A radioimmunoassay (RIA) technique has been devised for the measurement of human fibrinopeptide A (FPA). The system utilizes rabbit antiserum to native human FPA and a synthetic fibrinopeptide, with tyrosine substituted for phenylalanine in amino acid position 8. The test detects native human FPA at a concentration of 0.1 ng/ml, but does not cross react with human fibrinopeptide B or with fibrinopeptides A from canine, porcine, or bovine fibrinogen. Fibrinogen and chemical or plasmic degradation products with 2 moles of FPA per mole react fully in this test system. This includes the large-molecular-weight intermediate fragments X and Y and the NH₂-terminal disulfide knot, and indicates that this antibody recognizes and reacts with FPA in the presence of the contiguous peptide structures present in fibrinogen. Fragment E, which is derived from the NH₂-terminal portion of fibrinogen, loses most of its FPA content after its liberation from its precursor derivative and reacts to a lesser extent in the RIA than do fragments X and Y. This correlates with the recovery of FPA-positive material from ultrafiltrates of extensive but not partial plasmic digests of fibrinogen. Although FPA immunoreactivity liberated from fibrinogen does not necessarily reflect thrombin activity and/or fibrin formation, only extensive plasmic degradation yields peptide material which reacts in this RIA system. This should not be a serious limitation to the application of the RIA in the detection of venous thrombosis.

THROMBIN-INDUCED CONVERSION of fibrinogen to fibrin is associated with the proteolytic cleavage of four arginyl-glycyl peptide bonds per molecule of substrate and the release of fibrinopeptides A and B, two of each from the Aα and Bβ polypeptide chains, respectively. The two types of fibrinopeptides have been isolated from numerous animal species, and their amino acid sequences have been determined. It has been found that the NH₂-terminal amino acids of degradation product fragments X, Y, and E do not differ significantly from those of undegraded fibrinogen and that direct electrophoretic measurements of their thrombin-cleavage peptides verify the pres-
ence of fibrinopeptide A (FPA). Furthermore, plasmin may liberate fibrino-
peptide A from fragment E, possibly as part of a slightly larger peptide
fragment. A specific and sensitive radioimmunoassay (RIA) for human FPA
has been devised and used to measure its concentration in deproteinized or
dialyzed plasma in various clinical syndromes associated with thrombosis.
However, if FPA that is present on degradation products or cleaved from them
by plasmin is detected by the RIA, this test might not clearly distinguish be-
tween fibrinogenolysis and fibrin formation.

To study this question, an RIA has been developed using synthetic, tyrosine-
substituted derivatives of human FPA radiolabeled with $^{125}$I and antiserum
raised in rabbits against native human FPA. The specificity of the assay for hu-
man FPA has been tested by comparison with animal counterparts, and the
reactivity of the assay with plasmic degradation products of human fibrinogen
has been assessed by quantitative studies using total digestion mixtures and
purified degradation products.

**MATERIALS AND METHODS**

**Human Fibrinopeptides**

Native human fibrinopeptides A and B. These were cleaved from 2500 ml of human fibrinogen
(4 mg/ml; Grade L, Kabi, Stockholm) by adding 7.5 ml of human thrombin (100 NIH U/ml;
kindly provided by Dr. David Aronson, Division of Biologics Standards, NIH, Bethesda, Md.).
The clot liquor was expressed manually through nylon gauze, and the peptides were purified by
Dowex 50WX2 column chromatography, according to the method of Blomback and colleagues. Animal fibrinopeptides were obtained from bovine, canine, and porcine fibrinogens purified from
their respective citrated plasmas by ethanol-glycine fractionation. A 1.0-mi portion of fibrinogen
(3 mg/ml) was mixed with 0.1 ml of human thrombin (10 NIH U/ml), incubated at 37°C for 4 hr,
and the clot liquor containing fibrinopeptides A and B was expressed through a nylon cloth and
tested directly in the RIA. A sample of human fibrinopeptides A and B was also prepared in this
way for control purposes.

Synthetic human fibrinopeptide A. Synthetic human fibrinopeptide A, with an amino acid se-
quence corresponding to that of human FPA, was prepared using the Merrifield method of solid-
phase peptide synthesis. Tyrosine-substituted derivatives of human fibrinopeptide A. In order to use radioactive carrier-
free iodine to label antigen in the RIA, tyrosine was incorporated into the synthetic polypeptide
chain of fibrinopeptide (human FPA does not contain this amino acid). The tyrosine-substituted
derivatives were prepared using the solid-phase method of peptide synthesis in essentially the
same manner as for synthetic human FPA. The synthetic peptides were purified by column gel
filtration on Bio-Gel P-2 (Biorad, Richmond, Calif.), preparative high-voltage electrophoresis on
paper (3 MM, Whatman, Great Britain) in 0.1 M pyridine-acetate buffer, pH 5.5, and preparative
paper chromatography developed in pyridine:n-butanol:acetic acid:water (10:15:3:12). The purity
of the isolated peptides was assessed on thin-layer cellulose sheets (Chromogram 6064, Eastman
Kodak Co., Rochester, N.Y.) by high-voltage electrophoresis and chromatography using the same
respective solvents as noted above (Fig. 1). Two substituted derivatives were prepared: (1) the
tyrosine residue attached by a peptide bond to NH$_2$-terminal alanine with the attached tyrosine
residue retaining the free NH$_2$-group (NH$_2$-Tyr-FPA) and (2) the tyrosine residue substituted
for the phenylalanine residue which occupies position 8 in the amino acid sequence (8Tyr-FPA).

**Amino Acid Analysis**

The concentration of native and synthetic fibrinopeptides was determined using a single-column
 technique in an amino acid analyzer (Model 119, Beckman, Palo Alto, Calif.). Samples con-
taining approximately 20 nmols (approximately 30 µg) of the peptide were hydrolyzed in 5.7 N
hydrochloric acid in sealed glass vials under vacuum at 110°C for 18, 30, and 48 hr. The content of
serine was calculated by extrapolation to zero hydrolysis time; for other amino acids, average
values were calculated from 30- and 48-hr-hydrolysates. Fibrinopeptide concentration was obtained by summation of individual amino acid contents after allowance for water incorporated during hydrolysis. Amino acid analyses of native and synthetic human FPA and the synthetic tryosine-substituted derivatives are shown in Table 1.

**Fibrinogen Degradation Products**

Fibrinogen degradation products were obtained from digests of human fibrinogen incubated with streptokinase (Varidase, Lederle Laboratories, Pearl River, N.Y.) as described previously. Fragments X and Y were purified by gel filtration on Sephadex G-200 (Pharmacia, Piscataway, N. J.) columns and fragments D and E by block electrophoresis on Pevikon (C-870, Mercer, New York, N. Y.).

**Preparation of Antiserum Against Human Fibrinopeptide A.**

Native human FPA (5 mg) was coupled to chicken egg ovalbumin (5 mg) using 2, 4-toluene diisocyanate, according to the method of Schick and Singer. The conjugate was emulsified with an equal volume of complete Freund’s adjuvant (Difco Laboratories, Detroit, Mich.). White rabbits

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**Table 1. Amino Acid Analysis of Human Fibrinopeptide A and Synthetic Derivatives Prepared for Radioimmunoassay**

<table>
<thead>
<tr>
<th></th>
<th>Native</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FPA (mole/mole)</td>
<td>FPA (mole/mole)</td>
</tr>
<tr>
<td>ASP</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>SER</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>GLU</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>GLY</td>
<td>4.7</td>
<td>5.0</td>
</tr>
<tr>
<td>ALA</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>VAL</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>LEU</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>PHE</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>TYR</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>ARG</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>
received 0.25 mg of the immunogen per footpad and subcutaneous booster injections of approximately 0.1 mg every 4 wk. Ten days after each booster injection, the rabbits were bled from the marginal ear vein, and serum was obtained and tested for antibody to human FPA using the RIA. One of six rabbits immunized for 9 mo developed good antiserum (R 30), three produced weak antiserum, and two did not form antibodies detectable in the RIA. Double immunodiffusion in agar gel against native and synthetic human FPA in concentrations of 0.05-2.0 mg/ml did not form precipitin lines. Immunoprecipitates were not observed against human fibrinogen or fragments X, Y, D, or E.

**Labeling of Tyrosine Derivatives of Fibrinopeptide A**

Labeling of tyrosine derivatives of fibrinopeptide A was performed by a method similar to that of Hunter and Greenwood. The reaction was carried out in a 1-ml conical glass vial with a rubber seal (New England Nuclear, Boston, Mass.). Synthetic tyrosine-substituted FPA (5 μg) was mixed with 20 μl of 0.5 M sodium phosphate buffer, pH 7, and 5 mCi of carrier-free ¹²⁵I-sodium iodide. A 25-μl volume of chloramine T (1 mg/ml) was added, and 45 sec later, 100 μl of sodium metabisulfite (2.5 mg/ml), 50 μl of human serum albumin (Buminate 25%, Hyland, Costa Mesa, Calif.), and 5 μl of potassium iodide (40 mg/ml) were added. The mixture was passed through a Biogel P-2 column (0.8 x 25 cm) equilibrated with 0.15 M sodium chloride and 1 mg/ml ovalbumin (Sigma Chemical Company, St. Louis, Mo.) and eluted with the same solution. Fractions of 1 ml were collected, and radioactivity was counted in a gamma well scintillation counter (Model 530, Baird Atomic, Cambridge, Mass.). Two peaks were eluted, the first containing the labeled peptide and the second, unbound iodide. Only the single fraction with the highest radioactivity was saved from the first peak and used at 1:10,000 dilution in the RIA. The specific radioactivity obtained by this technique was of the order of 0.1 Ci/mg. The labeled tyrosine-substituted FPA was stored at -80°C. Binding affinity for antibody was unchanged after 2 wk storage, but preparations stored for 3 or more wk showed decreased binding with antibody and increased radioactivity which was not absorbed by charcoal.

**Radioimmunoassay Procedure**

The radioimmunoassay procedure is outlined in Table 2. The technique is based on the competition of human FPA with the binding of a radiolabeled tyrosine derivative of human FPA to antibody against the native peptide. The degree to which the test sample displaces the radioactive peptide reflects the concentration of FPA, which can be quantitated by use of a standard curve using known amounts of synthetic human FPA. Charcoal (2.5 mg/ml; Norit A, Pfanstiehl Laboratories, Inc., Waukegan, Ill.) coated with ovalbumin (3 mg/ml) absorbs 96%-99% of the radioactivity that is not bound to antibody within 5 min, but leaves the radioactive peptide-antibody complex in solution. Thus, the amount of radioactivity left after charcoal absorption is inversely related to the concentration of fibrinopeptide in the test sample.

<table>
<thead>
<tr>
<th>Table 2. Radioimmunoassay Procedure for Human Fibrinopeptide A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
</tr>
<tr>
<td>0.05 M Tris-HCl buffer pH 8.6, containing</td>
</tr>
<tr>
<td>0.1 M sodium chloride and 1 mg/ml ovalbumin</td>
</tr>
<tr>
<td>Sample to be tested</td>
</tr>
<tr>
<td>¹²⁵I-labeled tyrosinated synthetic human FPA (200,000 cpm/ml)</td>
</tr>
<tr>
<td>Rabbit antiserum (R 30) against human FPA (1:500 dilution in buffer)</td>
</tr>
<tr>
<td>Incubation at room temperature</td>
</tr>
<tr>
<td>2.5% charcoal in buffer containing 3 mg/ml ovalbumin</td>
</tr>
<tr>
<td>Stirring at room temperature</td>
</tr>
<tr>
<td>Centrifugation at 1600 g</td>
</tr>
<tr>
<td>Aliquots of supernatant solution for counting of radioactivity</td>
</tr>
</tbody>
</table>
For each experiment, four control samples were run: (1) Total radioactivity, with buffer and labeled fibrinopeptide only. (2) Nonabsorbable radioactivity, which is the supernatant of control 1 after absorption with charcoal. (3) Nonspecific binding of radioactivity, which is the increase in radioactivity in the supernatant after absorption with charcoal in the presence of preimmunization rabbit serum (at appropriate dilution), as compared with control 2. (4) Specific binding of radioactivity, which is the increase in radioactivity in the supernatant after absorption with charcoal in the presence of postimmunization antiserum (at the same dilution as in control 3) as compared with control 3.

**Binding of Labeled Peptide**

Binding of labeled peptide is calculated by the ratio of the radioactivity bound to antiserum (control 4) to the total radioactivity used in the assay (control 1), after correction of both values for the radioactivity not absorbable by charcoal (control 2). This sample is tested in the absence of unlabeled fibrinopeptide and is defined as $B_{\text{max}}$. Inhibition of binding is calculated from the ratio $\left(\frac{B_{\text{max}} - B}{B_{\text{max}}}\right) \times 100$ in which $B$ represents the binding in the presence of unlabeled FPA.

**Ultrafiltration**

Ultrafiltration was used to separate proteins of high molecular weight from low-molecular-weight peptides. Solutions (5 ml) were put into cones made from a semipermeable membrane with essentially no molecular retention below 50,000 daltons (Centriflo type 224-CF-SO, Amicon Corp., Lexington, Mass.) and centrifuged at 4°C at 2000 g for 15 min. Between 0.4 and 0.8 ml of the ultrafiltrate was obtained, depending on the protein concentration of the initial solution.

**RESULTS**

**Determination of Fibrinopeptide A in Purified Systems**

The binding of radiiodinated tyrosine-substituted peptides to antiserum against human FPA was approximately 1.5 times greater for $^8$Tyr-FPA than for $\text{NH}_2$-Tyr-FPA (Fig. 2). A similar increment occurred with different dilutions of antiserum and with different conditions of incubation. Binding was greater with a 24-hr incubation at 0°C than after 1 hr at room temperature. The relative increase of binding was greater at 1:1000 dilution of antiserum. Antiserum R 30

![Graph showing binding of two synthetic radiiodinated tyrosine-containing derivatives of human FPA ($\text{NH}_2$-Tyr-FPA and $^8$Tyr-FPA) to diluted rabbit antiserum (R 30). Labeled peptide was mixed with antiserum R 30 diluted to 1:100 or 1:1000 with buffer and incubated either at room temperature for 1 hr or in ice-cold water for 24 hr.](image)
bound better with \(^{6}\)Tyr-FPA, allowing for a lowering of the concentration of this component in the RIA; this synthetic peptide was used in subsequent experiments.

Binding was clearly decreased by native FPA at a final concentration of only 0.1 ng/ml and almost entirely abolished at a concentration of 10 ng/ml (Fig. 3). The slightly sigmoidal curve allowed for the quantitative measurement of human FPA. Since the absolute amount of binding in the absence of unlabeled peptide varied from day to day even under standard incubation conditions, especially when a new batch of labeled peptide was tested, the displacement curves have been normalized and expressed as per cent of inhibition of binding in the absence of unlabeled peptide.

Figure 4 shows the average and standard deviation of 11 such experiments obtained over a 4-wk period using the same batch of radioiodinated \(^{6}\)Tyr-FPA. There was no progressive change in experimental data, indicating that the observed variation was not related to radiolytic damage of the labeled peptide during storage. However, the standard deviation of this accumulated data was excessive at FPA concentrations below 1 ng/ml, and for this reason experimental samples were compared with standard curves performed the same day. The lowest concentration of human FPA detected by this assay was 0.1 ng/ml final concentration.
Table 3. Reaction of Various Fibrinopeptides in the Radioimmunoassay of Human Fibrinopeptide A

<table>
<thead>
<tr>
<th>Fibrinopeptide</th>
<th>Concentration (ng/ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human A</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>Human *Tyr-FPA</td>
<td>1.5</td>
<td>50</td>
</tr>
<tr>
<td>Human NH$_2$-Tyr-FPA</td>
<td>2.0</td>
<td>50</td>
</tr>
<tr>
<td>Human B</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>Human A + B</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Bovine A + B</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Canine A + B</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Porcine A + B</td>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

*Incubation of labeled *Tyr-FPA with R 30 antiserum at 1:1000 dilution at room temperature for 1 hr. Mixtures of fibrinopeptides A and B obtained from purified fibrinogens by clotting with thrombin and ultrafiltration.

Table 3 shows the reactions of various peptides related to FPA in the RIA utilizing labeled *Tyr-FPA as the marker. In these experiments, the RIA inhibition curves were obtained for each peptide, similar to those shown for native human FPA in Fig. 4. The peptide concentration corresponding to 50% inhibition was calculated from these curves. Native human FPA showed 50% inhibition at a final concentration of 2.5 ng/ml, more than was required for the two synthetic nonlabeled tyrosine derivatives. Human fibrinopeptide B did not inhibit, even at a concentration of 500 ng/ml. A mixture of human fibrinopeptides A and B showed 50% inhibition at 5 ng/ml, consistent with the results for purified peptides, and peptides obtained from bovine, canine, and porcine fibrinogens showed no appreciable inhibition of binding at concentrations of 30 ng/ml.

Fig. 5. Inhibition of binding of labeled *Tyr-FPA by increasing concentrations of human fibrinogen and by purified human FPA (○). Experimental conditions as in Fig. 4. Fibrinogen was tested without (e) thrombin, and the supernatant fluid was tested after prior incubation (○) with thrombin.
Comparison of human fibrinogen with native human FPA showed that lower molar concentrations of fibrinogen inhibit the binding of R 30 with Tyr-FPA, but the differing slopes of these inhibition curves (Fig. 5) did not allow a precise quantitative relation. No significant difference in inhibition between thrombin-treated fibrinogen and untreated fibrinogen was observed.

Table 4. Reaction of Plasmic Digests of Human Fibrinogen in the Radioimmunoassay of Human Fibrinopeptide A*  

<table>
<thead>
<tr>
<th>Total Sample (%)</th>
<th>Ultrafiltrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen Digest</td>
<td>65.9</td>
</tr>
<tr>
<td>20 min</td>
<td>118.2</td>
</tr>
<tr>
<td>40 min</td>
<td>125.0</td>
</tr>
<tr>
<td>60 min</td>
<td>110.2</td>
</tr>
<tr>
<td>24 hr</td>
<td>122.2</td>
</tr>
</tbody>
</table>

*Total protein concentration of undegraded fibrinogen was 20 mg/ml. Digestion was started by the addition of streptokinase (Varidase, Lederle Laboratories, Pearl River, N.Y.; 200 U/ml final concentration) and terminated by the addition of soybean trypsin inhibitor (Worthington, Freehold, N.J.; 0.05 mg/ml final concentration). The 20-, 40-, and 60-min digests correspond to stage 2 digests and the 24-hr digest to a stage 3 digest. The total digests and the ultrafiltrates were tested with R 30 anti-FPA antiserum. A series of double dilutions of each sample was tested, and the content of FPA was calculated for those dilutions which showed between 10% and 80% inhibition of the RIA. The average FPA content (ng/ml) was divided by the expected content of FPA in the starting fibrinogen preparation (176,000 ng/ml), and the results are expressed as per cent of expected FPA content.

Comparison of human fibrinogen with native human FPA showed that lower molar concentrations of fibrinogen inhibit the binding of R 30 with Tyr-FPA, but the differing slopes of these inhibition curves (Fig. 5) did not allow a precise quantitative relation. No significant difference in inhibition between thrombin-treated fibrinogen and untreated fibrinogen was observed.

Reaction of Plasmic Degradation Products of Fibrinogen in the Radioimmunoassay for Fibrinopeptide A

The effect of fibrinogen degradation on the determination of FPA was assessed using plasmic digests of fibrinogen and purified derivatives. The reaction of antiserum with plasmic digests was approximately 110%-120% of the expected value, independent of the time of proteolysis (Table 4). Approximately
66% of the expected content of FPA was found in fibrinogen in this experiment, and only 0.36% passed the ultrafilter membrane. Similarly, only a trace amount of reactive material was present in the ultrafiltrate of digests after 20 and 40 min. However, about 11% of the reactive material in the 60-min digest and 47% of the 24-hr digest were recovered in the respective ultrafiltrates.

Purified preparations of human fibrinogen, plasmic derivatives fragments X, Y, D, and E, and the NH2-terminal disulfide knot28 were compared for reactivity in the RIA (Fig. 6). The molar reactivity of all products was approximately the same in this test system except for that of fragment E, which was slightly less reactive, and fragment D, which required 1000-fold higher concentrations to show the same extent of inhibition. Ultrafiltrates of these proteins after treatment with thrombin (120 min at 37°C, 10 NIH U/ml final concentration) showed the same absolute and relative reactions in the RIA, indicating complete liberation and recovery of FPA from the respective degradation products.

DISCUSSION

As first demonstrated by Nossel and colleagues,14 the RIA procedure provides a method for the quantitative determination of nanogram amounts of human FPA (Figs. 3 and 4). With the technique described here, 0.1 ng/ml of purified FPA resulted in a detectable (10%) degree of inhibition (Fig. 4), which is comparable to findings already reported.14-16 In the assessment of unknown mixtures, a 0.1-ml sample containing FPA at a starting concentration of 1.0 ng/ml can be reliably measured, and by increasing the test sample to 0.35 ml (see Table 2), the concentration can be as low as approximately 0.6 ng/ml. The system is highly specific, since purified human fibrinopeptide B and fibrinopeptides A and B derived from bovine, canine, and porcine fibrinogens are not inhibitory (Table 3). Since the amino acid sequence of the six terminal carboxy residues of the fibrinopeptides A from these four species is identical and that of the eleven terminal carboxy residues is very similar (Fig. 7), the immunologic reactive site for this antiserum appears to reside in the amino terminal portion of human FPA. With the aromatic amino acid tyrosine at the amino terminus (NH2-Tyr-FPA) instead of position 8 (8-Tyr-FPA), there is less reactivity with antibody against native human FPA, further indicating that the structure of the NH2-terminal portion of the molecule appears to contribute to the reaction (Fig. 2, Table 3). The specificity of the antibody for human FPA agrees with the results of Nossel and colleagues on human fibrinopeptide B and on bradykinin,14 but is at variance with results obtained by others29 who found cross-reactions with fibrinogen of several animal species. In our double immunodiffusion studies, nonabsorbed antiserum against human FPA did not precipitate

![Fig. 7. Amino acid sequences of human, bovine, porcine, and canine FPA, as provided by reference 8.](image-url)
with purified homologous FPA or with fibrinogen, in contrast to the results of others.\textsuperscript{29,30} The antigen-antibody complex between human FPA and the heterologous antibody is apparently a nonprecipitable one, as are other complexes of antibody with low-molecular-weight polypeptide antigens.\textsuperscript{31}

Nossel and colleagues\textsuperscript{15} suggest that the reactivity of the FPA region of degraded fibrinogen with some antisera may vary according to the peptide structure of contiguous regions of the molecule. With our antiserum, reactivity was essentially the same with all products containing FPA, and reactivity before and after thrombin exposure was unchanged (Figs. 5 and 6). FPA immunoreactivity was recovered in the ultrafiltrate of plasmic digests only after the longer digestion (stage 3) periods (Table 4). This reflects the fact that the FPA region is intact in the larger plasmic derivatives (fragments X and Y),\textsuperscript{12} which constitute the major portion of the incomplete digests. As the content of fragment E in the digest increases,\textsuperscript{11} more FPA is recovered in the ultrafiltrate (Table 4). This reflects the fact that FPA or an FPA-containing peptide is liberated by plasmin from fragment E.\textsuperscript{13} Since only half of the reactive material in the 24-hr digest is recovered, we can conclude that plasmic cleavage of the FPA material is still incomplete. The 50\% inhibition level of fragment E was $7 \times 10^{-9} \text{M}$, as compared with $4 \times 10^{-9} \text{M}$ for fibrinogen, indicating the loss of approximately 40\% of FPA from fragment E. This agrees with the results of Table 4 (24-hr digest) and with prior studies showing that fragment E contains low, but detectable, amounts of FPA as determined by a nonimmunologic technique.\textsuperscript{13} The data of Gerrits and colleagues\textsuperscript{16} also show approximately 50\% recovery of FPA immunoreactivity from dialyzed, streptokinase-treated plasma fibrinogen, as compared with thrombin-treated plasma fibrinogen, although most of the FPA appeared after short, rather than prolonged, incubation periods.

The NH\textsubscript{2}-terminal disulfide knot reacted as well as fibrinogen and fragments X and Y, all of which had approximately twice the molar reactivity of purified FPA (Figs. 5 and 6). Fragment D should not contain FPA since it derives from the carboxyl-terminal portion of fibrinogen,\textsuperscript{12} and this is reflected by the inordinately high concentration ($2 \times 10^{-6} \text{M}$) required to demonstrate 50\% inhibition of the RIA (Fig. 6).

These results indicate that only extensive plasmic degradation of plasma fibrinogen would produce ultrafiltrable material that reacts in the RIA for FPA. Since this possibility is likely to occur only under extreme circumstances of plasminogen activation, such as with streptokinase or urokinase administration, the RIA of clinical samples of plasma should reflect primarily thrombin-induced FPA liberation from fibrinogen. Furthermore, the processing of plasma described by Nossel and colleagues\textsuperscript{15} favors the elimination of large-molecular-weight degradation products which contain FPA, further avoiding confusion of plasmin fibrinogenolysis with fibrin formation. Clinical states which reflect sequential thrombin and plasmin action on fibrinogen, such as disseminated intravascular coagulation, would give clearly positive results in this RIA. Only the fibrinolytic states with obvious shortening of the euglobulin lysis time but without thrombotic events might be expected to give false-positive tests. The major theoretical usefulness of the assay is in the detection of subclinical or early venous thrombosis, and there appears to be no theoretical limitation for such an application based on the studies reported here.
ACKNOWLEDGMENT

The excellent technical assistance of Miss Margery E. Parker and Mrs. Eugenia C. Rogers is gratefully acknowledged.

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Reaction of plasmin degradation products of fibrinogen in the radioimmunoassay of human fibrinopeptide A

AZ Budzynski, VJ Marder and S Sherry