Demonstration of In Situ Fibrin Degradation in Pathologic Thrombi

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Fibrin prepared from 15 pathologic thrombi was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to determine the extent and pattern of fibrinolysis that occurs in vivo. Two groups of patients could be distinguished on the basis of the polypeptide chain composition of fibrin in their thrombi. Those patients who presented with acute vascular obstruction, either arterial or venous, showed a minimal degree of fibrin degradation, with a dominance of intact, undegraded crosslinked γ-γ dimers. On the other hand, patients with long-standing symptoms associated with chronic aortic aneurysms had thrombi containing extensively degraded fibrin. Thrombi in large aortic aneurysms were dissected into concentric layers that showed different degrees of fibrinolysis. The luminal surface consisted of fresh, red thrombus and contained undegraded crosslinked fibrin similar to that found in patients with acute occlusive disease. Deeper layers of the thrombus showed γ-γ chain degradation throughout, indicating that this portion was undergoing active thrombolysis. The findings demonstrate that the variability in the pathophysiologic balance between coagulation and fibrinolysis is reflected in vivo by the polypeptide chain composition of crosslinked fibrin in thrombi. The results support the hypothesis of a dynamic equilibrium between clotting and lysis, but indicate that the balance between these two processes may be distinctly different in separate areas of a single clot.

Fibrin formation and dissolution are thought to occur simultaneously during the evolution of pathologic thrombi, and the balance between these two opposing processes may influence the occurrence and clinical course of thrombotic disease. Nossel has postulated that the competing actions of thrombin and plasmin for the "fibrin I" substrate (fibrinogen minus fibrinopeptide A) determines whether the molecule will polymerize into fibrin II fibers or be degraded into the soluble fragment X. The balance of opposing forces probably pertains during the entire period of thrombus formation, organization, and resolution, with a supply of appropriate enzymes, proenzymes, and activators provided in the form of fibrin-bound thrombin, plasmin, and α-antiplasmin, endothelial cell and plasma Hageman factor-derived plasminogen activators, and leukocyte-derived proteolytic enzymes. Thrombosis could result from an imbalance of these components, such as that due to impaired fibrinolysis resulting from deficient vascular plasminogen activator or an abnormal plasminogen, or excessive coagulation caused by deficiency of the inhibitors antithrombin III or protein C.

The molecular events that produce fibrin and then remove it from thrombi have been studied extensively. Fibrin formation is mediated through thrombin action, which cleaves the fibrinopeptides from fibrinogen, activates factor XIII, and leads ultimately to the formation of fibrin fibers that are stabilized by intermolecular isopeptide bonds between γ-chain pairs and α-chain polymers. Fibrin degradation results from plasmin action on the same susceptible sites as exist in fibrinogen, proceeding first through extensive degradation of the α-polymer chains prior to significant clot lysis. Solubilization occurs following cleavages of the "coiled coils" in the protofibrils, resulting in the liberation of large complexes into solution and progressive thrombolysis.

Despite the extensive information on in vitro fibrin structure and degradation, there have been few studies of fibrin molecular structure in pathologic thrombi. Gaffney and colleagues showed a predominance of γ-γ dimers in reduced fibrin obtained from a surgically removed pulmonary embolus, indicating virtually complete fibrin crosslinking. Fretto and McKee studied a femoral artery embolus removed at surgery and also demonstrated full crosslinking of the α-chains in the thrombus. Degradation of these thrombi in vitro with either plasmin or with cyanogen bromide produced the expected crosslinked derivatives, namely D dimer from plasmin action and an α-polymer remnant of 29,000 mol wt with cyanogen bromide. Neither of these studies demonstrated significant degrees of fibrinolysis in situ of these relatively acute thrombi, and there are no reported studies that compare the extent of degradation in different types of pathologic thrombi or in different areas of a single thrombus.

In this article, we determine the polypeptide chain composition of fibrin in pathologic thrombi from 15 patients and demonstrate in situ fibrinolysis. The extent of polypeptide chain degradation is correlated with the clinical presentation of the patients and is compared in different areas of large arterial clots. The results allow tentative conclusions regarding the natu-
al history of pathologic thrombi and the physiologic role of plasmin in dissolving thrombi and liberating specific crosslinked fibrin derivatives.

**MATERIALS AND METHODS**

Human fibrinogen (grade L) was purchased from AB Kabi (Stockholm, Sweden). Human thrombin (99.1% α-thrombin, 3468 NIH U/mg) was kindly supplied by Dr. John Fenton (Albany, NY), and human plasmin was provided by Dr. David Aronson (Bureau of Biologics Standards, Bethesda, MD). Nitrocellulose paper with a pore size of 0.45 μm (type HA) was supplied by Millipore Corp. (Bedford, MA). Acrylamide/bisacrylamide in a ratio (w/w) of 29:1 was obtained from Bio-Rad Laboratories (Richmond, CA). Coomassie blue R-250, HEPES, O-dianisidine, soybean trypsin inhibitor, bovine serum albumin (fraction V), Tween 20, and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. (St. Louis, MO), and the latter was recrystallized from ethanol for use. Urokinase (Abbokinase) was obtained from Abbott Laboratories (North Chicago, IL). Goat anti-human fibrinogen (lot 167744) and peroxidase-conjugated IgG fraction rabbit anti-goat IgG (lot 18065) were obtained from Cappel Laboratories (Cochranville, PA). Fetal bovine serum was from GIBCO Laboratories (Grand Island, NY) and hydrogen peroxide from Fischer Scientific Corp. (Fairlawn, NJ). Human factor XIII (fraction V) was prepared according to the method of Loewy et al. and was assayed by the method of Lorand et al.

**Preparation of Crosslinked Fibrin**

Lyophilized fibrinogen was dissolved in water to a concentration of 20 mg/ml and diluted to 2.5 mg/ml with 0.15 M sodium chloride, 0.05 M Tris-hydrochloric acid buffer, pH 7.6, containing 15 mM calcium chloride and 3 U/ml factor XIII. Clotting was initiated by adding thrombin (1.24 NIH U/ml, final concentration), and the clot was incubated at 25°C for 18 hr. Entrained fluid was removed by squeezing the clot between nylon mesh, after which it was washed extensively with 0.15 M sodium chloride, lyophilized, and finely ground with a glass rod.

**Plasmic Digestion of Fibrin and Fibrinogen**

Fibrinogen was dissolved in water to a concentration of 20 mg/ml and digested with urokinase (200 CTA U/ml final concentration) at 37°C for 60 min; the reaction was terminated by the addition of soybean trypsin inhibitor to a concentration of 0.4 mg/ml. Lyophilized crosslinked fibrin was suspended in 0.15 M sodium chloride, 0.05 M Tris-hydrochloric acid buffer, pH 7.6, to a concentration of 25 mg/ml, after which plasmin was added to a final concentration of 0.15 CTA U/ml. The mixture was incubated at 37°C with magnetic stirring, and digestion was terminated at 1 hr by the addition of soybean trypsin inhibitor to a final concentration of 0.2 mg/ml. Undigested fibrin was removed following centrifugation at 2,300 g for 10 min at 4°C.

**Collection and Processing of Thrombi**

Thrombi removed at operation were placed into 0.15 M sodium chloride or into 0.15 M sodium chloride, 0.05 M Tris-hydrochloric acid buffer, pH 7.6, or transported fresh to the pathology department for examination, after which they were obtained for processing (1–12 hr after surgical removal). The specimens from two patients (nos. 11 and 5, Table 1) were placed into 0.15 M sodium chloride, 0.05 M Tris-hydrochloric acid, pH 7.6, 0.1% disodium ethylenediaminetetraacetate (EDTA), 0.02 M epsilon aminocaproic acid, and 0.02% sodium azide immediately following surgical removal. Specimens were compressed to remove entrapped fluid, then washed for 12–24 hr in 0.15 M sodium chloride, 0.05 M Tris-hydrochloric acid buffer, pH 7.6, containing 0.02% sodium azide, lyophilized, and ground with a glass rod. Three thrombi were obtained at postmortem examination 12–24 hr after death. These were placed into buffer (0.15 M sodium chloride, 0.05 M Tris-hydrochloric acid, pH 7.6), then processed as for surgical specimens. The thrombus from patient 15 was removed from the filter in the line from a hemodialysis apparatus to the patient and was placed into 0.15 M sodium chloride for 24 hr and then processed as above. Prior to electrophoresis, the lyophilized protein was dissolved by incubation at 60°C for 18 hr in a solution of 3.75% SDS, 0.38 M sucrose, 0.025% disodium EDTA, 5% β-mercaptoethanol, and 0.1 M boric acid-Tris buffer, pH 8.6.

**Electrophoresis**

SDS-polyacrylamide gradient gel electrophoresis (SDS-PAGE), utilizing the sulfate-borate discontinuous buffer system described by Neville, was performed as previously described.

**Blotting**

Western blotting was performed by a modification of the method of Towbin and colleagues, using a Trans-Blot cell (Bio-Rad Laboratories, Richmond, CA). Transfer to nitrocellulose paper was done in 0.1 M Tris-hydrochloric acid buffer, pH 8.3, containing 0.096 M glycine and 20% (v/v) methanol at 60 V for 2 hr at 20°C. The nitrocellulose paper was incubated at 3°C for 18 hr in 0.05 M phosphate buffer, pH 7.3, containing 0.15 M sodium chloride and 3% (w/v) bovine serum albumin. Five milliliters of goat anti-human fibrinogen antibody (diluted 1:10 with 0.05 M phosphate buffer, pH 7.3, containing 0.15 M sodium chloride and 0.05% (v/v) Tween 20) was incubated with the paper for 2 hr at 20°C in the heat-sealed plastic bag with end-over-end mixing. The nitrocellulose paper was then washed with 6 columns of 40 ml each of 0.05 M phosphate buffer, pH 7.5, containing 0.15 M sodium chloride and 0.05% (v/v) Tween 20 using gentle agitation on a platform shaker for 10 min for each wash. The paper was then incubated with end-over-end rotation for 2 hr at 20°C in a sealed plastic bag with 5 ml of peroxidase-conjugated rabbit anti-goat IgG fraction diluted 1:1,000 with 0.05 M phosphate buffer, pH 7.3, containing 0.15 M sodium chloride 3% (w/v) bovine serum albumin, 20% (v/v) fetal bovine serum, and 0.05% (v/v) Tween 20. Following this, it was washed 6 times, as following the primary antibody. The paper was then covered with 250 ml of 10 mM HEPES buffer, pH 7.4, containing 0.75 ml of 1% O-dianisidine in methanol and 0.06 ml of hydrogen peroxide, for 20 min at 20°C, after which it was washed with water.

**RESULTS**

Thrombi were obtained from 15 patients and included 11 that were removed at operation, 3 obtained at autopsy, and 1 taken from a hemodialysis apparatus (Table 1). Five thrombi (nos. 1–5) were removed from abdominal aortic aneurysms at the time of surgical repair; two thrombi (nos. 1 and 3) were large enough to be dissected into layers (see Fig. 3), and the other three consisted of a 1–2-mm layer of red clot on the luminal surface overlying 2–5 mm of whitish-tan material and layers of friable fatty atheroma. Of the five aortic aneurysms, one patient was asymptomatic, three had chronic back or leg pain, and one had symptoms attributable to aneurysm rupture.
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age</th>
<th>Sex</th>
<th>Procedure</th>
<th>Specimen</th>
<th>Symptoms</th>
<th>Note</th>
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<tr>
<td>1</td>
<td>80</td>
<td>F</td>
<td>Abdominal aortic aneurysmectomy</td>
<td>Thrombus from aneurysm</td>
<td>Asymptomatic</td>
<td>Hypertension, history of myocardial infarction 1963</td>
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<td>2</td>
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<td>M</td>
<td>Abdominal aortic aneurysmectomy</td>
<td>Thrombus from aneurysm</td>
<td>Low back pain, 10 days</td>
<td>Hypertension, history of myocardial infarction early 1970s</td>
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<td>Abdominal aortic aneurysmectomy</td>
<td>Thrombus from aneurysm</td>
<td>Leg pain, several months</td>
<td>Claudication, hypertension history of myocardial infarction 1972</td>
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<tr>
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<td>68</td>
<td>M</td>
<td>Abdominal aortic aneurysmectomy</td>
<td>Thrombus from aneurysm</td>
<td>Abdominal pain, loss of consciousness, 1 day (ruptured)</td>
<td>—</td>
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<tr>
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<td>74</td>
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<td>Thrombus from aneurysm</td>
<td>Low back pain, 3 mo</td>
<td>Duodenal ulcer, chronic atrial fibrillation, diabetes mellitus</td>
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<td>6</td>
<td>71</td>
<td>M</td>
<td>Femoral-popliteal bypass graft thrombectomy</td>
<td>Thrombus from graft</td>
<td>Leg pain, 6 hr</td>
<td>Diabetes mellitus, hypertension, congestive heart failure</td>
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<td>7</td>
<td>57</td>
<td>M</td>
<td>Aorto-femoral bypass graft thrombectomy</td>
<td>Thrombus from graft</td>
<td>Leg pain, 1 day</td>
<td>Coronary artery bypass graft 3 days previously; hypertension, aorto-femoral bypass graft 1975</td>
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<td>8</td>
<td>60</td>
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<td>Femoral-popliteal bypass graft thrombectomy</td>
<td>Thrombus from graft</td>
<td>Leg pain, 3 days</td>
<td>Hypertension, coronary artery bypass graft 1976; femoral-popliteal bypass graft 1982</td>
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<td>76</td>
<td>M</td>
<td>Femoral artery thrombectomy</td>
<td>Femoral artery thrombus</td>
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<td>10</td>
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<td>Above-knee amputation</td>
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<td>Leg pain, 3 wk</td>
<td>Infected and nonhealing below-knee amputation, diabetes mellitus</td>
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<td>M</td>
<td>Femoral artery thrombectomy</td>
<td>Femoral artery thrombus</td>
<td>Leg pain, 12 hr</td>
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<td>Autopsy</td>
<td>Thrombus on aortic atherosclerotic plaque</td>
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<td>Hypertension, congestive heart failure, emphysema, nephrotic syndrome</td>
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<td>13</td>
<td>66</td>
<td>M</td>
<td>Autopsy</td>
<td>Thrombus on aortic atherosclerotic plaque</td>
<td>—</td>
<td>Alcoholism, cardiomyopathy, cirrhosis, pneumonia</td>
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<td>Clot on efferent filter</td>
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<tr>
<td>15</td>
<td>32</td>
<td>F</td>
<td>Autopsy</td>
<td>Pulmonary embolus</td>
<td>Dyspnea, chest pain, 5 days</td>
<td>Died during cesarean section</td>
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</table>

Six patients (nos. 6–11) presented with acute symptoms of leg ischemia due to thrombotic occlusion of the femoral artery (nos. 9–11), femoral-popliteal bypass graft (nos. 6 and 8), or aorto-femoral bypass graft (no. 7). Most patients had symptomatic cardiovascular disease in addition to the presenting problem. Thrombus from these occluded arteries consisted almost entirely of homogeneous red clot, 3–5 mm diameter and 2–40 cm length. Postmortem samples of thrombus adherent to atherosclerotic aortic plaque were obtained from patients 12 and 13. Patient 14 was undergoing hemodialysis following renal transplant rejection, and a 0.2 x 2 cm white thrombus that occluded the filter from the dialysis machine was removed for study. Patient 15 died suddenly during delivery by cesarean section, and a large pulmonary embolus was obtained at postmortem 8 hr after death.

Protein prepared from the clots was reduced, and the polypeptide chains were separated by SDS-PAGE (Fig. 1). Multiple bands were present, representing the polypeptide chains of fibrin and other proteins associated with the thrombi. Prominent bands corresponding in migration to γγ and β-chains can be seen in each sample. Individual bands were identified by reaction with antifibrinogen antibody following Western blotting (Fig. 2) and by their migration position in comparison with purified standards. In the sample of purified fibrinogen, the Aα, Bβ, and γ-chain bands were identi-
Fig. 1. SDS-polyacrylamide gel patterns of reduced protein from pathologic thrombi. Electrophoresis was toward the anode (bottom) in 5%-14% SDS-polyacrylamide gradient gels with a 5% stacking gel. The protein-stained gel strips represent lanes from several electrophoretic runs, with the bands corresponding to γγ-chains aligned for comparison. The numbers below the lanes refer to the patients in Table 1. Crosslinked fibrin prepared from purified fibrinogen and a stage 1 plasmic digest of crosslinked fibrin are shown for comparison.

fied by the antibody ("blot"), whereas other protein-stained bands in the fibrinogen preparation, such as fibronectin, did not react with the antibody. Comparison of the immunoblots and Coomassie-stained gel strips of samples from patients 15 and 3 showed that most of the protein was fibrin-derived, although the percentage of total protein in the thrombi that was fibrin was not determined. The γγ, /γγ, and β-chains were unequivocally identified by their characteristic migration positions, and protein at the top of the stacking gels represented α-polymer chains.

On the basis of clinical presentation, the thrombi were divided into two groups, those associated with rapid onset of symptoms and those with long-standing...
or no symptoms. Figure 3A shows segments of the SDS-PAGE electrophoretic patterns of fibrin from thrombi that caused death or symptoms of acute ischemia that necessitated emergency surgery to relieve obstruction. Patient 14 was included in this group, as the thrombus developed acutely during hemodialysis. Because the progress of crosslinked fibrin degradation can be monitored by $\gamma\gamma$-chain conversion to $/\gamma\gamma$-remnants of mol wt 96,000–76,000, this portion of the gels has been isolated for clarity. In each case shown in Fig. 3A, the $\gamma\gamma$-chains were almost completely intact, with only a trace of degraded $/\gamma\gamma$-chains present. The $\beta$-chains (not shown) were also mostly undegraded. The second group of thrombi were from patients with aortic aneurysms or atherosclerotic plaques. Figure 3B shows the gel patterns of fibrin from three aortic aneurysm thrombi and from the two atherosclerotic plaques. There was extensive conversion of the $\gamma\gamma$-bands to $/\gamma\gamma$-derivatives, as well as an increased proportion (not shown) of the $/\beta$-remnant.

Thrombi from patients 1 and 3 were large, allowing preparation of fibrin samples from different layers. That from patient 1 was a cylindrical hollow cast of the aorta (Fig. 4A), with 2–4 mm of friable red clot on the luminal surface, overlying several millimeters of whitish-tan homogeneous material, and a deeper layer of atheromatous tissue that formed the plane of dissection when the sample was removed from the aortic wall. A photomicrograph of a histologic section taken from the luminal surface (Fig. 5, upper panel) showed a thrombus with abundant erythrocytes and some polymorphonuclear leukocytes within a fibrin matrix. A section from deep within the white portion of the thrombus (Fig. 5, lower panel) showed a large, relatively acellular mass, consisting mostly of fibrin and an...
area of organization containing some fibroblasts and collagen in the lower portion of the section. The SDS-PAGE pattern of the fibrin from the deep white layer showed extensive degradation of \( \gamma \gamma \)-chains, whereas that from the luminal red surface clot showed a predominance of intact \( \gamma \gamma \)-chains (Fig. 6).

The gross appearance of the clot from patient 3 was similar to that from patient 1, but the thrombus was thicker and could be divided into four layers: red clot from the luminal surface, white fibrin immediately beneath, a deeper layer of white clot, and material from the friable atheromatous area adjacent to the vessel wall. The fibrin polypeptide chain composition of samples taken from these areas is shown in Fig. 6. Luminal material (layer 1) was the least degraded, as indicated by the predominantly intact \( \gamma \gamma \)-chains. Deeper layers all showed intermediate and small \( \gamma \gamma \)-chain derivatives, with relatively less intact \( \gamma \gamma \)-dimers, indicating that significant degradation of the crosslinked fibrin had occurred.

Because thrombi were processed for variable periods prior to electrophoretic analysis, the possibility of in vitro fibrin degradation affecting the polypeptide chain composition was assessed. The polypeptide chain pattern of processed thrombi remained constant during storage at \(-20^\circ C\) for as long as 1 yr. The pattern of \( \gamma \gamma \) and \( /\gamma \gamma \)-chains of fibrin from patient 8 showed no change after incubation in buffer for 48 hr (Fig. 7). The pattern from patient 2 showed extensive degradation prior to such in vitro incubation, and therefore little further change could have occurred without frank dissolution of the fibrin matrix. However, only 1% of the matrix fibrin from patient 2 appeared in the buffer after 48-hr incubation, as measured by the increase in OD\(_{280}\), indicating that significant fibrinolysis during in vitro processing of the specimen did not occur.

**DISCUSSION**

We have examined the polypeptide chain composition of crosslinked fibrin in pathologic arterial and venous thrombi in order to determine the proportion, degree, and pathway by which fibrin degradation proceeds in vivo. The principal criteria for fibrin degradation were the conversion of crosslinked \( \gamma \gamma \)-dimers (mol wt 100,000) to \( /\gamma \gamma \)-derivatives of molecular weight 96,000–76,000\(^2\) and the proportion of \( \beta \beta \)-chains that were converted to the \( /\beta \beta \)-remnant of 43,000.\(^2\) Based on these criteria, the 15 pathologic thrombi (Table I) fell into two distinct groups, namely, those with intact or only slightly degraded \( \gamma \gamma \)-chains and those with a predominance of \( /\gamma \gamma \)-derivatives. Those thrombi that showed little or no evidence of...
Degradation were obtained from patients with acute symptoms of vascular obstruction (Fig. 3A), suggesting either that thrombus formation was proceeding much more rapidly than dissolution or that only a very small portion of a stable thrombus was currently undergoing dissolution.

In contrast, fibrin from thrombi obtained from patients with more chronic conditions, such as aortic aneurysm, was found to be extensively degraded (Fig. 3B). However, the regional differences in aneurysmal fibrin degradation (Fig. 6) indicated that the overall proportion of γγ and γγ-β-derivative did not reflect a uniform process throughout the thrombus. Rather, the luminal surface of the thrombus, which had a fresh red appearance (Fig. 5), showed mostly intact γγ-chains, whereas deeper layers of the thrombus, which were white and laminated, showed a preponderance of γγ-derivatives. This variation in polypeptide chain content suggests that the surface region was the site of more active fibrin formation, possibly the result of persistent thrombin activity in association with the fibrin3,4,47 and accounting for the chronic local consumptive coagulopathy of aneurysms48,49 and the presence of fibrinopeptide A in the blood of such patients.50 The deeper layers would be the site of a predominant fibrinolytic process, presumably accounting for the associated increase in circulating fibrin degradation products. Thus, the complex polypeptide chain patterns shown in Figs. 3B and 6 resulted less from a uniform balance of prothrombotic and thrombolytic tendencies on the same or contiguous fibrin molecules, and more from the cumulative total of clearly disparate degrees of thrombosis and thrombolysis in superficial and deep layers of the chronic thrombus. These conclusions are shown schematically in Fig. 8.

Fig. 7. Polypeptide chain composition of fibrin during in vitro incubation. A 3.5-mg portion of fibrin from patient B was incubated in 1.0 ml buffer (0.15 M sodium chloride, 0.05 M Tris-hydrochloric acid, pH 7.6, containing 0.02% sodium azide) at 25°C for 24 and 48 hr, after which the fibrin was lyophilized, dissolved, and reduced for electrophoresis as in Fig. 2. Protein loading was 30 μg for each sample. No degradation of γγ or β-chains to γγ or β-derivatives was evident at 24 or 48 hr of in vitro incubation.

Fig. 8. Schematic representation of vessels occluded with recent or chronic thrombi. (A) Complete occlusion producing acute symptoms: the red thrombus contains predominantly undegraded crosslinked fibrin. A very small proportion of degraded material present on or in such a thrombus would not significantly alter the electrophoretic patterns shown in Fig. 2A. (B) Small nonocclusive arterial thrombus, consisting only of a thin layer of red thrombus adherent to the vessel endothelial surface or to underlying white clot. (C) Large thrombus removed from an aortic aneurysm with a laminated structure containing layers of thick white fibrin clot underlying a red thrombus on the luminal surface. Minimal fibrinolysis would be present in the luminal layer, whereas deep portions would show extensive fibrin degradation.
In this representation of vessels containing thrombi that led to symptoms of acute vascular obstruction (Fig. 8A), we postulate that fibrin formation proceeded at a rapid rate and that fibrinolysis was minimal. A slight degree of degradation (Fig. 3A) may have been the result of local areas of partial lysis adjacent to the vessel wall, where endothelial cell plasminogen activator was released and locally active. Nonobstructing chronic arterial thrombi would have a more complex composition (Figs. 8B and 8C). Thrombus in smaller aneurysms (Fig. 8B) may be composed of a thin red thrombus alone or, as shown, thin layers of luminal red thrombus and underlying older white thrombus. This phase of aneurysmal thrombus formation may antedate the laminated thrombus in large aneurysms shown in Fig. 8C. The latter also have luminal red clot, but, in addition, contain several layers of white thrombus. In these thrombi, the overall rate of fibrin deposition and lysis would be nearly equal (Figs. 3B and 6), but the balance between coagulation and fibrinolysis would be quite different in the superficial and deeper layers. The relative lack of degradation in the luminal layer suggests that thrombin action and fibrin formation predominated there; this would be balanced by correspondingly greater plasmin action and fibrinolysis in the deeper layers. The progressive alteration of intact crosslinked fibrin present in the luminal portion to the degrading fibrin present in the underlying layers may contribute to the laminated appearance of the thrombus.

Several lines of evidence indicate that the results reflect actual fibrin structure and that the gel electrophoretic patterns accurately represent the in situ composition in the thrombi. First, the immunoblot experiments (Fig. 2) with antifibrinogen antiserum identified the electrophoretic bands as fibrin-derived, and, together with the electrophoretic mobilities, allowed us to firmly identify specific intact and degraded γγ and β-chains. Second, in vitro delays in processing the thrombi had no effect on the observed polypeptide chain structure. Precautions were taken by adding fibrinolytic inhibitors to the buffer in which some thrombi were stored immediately after removal at surgery, but, in addition, no change in polypeptide chain composition was observed, even when the thrombi were incubated in vitro in buffer without inhibitors (Fig. 7). Although samples obtained at autopsy could have undergone some postmortem degradation, one of the three postmortem samples (no. 15) showed little evidence of degradation, and the polypeptide chain structure of postmortem aortic atherosclerotic plaque thrombi was similar to that of aortic aneurysm thrombi obtained at surgery. Although we cannot entirely exclude artefactual in vitro or postmortem change as an influence on the polypeptide chain composition of fibrin, the evidence suggests that the observed data reflect the in situ composition.

The characteristic pattern of polypeptide chain composition seen in pathologic thrombi was entirely consistent with the sequence of plasmic degradation of crosslinked fibrin in vitro. Although the degree of α-polymere cleavage in the thrombi could not be determined, as these large chains did not enter the SDS-polyacrylamide gels, the degraded forms of γγ and β-chains seen in fibrin from pathologic thrombi (Figs. 3 and 6) were identical in molecular weight to those already described. Although a contribution by other proteolytic systems, such as that of leukocyte proteases, cannot be ruled out, our findings are consistent with the importance of the plasmin/plasminogen system in physiologic thrombolysis.

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