for 30 minutes; *5,* plasmin digestion for 60 minutes; *M,* molecular-weight standard. **B,** 36- and 43-kd bands appeared after digestion with extraction of neutrophil solution for 60 minutes (lane 3, *arrows*). *1,* Plasmin digestion; *2,* elastase digestion.

fibrin sample was subjected to SDS-PAGE (gel concentration at 4%-20%) (Tefco, Tokyo, Japan), in accordance with the method reported by Laemmli.[13] In the analysis of the samples, 20- to 28-mg wet-weight fibrin samples were obtained from several layers of each mural fibrin sample. Likewise, each sample was placed in an incubation well containing 1 mL of the fibrin-dissolving buffer, incubated in the same manner, and subjected to SDS-PAGE. Two-percent mercaptoethanol was added to disrupt the S-S binding sites, so that the subunits composing the fibrin molecule could be examined. The protein bands were stained with Coomassie Blue containing a mixture of H_2O-methanol-acetic acid (10:8:2, vol/vol/vol) and then destained with the mixture of H_2O-methanol-acetic acid solution (19:7:2, vol/vol/vol).

We further examined the specificity of the bands by using immunoblot analysis[14] (Clear blot P membrane,

ATTO, Tokyo, Japan), with peroxidase-labeled anti-human fibrinogen antibody (PPO56, The Binding Site, Birmingham, United Kingdom). The column was stained with a 1:100 dilution of the antifibrinogen antibody and made visual with 4-chloro-1-naftol (Tokyo Chemical Industry, Tokyo, Japan).

Histologic examination and scanning electron microscopy. The mural thrombi from the aneurysms were cross-sectioned and divided into two to three layers (surface, middle, outer), whereas the vascular grafts were cross-sectioned, longitudinally sectioned, or both. Each sectioned specimen was fixed with 10% neutral buffered formalin solution for 24 hours at 4°C. After fixation, the specimens were embedded in paraffin, sectioned, and stained with the hematoxylin-eosin, azan, phosphotungstic acid-hematoxylin, and Masson trichrome methods.
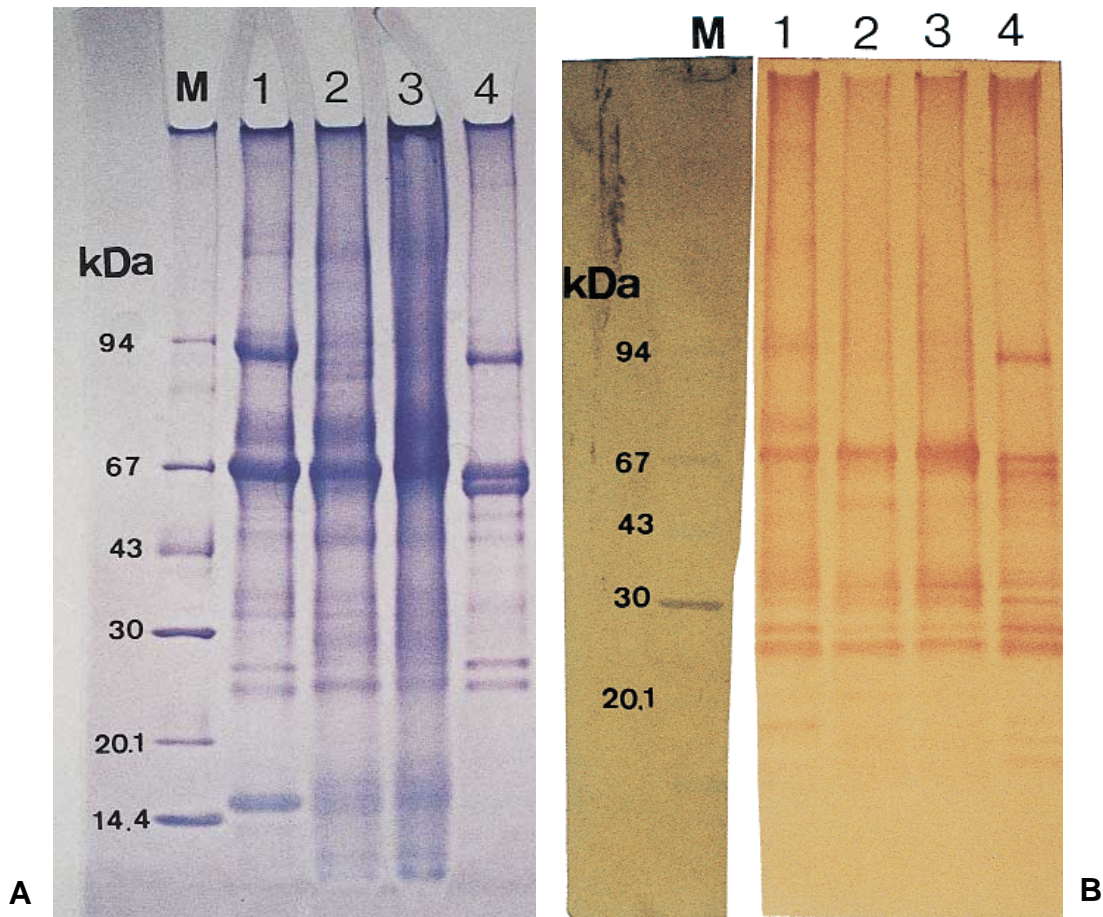
Fig 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis/immunoblot of the mural fibrin mass obtained from an aortic arch aneurysm. **A,** 25- and 27-kd bands were more clearly visible in lane 1 when compared with those in lanes 2 and 3, confirming that the flow surface of the mural fibrin had been digested by endogenous plasmin. A 43-kd band was recognized in lanes 2 to 3, suggesting digestion with proteases derived from neutrophils. **B,** Immunoblot, revealing that all specific protein bands that appeared in Fig 3*A* were fibrin fragments. *1 through 3,* Mural fibrin; *1,* flow surface; *2,* middle layer; *3,* outer layer; *4,* plasmin treated experimental cross-linked fibrin; *M,* molecular-weight standard.

The surfaces of the fibrin specimens were observed with SEM. Each specimen was fixed overnight with 1% glutaraldehyde in PBS. After rinsing in PBS, the specimen was postfixed in 1% osmium tetroxide, dehydrated, dried in the critical point dryer, and sputter-coated with platinum.

Immunohistochemistry. To investigate the distribution of fibrinolytic activity in the specimens, we performed immunohistochemical analysis, by using the labeled avidin-biotin immunoperoxidase technique, with antihuman plasmin/plasminogen and anti-tPA mouse monoclonal antibodies (murine MAb #3642, American Diagnostica, Greenwich, Conn, and PAM-1, Biopool AB, Umeà, Sweden, respectively), and antifibrin degradation product (D-

dimer) monoclonal antibody (2F7#84911, Celsus Laboratories, Cincinnati, Ohio) as the primary antibodies. These three types of antibodies were applied to three different continuous sections (ie, one section for each antibody), and a negative control was used for each staining.

After deparaffinization of the paraffin sections, blockage of endogenous peroxidase activity was performed for 30 minutes with 0.3% H_2O_2 in methanol as a diluent; the sections were then incubated in a 1:200 or 1:500 dilution of each primary monoclonal antibody for 60 minutes at room temperature. After being washed twice with PBS for 5 minutes each, the sections were incubated in biotinylated secondary antibody for 30 minutes at room temper-
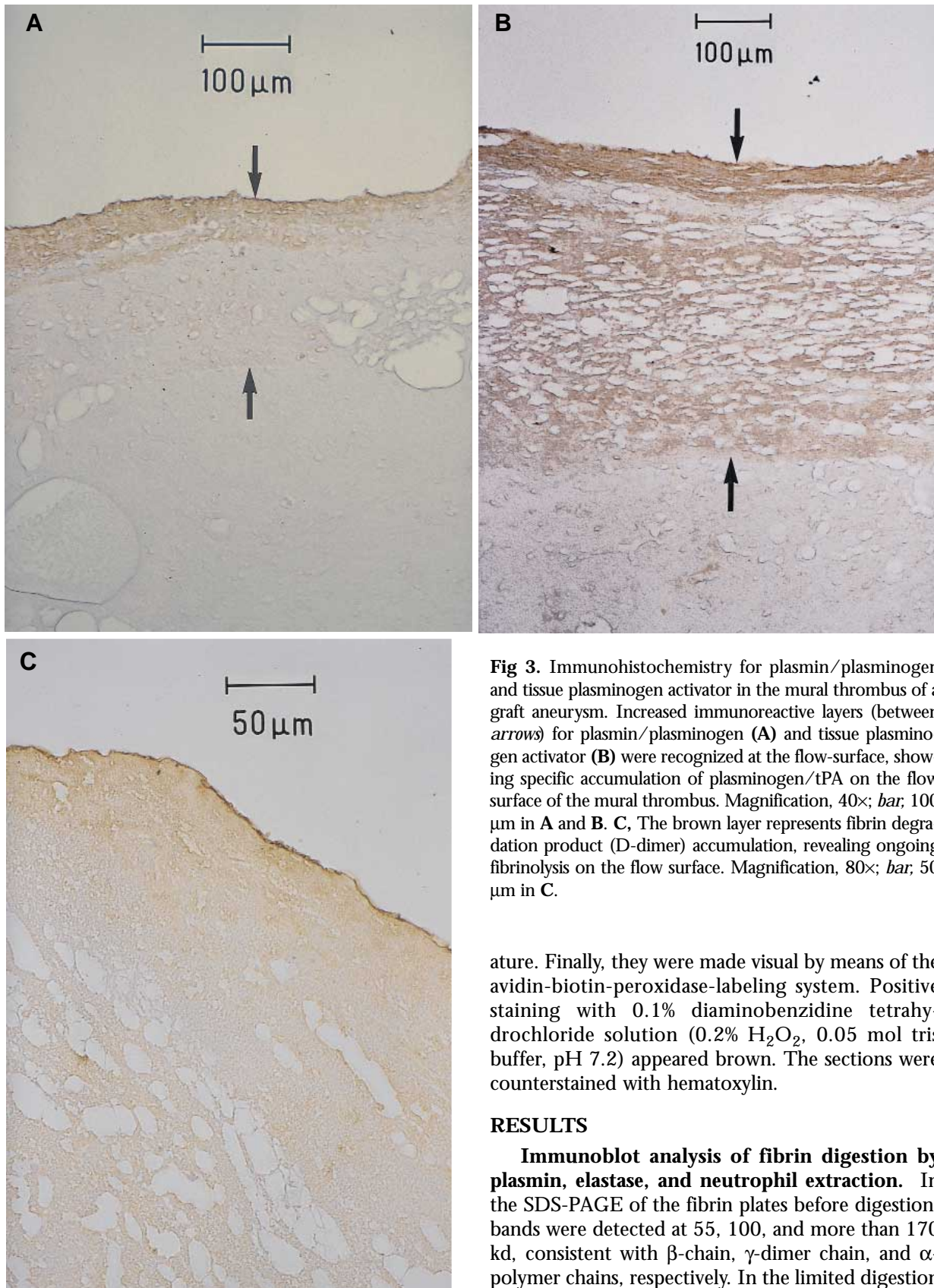
Fig 3. Immunohistochemistry for plasmin/plasminogen and tissue plasminogen activator in the mural thrombus of a graft aneurysm. Increased immunoreactive layers (between *arrows*) for plasmin/plasminogen **(A)** and tissue plasminogen activator **(B)** were recognized at the flow-surface, showing specific accumulation of plasminogen/tPA on the flow surface of the mural thrombus. Magnification, 40×; *bar,* 100 μm in **A** and **B**. **C,** The brown layer represents fibrin degradation product (D-dimer) accumulation, revealing ongoing fibrinolysis on the flow surface. Magnification, 80×; *bar,* 50 μm in **C**.

ature. Finally, they were made visual by means of the avidin-biotin-peroxidase-labeling system. Positive staining with 0.1% diaminobenzidine tetrahydrochloride solution (0.2% H_2O_2, 0.05 mol tris buffer, pH 7.2) appeared brown. The sections were counterstained with hematoxylin.

RESULTS

Immunoblot analysis of fibrin digestion by plasmin, elastase, and neutrophil extraction. In the SDS-PAGE of the fibrin plates before digestion, bands were detected at 55, 100, and more than 170 kd, consistent with β-chain, γ-dimer chain, and α-polymer chains, respectively. In the limited digestion
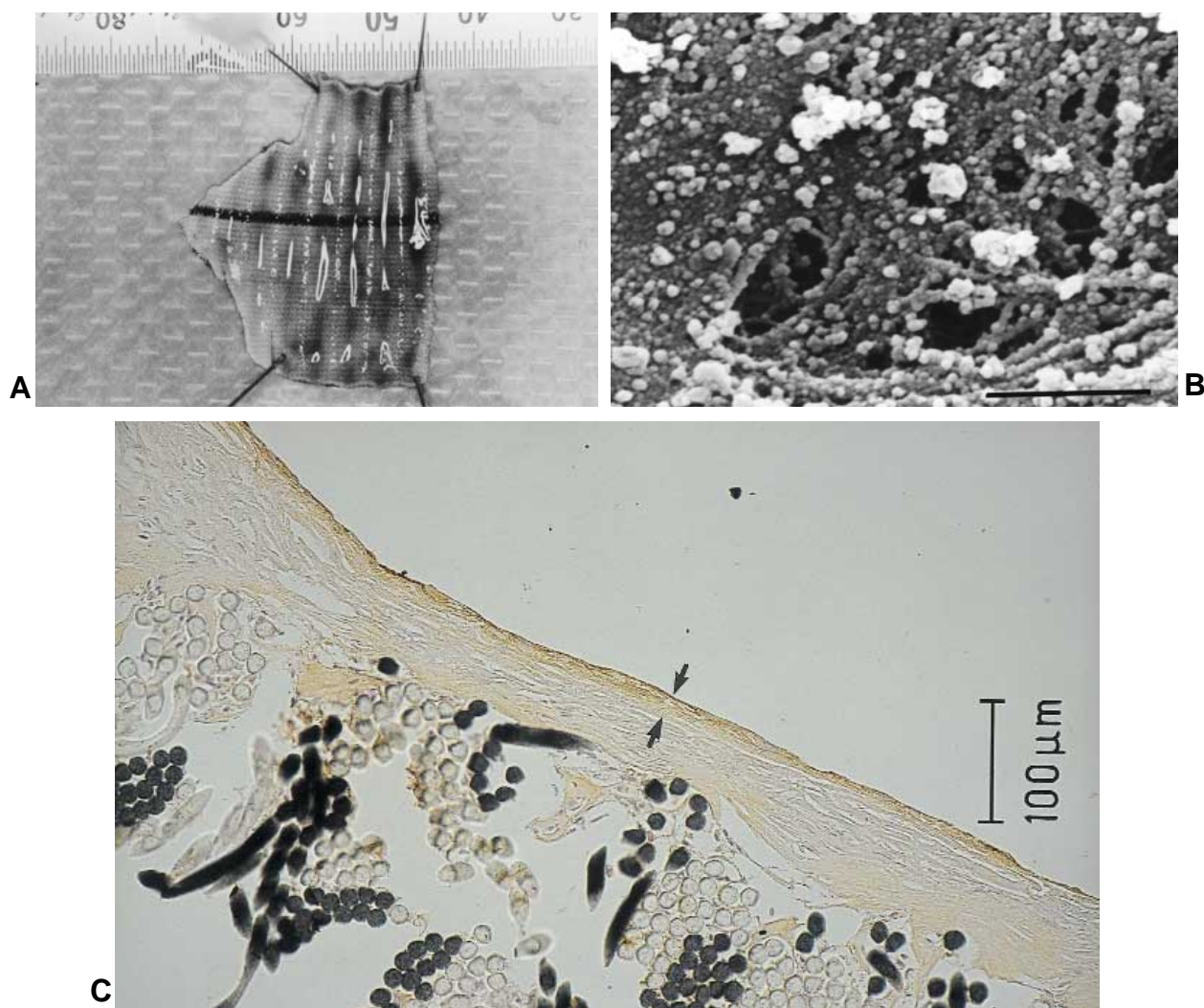
Fig 4. Immunohistochemistry for plasmin/plasminogen, tissue plasminogen activator, and fibrin degradation product (D-dimer) in a knitted Dacron graft with a well-healed inner capsule retrieved 8 months after implantation. **A,** The flow surface of the graft. **B,** Scanning electron microscopy for the flow surface showing compact fibrin. Magnification, 35,000×; *bar,* 857 nm. Increased immunoreactivities (between *arrows*) for plasmin/plasminogen **(C).**

by each enzyme, 25- and 27-kd bands were recognized specifically only after the plasmin treatment (Fig 1), suggesting that these bands represented the fibrin fragments produced by the plasmin digestion. The 36- and 43-kd bands were observed when the neutrophil extraction was applied to the fibrin plate.

Immunoblot analysis of specimens. In the SDS-PAGE for the mural thrombi obtained from the aorta or pseudointima of the grafts, several bands were observed. According to the analysis of the experimental fibrin plates, the bands that appeared at 55, 100, and more than 170 kd were consistent with the β-chain, γ-dimer chain, and α-polymer chains, respec-

tively, suggesting that the mural thrombus of aneurysms is mainly composed of cross-linked fibrin polymer. The bands at 25 and 27 kd were most prominent in sections near the flow surface layer (Fig 2, *A*). All the proteins that appeared in the SDS-PAGE of the mural thrombi were demonstrated by means of the immunoblot to be fibrin fragments (Fig 2, *B*). The presence of a 43-kd band was more prominent in the middle and outer layers of the mural thrombus. No qualitative differences were found among the results of the SDS-PAGE/immunoblot in the samples obtained from the abdominal aortic aneurysms, thoracic aortic aneurysms, and vascular graft lining.
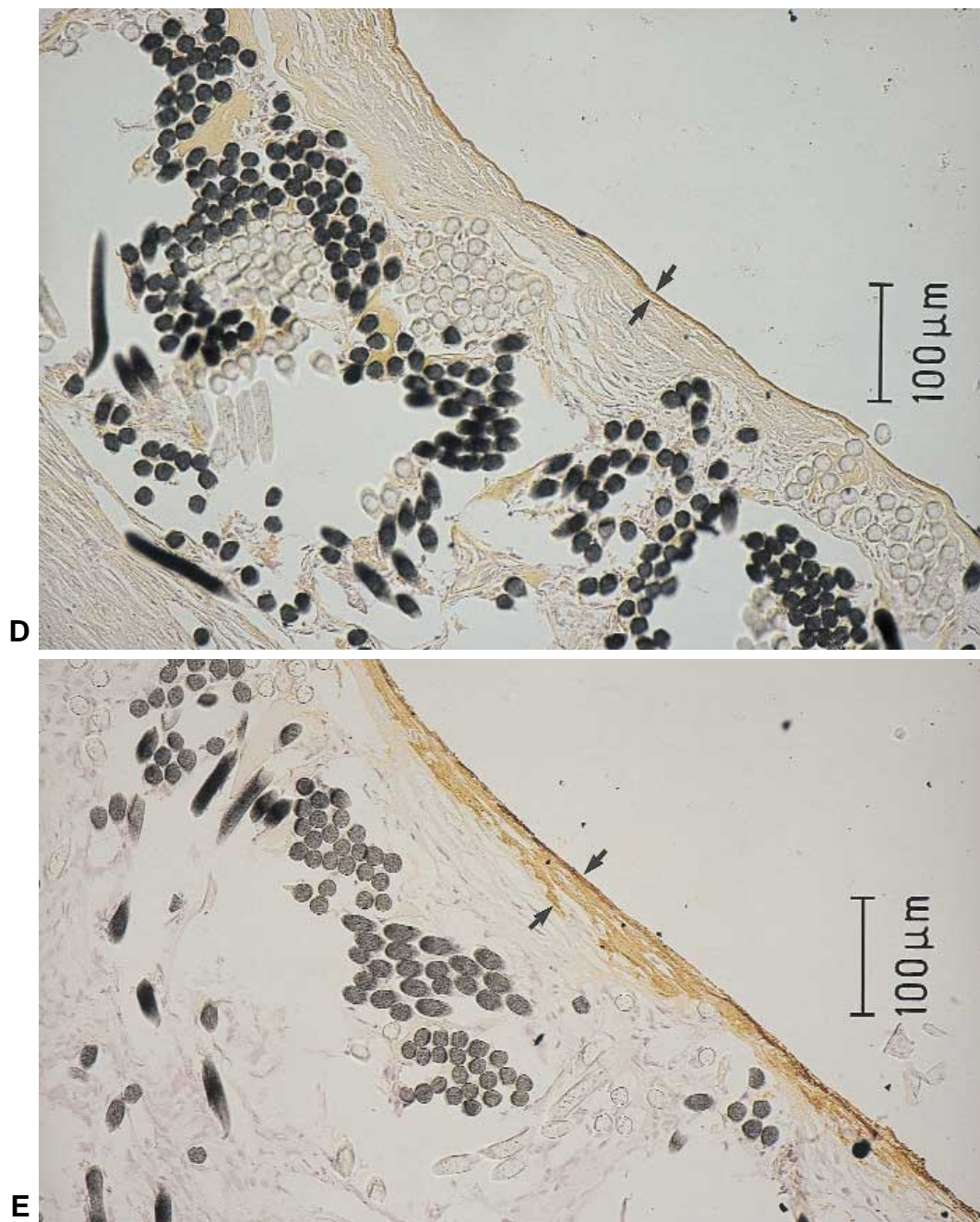
Fig 4 Cont'd. Tissue plasminogen activator **(D)**, and D-dimer **(E)** on the fibrin lining of the pseudointima, suggesting fibrinolysis on the flow surface. Magnification, 40×; *bar,* 100 μm in **C** through **E**.

Histologic examination, scanning electron microscopy, and immunohistochemistry. The samples obtained from the aneurysm wall thrombus were stained uniformly; however, the middle and outer layers had a variable staining pattern, suggesting exposure to various enzymes for a long period. The flow surface layer was revealed by means of SEM to consist of compact fibrin. Positive uniform stain-

ing with a thickness of approximately 10 to 400 μm at the flow surface was demonstrated by means of immunohistochemical staining for plasmin/plasminogen, tPA, and D-dimer (Fig 3). This pattern of staining, with high accumulations of both plasminogen and tPA and with D-dimer as a consequential product of fibrinolysis, is consistent with our hypothesis.

The formation of well-healed pseudointima was demonstrated by means of hematoxylin-eosin staining of the graft samples; however, the flow surface was lined with a thin compact fibrin layer, the observation of which was confirmed by means of both histologic examination and SEM (Fig, 4, *A* and *B*). Accumulations of plasmin/plasminogen and tPA, with a thickness of 10 to 20 μm, and an increased immunoreactivity for D-dimer, with a thickness of 20 to 50 μm, was demonstrated by means of immunohistochemical analysis of the fibrin lining (Fig 4, *C, D,* and *E*), suggesting fibrinolysis on the flow surface.

DISCUSSION

More than 40 years ago, Todd[15] and Astrup[16] recognized that the intima of vessels possessed intrinsic fibrinolytic activity. Almost 20 years later, Levin and Loskutoff[17] demonstrated that cultured bovine endothelial cells had the capacity to produce and bind both urokinase and tPA, and Yao et al[11] demonstrated fibrinolytic activity on the luminal surface of unoccluded Dacron grafts. Underwood et al observed that the intrinsic fibrinolytic activity ranked in the order of endarterectomy cores, then internal mammary artery, then saphenous vein[18] and that the impaired activity may contribute to early graft thrombosis.[19] These observations suggest that fibrinolytic activity on the flow surface plays an important role in maintaining the antithrombogenicity of the vessels.

Fibrinolysis in the vascular system is regulated by various pathways of plasminogen to plasmin.[6,7,20] The major plasminogen activator inhibitor (PAI), PAI-1, is released by endothelial cells and is inactivated rapidly by incorporation into the plasminogen activator. A constant process of fibrinolysis and clot formation exists on the endothelial surface, which is regulated by the balance of tPA and PAI-1. The plasma concentration of PAI-1 in normal conditions is only approximately 20 ng/mL,[21] whereas the binding of plasminogen and tPA increases progressively as a function of the degradation of the fibrin surface.[22] When fibrin is treated with plasmin, the binding of plasminogen to the superficial layer of a fibrin clot markedly increases.[9,23-26] The binding of tPA to fibrin also increases after limited plasmin digestion, depending on the generation of carboxyl-terminal lysin residues.[27]

The enhancing mechanism of the fibrinolysis on the fibrin surface has been studied in experimental fibrin clot models. In the presence of fibrin, tPA strikingly enhances the activation rate of plasminogen.[8] Furthermore, because the inhibition of fibrinolytic enzymes proceeds only in the fluid phase,[22,28] PAI-1 is unable to inhibit fibrin-bound tPA in a fibrin layer. On the basis of these reports, we speculated that, although PAI-1 inactivates tPA only on the fibrin surface, impairing further degradation of the fibrin, PAI-1 on the fibrin surface may be insufficient for continued inactivation of the tPA in normal conditions. Thus, the fibrinolysis proceeds slowly. In the current study, the high accumulation of plasminogen/tPA on the mural fibrin surface was recognized, but it was difficult to prove the presence of ongoing fibrinolysis conclusively. The accumulation of D-dimer, which is an important marker for fibrinolysis of the thrombus, was demonstrated by means of immunohistochemistry.[29] Although the detailed regulation mechanism of tPA and PAI-1 at the interface remains unclear, we considered the accumulation of D-dimers, although indirect, to be evidence of ongoing fibrinolysis in the plasminogen/tPA-rich layer.

The initial digestive products of fibrin treated with plasmin were demonstrated by means of the SDS-PAGE to be proteins of 25 and 27 kd, which Vries et al[27] and Pizzo et al[30] also recognized as aminoterminal fragments of the α-chains. The presence of these bands in the mural fibrins, which were most prominent in sections near the flow surface layer, suggested the presence of fragments of the α-chains and was consistent with the hypothesis that the mural fibrin was digested by the endogenous plasmin. Because the human fibrinogen used in this study contained a significant amount of plasminogen and tPA, the untreated fibrin plate was gradually digested by the inherent plasmin. This explains the presence of a dim 27-kd band in the SDS-PAGE of the untreated fibrin plate (Fig 1, *A*).

Elastase and cathepsin G, which were identified as the major fibrinolytic enzymes of leukocytes,[31] are possible contributors of other enzymes to fibrinolysis. Plasminogen is a substrate of elastase and cathepsin G, and the product of its limited digestion is miniplasminogen. Kolev et al[32] performed a quantitative comparison of the degradation of cross-linked fibrin with plasmin, miniplasmin, neutrophil leukocyte elastase, and cathepsin G; no changes in their contribution to fibrinolysis were observed, whereas the most efficient fibrinolytic enzymes were found to be miniplasmin and elastase when the plasmin inhibitor was cross-linked to fibrin. In this study, the presence of a 43-kd band, which was more prominent in the middle and outer layers of the mural thrombus, suggested that the fibrin of the middle and outer layers is digested not only by plasmin, but also by proteases derived from neutrophils.

In the analysis of vascular grafts, it is essential to obtain the samples from patent grafts. When graft occlusion occurs, the lumen is occupied by thrombi, which may abruptly alter the characteristics of the flow surface. Yao et al[11] reported that fibrinolytic activity could be measured only in vascular grafts that had maintained patency at the time of harvesting. However, because of the obvious difficulties in obtaining patent grafts from living patients, the number of specimens available for the evaluation was limited.

In conclusion, the results of this study are consistent with our hypothesis that the fibrin surface of aneurysms or prosthetic grafts has a thin plasminogen and tPA-rich layer, formed by digestion with endogenous plasmin. This potentiates the fibrinolytic process on the flow surface and may, in part, be responsible for maintaining its antithrombogenicity.

REFERENCES

1. Rosenberg N. Physiologic factors in arterial grafting. In: Dardik H, editor. Graft materials in vascular surgery. Miami: Symposia Specialists; 1978. p. 15-28.
2. Mohr LL, Smith LL. Polytetrafluoroethylene graft aneurysms: a report of five aneurysms. Arch Surg 1980;115:1467-70.
3. Yates SG II, Nakagawa Y, Berger K, Sauvage LR. Surface thrombogenicity of arterial prostheses. Surg Gynecol Obstet 1973;136:12-6.
4. Puckett CL, Gervin AS, Denton JG, Silver D. Prosthetic "intimal" fibrinolysis. Surg Forum 1973;24:262-4.
5. Guidoin R, Gosselin C, Martin L, Morois M, Laroche F. Polyester prostheses as substitutes in the thoracic aorta of dogs. I. evaluation of commercial prostheses. J Biomed Materials Res 1983;17:1049-77.
6. Collen D. On the regulation and control of fibrinolysis: Edward Kowalski memorial lecture. Thromb Haemost 1980;43:77-89.
7. Lijnen HR, Collen D. Mechanism of physiological fibrinolysis: Bailliere's clinical haematology. In: Lijnen HR, Collen D, editors. Fibrinolysis. Volume 8. London: Bailli re Tindall; 1995. p. 277-90.
8. Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator: role of fibrin. J Biol Chem 1982;257:2912-9.
9. Suenson E, Lützen O, Thorsen S. Initial plasmindegradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. Eur J Biochem 1984;140:513-22.
10. Sakharov DV, Rijken DC. Superficial accumulation of plasminogen during plasma clotlysis. Circulation 1995;92:1883-90.
11. Yao JST, Bergan JJ, Kwaan HC. Quantitation of fibrinolytic activity in venous and prosthetic arterial grafts. Arch Surg 1974;109:163-7.
12. Böyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968;21 (Suppl):77-89.
13. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-2.
14. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci 1979;76:4350-4.
15. Todd AS. Histological localisation of plasminogen activator. J Pathol Bacteriol 1959;78:281-7.
16. Astrup T. The biological significance of fibrinolysis. Lancet 1956;15:565-8.
17. Levin EG, Loskutoff DJ. Cultured bovine endothelial cells produce both urokinase and tissue-type plasminogen activators. J Cell Biol 1982;94:631-6.
18. Underwood MJ, De Bono DP. Increased fibrinolytic activity in the intima of atheromatous coronary arteries: protection at a price. Cardiovasc Res 1993;27:882-5.
19. Underwood MJ, More R, Weeresena N, Firmin RK, De Bono DP. The effect of surgical preparation and in-vitro distension on the intrinsic fibrinolytic activity of human saphenous vein. Eur J Vasc Surg 1993;7:518-22.
20. Loskutoff DJ, Curriden SA. The fibrinolytic system of the vessel wall and its role in the control of thrombosis. Ann NY Acad Sci 1990;598:238-47.
21. Van Mourik JA, Lawrence DA, Loskutoff DJ. Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. J Biol Chem 1984;259:14914-21.
22. Angles-Cano E. Overview on fibrinolysis: plasminogen activation pathways on fibrin and cell surfaces. Chem Phys Lipids 1994;67/68:353-62.
23. Harpel PC, Chang TS, Verderber E. Tissue plasminogen activator and urokinase mediate the binding of glu-plasminogen to plasma fibrin I. J Biol Chem 1985;260:4432-40.
24. Bok RA, Mangel WF. Quantitative characterization of the binding of plasminogen to intact fibrin clots, lysine-sepharose, and fibrin cleaved by plasmin. Biochemistry 1985;24:3279-86.
25. Christensen U. C-terminal lysine residues of fibrinogen fragments essential for binding to plasminogen. FEBS Lett 1985;182:43-6.
26. Tran-Thang C, Kruithof EKO, Atkinson J, Bachmann F. High-affinity binding sites for human glu-plasminogen unveiled by limited plasmic degradation of human fibrin. Eur J Biochem 1986;160:599-604.
27. de Vries C, Veerman H, Pannekoek H. Identification of the domains of tissue-type plasminogen activator involved in the augmented binding to fibrin after limited digestion with plasmin. J Biol Chem 1989;264:12604-10.
28. Chmielewska J, Rànby M, Wiman B. Kinetics of the inhibition of plasminogen activators by the plasminogen-activator inhibitor: evidence for "second-site" interactions. Biochem J 1988;251:327-32.
29. Wakefield TW. Coagulation and disorders of hemostasis. In: Sidawy AN, Sumpio BE, DePalma RG, editors. The basic science of vascular disease. New York: Futura Publishing; 1997. p. 477-501.
30. Pizzo SV, Schwartz ML, Hill RL, McKee PA. The effect of plasmin on the subunit structure of human fibrin. J Biol Chem 1973;248:4574-83.
31. Plow EF. The major fibrinolytic proteases of human leukocytes. Biochem Biophys Acta 1980;630:47-56.
32. Kolev K, Komorowicz E, Owen WG, Machovich R. Quantitative comparison of fibrin degradation with plasmin, miniplasmin, neurophil leukocyte elastase and cathepsin G. Thromb Haemost 1996;75:140-6.