The Fate of Soluble Fibrin Monomer in Relation to Intravascular Fibrin Formation and Degradation in Rabbits

By Victor Gurewich, Robert Wetmore, Andrzej Nowak, and Boguslaw Lipinski

125I-labeled fibrinogen or fibrin monomer (FM) were infused into rabbits, and the radioactivity in the blood, certain organs, and urine was followed. In the FM rabbits, a progressive, relatively rapid loss of blood radioactivity occurred which was accompanied by radioactive deposits in the organs and the excretion of radioactivity in the urine. Extraction of radioactive material from homogenized organ tissues of FM-infused rabbits showed that most of it had the characteristics of stabilized fibrin. Administration of EACA did not change any of the measurements in the fibrinogen animals but increased the rate and extent of fibrin deposition in the FM animals. In animals made leukopenic with HN2, fibrin deposition was inhibited. The findings indicate that a major pathway of FM clearance from the blood involves fibrin formation and deposition with subsequent degradation and excretion. The reticuloendothelial system appeared to play a major role in FM clearance, since most of the fibrin deposits were found in the liver, and the highest concentration was in the spleen. A nonenzymatic mechanism of fibrin formation from soluble FM involving leukocytes is postulated.

FIBRIN MONOMER (FM) is formed as a result of thrombin action on fibrinogen. Polymerization of FM to fibrin in blood is inhibited by the formation of soluble complexes with fibrinogen1 and fibrin-degradation products.2 Soluble complexes of FM have been demonstrated in the plasma in pregnancy and the puerperium,3 in women on oral contraceptives4,5 and in certain pathologic states, particularly disseminated intravascular coagulation.6-8 Certain in vitro characteristics of soluble FM have been described, such as the nonenzymatic formation of fibrin in the presence of ethanol6 or protamine sulfate.9,10 In addition, FM shortens the thrombin time of plasma12 and potentiates platelet aggregation.13 However, little is known about the clearance of FM from the circulation or its biologic properties in vivo.

In the present study, the fate of FM was investigated in rabbits infused with 125I-labeled soluble fibrin by performing measurements of radioactivity in the blood, in certain organs, and in the urine. The results were compared with similar measurements in animals given 125I-labeled fibrinogen. The effects of fibrinolytic inhibition, streptokinase (SK), and leukopenia were evaluated. The findings indicate that the clearance of FM from the blood involves fibrin deposition in organs and subsequent degradation and excretion. Granulocytes appear to be involved in the precipitation of soluble FM in the organs.

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MATERIALS AND METHODS

Purification of Rabbit Fibrinogen

Rabbit fibrinogen (Fraction I, Miles Laboratories, Inc., Kankakee, Ill.) was purified as follows: 1 g fibrinogen was dissolved in 100 ml 0.85% saline and centrifuged (5000 g) for 20 min in the cold (4°C). The supernatant was precipitated with ammonium sulfate at 0.25 saturation. The precipitate was redissolved in 100 ml saline and centrifugation and precipitation with ammonium sulfate repeated. The final product was dialyzed at 4°C against Tris-buffered saline (pH 7.9). The cold insoluble material formed during dialysis was discarded. The final product was 90% clottable and was homogeneous in 3.5% SDS polyacrylamide gel electrophoresis.14

Preparation of 125I-labeled Fibrinogen

A 1% solution of purified fibrinogen was labeled with 125-I-iodine according to the method of McFarlane.15 The specific radioactivity of the final material was 10 MCi/mg. One-tenth milliliter of the labeled material was mixed with a solution of fibrinogen for infusion. The radioactive material was 90% clottable.

Preparation of 125I-labeled FM

One-tenth milliliter of the 125I-fibrinogen was added to 4 ml of a saline solution of purified rabbit fibrinogen. The final fibrinogen concentration of the solution was 20 mg/ml. The solution was clotted with 0.05 ml thrombin (100 NIH U/ml). The clot was incubated for 20 min at 37°C and then was dissolved in 4 ml of 2 M NaBr. After incubation (37°C) for 1 hr, complete dissolution had occurred. The solution of soluble fibrin was made up to a final volume of 15 ml by the addition of 1 M NaBr (pH 5.1–5.3).

An aliquot of the soluble fibrin solution was diluted out fivefold with Tris-buffered saline (pH 7.4) and the radioactivity in the precipitated fibrin determined. From this value the clottability of the solution was calculated to be 88%.

Infusion of 125I-fibrinogen or 125I-FM

The solutions of fibrinogen or soluble fibrin were infused into rabbits (20 mg/kg) in a 15-ml 1 M NaBr solution. All infusions were given through a marginal ear vein with an infusion pump (Harvard Apparatus Co., Dover, Mass.). Unless otherwise stated, a 30-min infusion period was used.

Laboratory Determinations

Platelets and leukocytes in blood collected on EDTA were counted with a Coulter Counter (Model Fn., Coulter Electronics, Inc., Fla.).

Fibrinogen was measured in blood collected on 4.8% citrate (9:1) by the thrombin clottable protein method of Swain and Feders.16

Ethanol gelation test (EGT) was determined by the method of Godal.6

Serial dilution protamine sulfate (SDPS) test was determined by the method of Gurewich and Hutchinson.8 Only the presence of fibrin strands (fs) or a gel (g) after 30 min standing at room temperature was considered a positive reaction. Protamine sulfate (Eli Lilly Co., Indianapolis, Ind.) dilutions ranging from 1:10 to 1:80 were used.

Euglobulin clot lysis was determined by the method of Kowalski et al.17 Preparation of the euglobulin fraction was performed in the cold (4°C).

In the animals infused with EACA, SK-activated clot lysis was performed after the initial infusion and at 3 and 5 hr. One-tenth milliliter ml thrombin (100 U/ml) and 0.1 ml SK (2000 U/ml) were mixed and added to 0.8 ml citrated whole blood in an ice bath. After complete clotting had occurred, the clot was incubated (37°C) and observed for 24 hr.

Radioisotope Counting

A well scintillation counter connected to a scaler (Spectrometer Model 530, Baird Atomic) was used. Freshly removed organs were rinsed thoroughly in saline and blotted with filter paper. One
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gram of tissue was suspended in 3 ml 0.85% saline containing heparin (10 U/ml) and homogenized in a high-speed homogenizer (Virtis “23,” the Virtis Co., Gardiner, N.Y.). The mixture was centrifuged and the radioactivity of the sediment determined.

One-gram portions of the following organs were prepared for counting: heart, kidney (cortex), liver, lungs, and spleen. The weight of each organ was determined and divided by the rabbit weight (kg).

The counts per minute (cpm) were invariably lowest for the heart. In each experiment, the radioactivity in this organ was used as a reference standard (cpm/g heart = 1 U) to which the radioactivity in the other organs was compared. This procedure was followed in order to provide a system which is not influenced by changes in the potency of the radioisotope and unavoidable differences in the amount of radioactivity infused into each animal.

The results were expressed in units for each organ per kilogram of rabbit.

One-milliliter samples of whole blood were counted. The whole blood counts were expressed as a per cent of the absolute count obtained immediately after each infusion (baseline).

The radioactivity in 1 ml of urine was multiplied by the total volume excreted by the rabbit during the experiment. It was expressed as a per cent of the total radioactivity initially infused into the animal.

**Extraction of Radioactive Material From the Organs**

One-gram portions of the five organs from each of five rabbits infused with $^{125}$I-fibrinogen and from five rabbits infused with $^{125}$I-FM were homogenized with 0.85% saline (3 ml/g) and centrifuged (5000 g for 10 min). The sediment was resuspended in 3-ml portions of 2%, acetic acid. Between steps, the saline and acetic acid suspensions were incubated (37°C) for 30 min. After centrifugation, the radioactivity of the supernatants and sediment was determined. The results were expressed as a percentage of the total radioactivity in the extracts and sediment from each organ.

**Animal Models**

All experiments were performed on white New Zealand rabbits weighing 2 3 kg and anesthetized with intravenous Nembutal. A polyethylene catheter (PE 90) was inserted into a carotid artery for blood sampling unless otherwise stated. In all of the 5-k hr experiments, blood samples were taken immediately after infusion (baseline) and at 1, 1 2, 3 4, and 5 5 hr from the time the infusion was started. Eight-milliliter volumes were removed for the first and last samples and 2-ml volumes for the remaining samples.

Urine was collected into a plastic bag and was kept free of fecal contamination. At the end of the experiment, the bladder was manually emptied through an incision in the lower abdomen. Animals were given 4 ml of 25% EACA in saline containing heparin (50 U/ml) before being killed and the organs removed.

**Fibrinogen (5 5 hr)**. Six animals were infused with $^{125}$I-fibrinogen solution and killed 5 5 hr later. Twenty milliliters saline was infused over the 5-k hr period of the experiment.

**Fibrinogen (24 hr)**. In six animals, after the infusion of $^{125}$I-fibrinogen solution, a blood sample was collected from the central artery of the ear through a No. 19 needle after rubbing the ear lightly with xylene. These animals were lightly anesthetized but not cannulated. After this, the rabbit was returned to its cage, and 24 hr later blood was collected by cardiac puncture and the animal killed and autopsied.

**FM (10 min)**. In six animals the standard solution of $^{125}$I-FM was infused over a 10 min period and the animals killed immediately following the infusion.

**FM (30 min)**. Six animals were infused with $^{125}$I-FM solution and killed immediately following the infusion.

**FM (5 5 hr)**. Six animals were infused with $^{125}$I-FM solution and killed 5 5 hr later. Twenty milliliters saline was infused over the last 5 hr of the experiment.

**FM (24 hr)**. In six animals, after the infusion of $^{125}$I-FM solution, a blood sample was collected from the central artery of the ear and the same procedure was followed as described above in the fibrinogen animals (group 2).

**FM and EACA (5 5 hr)**. Six animals were given 1 g of EACA (4 ml) intravenously over 2 min. Thereafter, $^{125}$I-soluble fibrin solution was infused. At the end of the infusion, EACA (5 g in 20 ml saline) was given by constant infusion over 5 hr.
FM and SK (5 1/2 hr). Six animals were infused with SK (10,000 U/kg/hr, Streptase, Hoechst Pharmaceutical Co., Somerville, N. J.) for 4 3/4 hr beginning 3 hr after the completion of the 125I-FM infusion.

FM and nitrogen mustard (HN2) (5 1/2 hr). Six animals were pretreated with HN2 (Mustargen, Merck Sharp & Dohme, West Point, Penn.), 2 mg/kg intravenously, 3 days before infusion of the 125I-soluble fibrin solution. These animals were infused with 20 ml saline for the last 5 hr of the experiment.

Statistical Analysis

The changes in blood radioactivity in the different groups were analyzed by performing a multiple comparison procedure (Dunnett's procedure) within an analysis of variance framework. A rank sum test was used for the remaining analyses with the p value obtained by the distribution of the rank sum T².

RESULTS

Laboratory Findings

The animals pretreated with HN2 had low leukocyte counts: 1653 (790-3150)/cu mm. In the remaining animals, the mean and range leukocyte counts were 5500 (3200-9800). A slight increase at 5 1/2 hr occurred in most rabbits, but there were no significant differences between groups in these changes. Platelets were not reduced in number by the HN2, and the counts were the same in all the groups of animals. In all rabbits the platelet count fell at 5 1/2 hr, but there were no significant differences between the groups. Between the baseline (BL) and 5 1/2-hr values, there was also a drop in hematocrit and fibrinogen in all the groups of animals. The fall in fibrinogen levels of all the 125I-FM groups except those given HN2 was significantly greater (p < 0.02) compared to the 125I-fibrinogen animals. The HN2-treated animals had significantly (p < 0.05) higher baseline fibrinogen levels (Table 1).

All of the animals infused with FM developed a positive SDPS test (1:20 g:1:80 g) and EGT. The tests remained positive for 5 1/2 hr but were negative at 24 hr. In the fibrinogen-infused animals, these tests were negative after the infusion, but after 5 1/2 hr some rabbits had a positive test. The 24-hr determinations were negative (Table 1).

The ELT in the SK-treated rabbits was 3.4 min compared to 2.6 hr in the

<table>
<thead>
<tr>
<th>Table 1. Mean Laboratory Values at Baseline (B.L.) and 5 1/2 Hours</th>
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<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>125I-Fibrinogen</td>
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<tr>
<td></td>
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<tr>
<td>125I-Fibrinogen - EACA</td>
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<tr>
<td></td>
</tr>
<tr>
<td>125I-FM</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>125I-FM + HN2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>125I-FM + EACA</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>125I-FM + SK</td>
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untreated animals. In the EACA-treated animals, the ELT was greater than 12 hr, and the whole blood clot lysis time after SK activation was greater than 24 hr (normal: <5 min).

Extraction of Radioactive Material

The percentage of radioactivity (mean and range) in the saline and acetic acid extracts and in the sediment (insoluble organ residue) for all the organs from the five rabbits in each group are given. In the fibrinogen-infused animals, about 35% (mean) of the total radioactivity was extractable with saline, and slightly less than half was in the soluble organ residue. By contrast, in the FM animals about 14% of the radioactive material was extractable with saline, and almost two-thirds of the radioactivity remained in the sediment (Table 2).

Animal Models

Radioactivity in Blood

In the fibrinogen animals, a slow loss of radioactivity occurred with 59% (51%–68%) still present at 5 hr and 36% (26%–49%) remaining at the end of 100 hours.

Table 2. Percentage Distribution of Radioactivity in Saline and Acetic Acid Extracts and in the Homogenized Tissue Residue of All the Organs Removed After 5 1/2 Hours

<table>
<thead>
<tr>
<th>Organ</th>
<th>Saline (%)</th>
<th>Acetic Acid (%)</th>
<th>Insoluble Organ Residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125I-Fibrinogen</td>
<td>34.8</td>
<td>16.5</td>
<td>48.7</td>
</tr>
<tr>
<td>(5 rabbits)</td>
<td>(14–55)</td>
<td>(8–33)</td>
<td>(26–77)</td>
</tr>
<tr>
<td>125I-FM</td>
<td>13.7</td>
<td>21.6</td>
<td>64.6</td>
</tr>
<tr>
<td>(5 rabbits)</td>
<td>(3–30)</td>
<td>(3–38)</td>
<td>(58–80)</td>
</tr>
</tbody>
</table>

Fig. 1. Blood radioactivity expressed as per cent baseline (postinfusion) count. Fibrinogen and fibrinogen + EACA significantly (p < 0.05 – < 0.002) different from FM, FM + EACA, and FM + SK. FM significantly (p < 0.05) different from FM + HN2 and FM + EACA.
24 hr. The infusion of EACA did not significantly effect the blood radioactivity in these animals.

In the FM group, the decay of blood radioactivity was significantly \((p < 0.001)\) more rapid at 3 hr, thereafter falling to 47\(^{\circ}\) (34\(^{\circ}\), 59\(^{\circ}\)) at 5\(\frac{1}{2}\) hr and to 9\(^{\circ}\) (5\(^{\circ}\), 12\(^{\circ}\)) after 24 hr. EACA caused a significant \((p < 0.05)\) acceleration in the decay of blood radioactivity, with only 33\(^{\circ}\) (14\(^{\circ}\), 52\(^{\circ}\)) remaining at 5\(\frac{1}{2}\) hr. The infusion of SK did not significantly alter the changes in blood radioactivity. Pretreatment with HN\(_2\) significantly \((p < 0.01)\) delayed the loss of blood radioactivity compared to the other FM groups, with 56\(^{\circ}\) (54\(^{\circ}\), 59\(^{\circ}\)) still remaining at 5\(\frac{1}{2}\) hr (Fig. 1).

**Organ Weights**

The mean and range weights of the organs in grams per kilogram of rabbit were as follows: heart, 2.5 (1.4, 3.8); kidneys, 7.9 (5.9, 10.3); liver, 35.6 (20.9, 55.0); lungs, 5.4 (3.5, 12.6); spleen, 0.8 (0.4, 1.5).

**Radioactivity in the Organs**

125I-fibrinogen. All three groups had a similar amount and distribution of radioactivity. EACA did not effect the results, and the radioactive counts at 24 hr were not significantly different from those at 5\(\frac{1}{2}\) hr (Table 3).

The concentration of radioactivity per gram of organ was approximately equal for the four organs (mean values, 1.2, 3.4 U) (Table 4).

125I-FM. (1) Saline. The amount of radioactive material deposited at 10 min, 30 min, and 5\(\frac{1}{2}\) hr was significantly \((p < 0.001)\) greater than in the fibrinogen-infused animals. By 24 hr, less than half of the radioactivity remained.

### Table 3. Total Radioactivity in Each Organ and Their Sum Total (Mean and Range)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (Hr)</th>
<th>Kidney (U)</th>
<th>Liver (U)</th>
<th>Lungs (U)</th>
<th>Spleen (U)</th>
<th>Total (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 I-Fibrinogen</td>
<td>5(\frac{1}{2})</td>
<td>11.8 (7.2-18.1)</td>
<td>65.2 (36.4-105.2)</td>
<td>7.9 (3.6-12.8)</td>
<td>1.6 (0.9-3.0)</td>
<td>86.5 (48.4-122.0)</td>
</tr>
<tr>
<td>EACA</td>
<td>5(\frac{1}{2})</td>
<td>12.0 (7.8-16.9)</td>
<td>63.6 (38.0-108.1)</td>
<td>10.5 (4.0-19.8)</td>
<td>2.4 (0.8-4.4)</td>
<td>88.5 (50.3-132.2)</td>
</tr>
<tr>
<td>24</td>
<td>12.2 (7.7-17.3)</td>
<td>59.2 (30.1-96.3)</td>
<td>9.8 (3.8-17.2)</td>
<td>1.5 (1.0-4.0)</td>
<td>82.7 (47.2-118.5)</td>
<td></td>
</tr>
<tr>
<td>125 I-FM</td>
<td>5(\frac{1}{2})</td>
<td>65.6 (16.8-32.0)</td>
<td>128.3 (98.1-184.5)</td>
<td>9.4 (4.1-16.8)</td>
<td>6.1 (4.8-9.2)</td>
<td>165.0 (129.8-208.3)</td>
</tr>
<tr>
<td>24</td>
<td>21.2 (15.0-31.2)</td>
<td>131.2 (101.6-194.2)</td>
<td>14.2 (3.5-28.1)</td>
<td>12.8 (4.6-20.3)</td>
<td>199.2 (129.8-225.0)</td>
<td></td>
</tr>
<tr>
<td>125 I-FM</td>
<td>5(\frac{1}{2})</td>
<td>19.2 (14.9-30.8)</td>
<td>131.2 (91.4-211.8)</td>
<td>14.8 (3.9-39.2)</td>
<td>24.0 (10.3-50.1)</td>
<td>189.2 (138.1-310.4)</td>
</tr>
<tr>
<td>24</td>
<td>10.2 (7.4-15.1)</td>
<td>44.5 (25.6-70.1)</td>
<td>11.5 (2.1-25.2)</td>
<td>2.8 (1.1-4.5)</td>
<td>69.0 (42.3-118.0)</td>
<td></td>
</tr>
<tr>
<td>125 I-FM</td>
<td>HN(_2)</td>
<td>16.1 (8.1-19.2)</td>
<td>65.6 (34.1-98.7)</td>
<td>9.7 (3.6-18.4)</td>
<td>5.9 (3.1-7.4)</td>
<td>102.7 (70.5-130.0)</td>
</tr>
<tr>
<td>EACA</td>
<td>5(\frac{1}{2})</td>
<td>78.2 (18.2-168.1)</td>
<td>200.5 (88.3-390.7)</td>
<td>22.5 (16.8-38.0)</td>
<td>49.7 (39.2-64.8)</td>
<td>350.9 (169.4-520.2)</td>
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<tr>
<td>SK</td>
<td>15.8 (9.2-18.0)</td>
<td>62.4 (38.1-78.7)</td>
<td>14.8 (4.1-24.0)</td>
<td>1.9 (1.2-2.8)</td>
<td>94.9 (65.1-112.8)</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Amount of Radioactive Material (Mean and Range) in One Gram of Each Organ

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (hr)</th>
<th>Concentration of Radioactivity in Organs (U/g)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
</tr>
<tr>
<td>125 I-Fibrinogen</td>
<td>5 1/2</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.2-2.1)</td>
</tr>
<tr>
<td>EACA</td>
<td>5 1/2</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.0-2.1)</td>
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<tr>
<td></td>
<td>24</td>
<td>1.5</td>
</tr>
<tr>
<td>125 I-M</td>
<td>1/4</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.1-4.3)</td>
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<td></td>
<td>1/2</td>
<td>2.8</td>
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<td>(1.1-4.2)</td>
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<td></td>
<td>5 1/2</td>
<td>2.4</td>
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<td></td>
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<td>(1.3-4.2)</td>
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<tr>
<td></td>
<td>24</td>
<td>1.3</td>
</tr>
<tr>
<td>125 I-M</td>
<td>24</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.1-2.1)</td>
</tr>
<tr>
<td>HN2</td>
<td>5 1/2</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.1-3.2)</td>
</tr>
<tr>
<td>EACA</td>
<td>5 1/2</td>
<td>9.6</td>
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<tr>
<td></td>
<td></td>
<td>(2.7-30.4)</td>
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<tr>
<td>SK</td>
<td>5 1/2</td>
<td>2.1</td>
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<tr>
<td></td>
<td></td>
<td>(1.2-2.6)</td>
</tr>
</tbody>
</table>

The amount of material deposited in the spleen increased progressively from 10 min to 5 1/2 hr (Table 3). The concentration of radioactivity per gram of spleen at 10 min, 30 min, and 5 1/2 hr was significantly \( p < 0.001 \) greater than for any of the other organs (mean values, 7.3–28.1 U compared to 1.6–4.7 U for the other organs) (Table 4).

(2) EACA—The infusion of EACA significantly \( p < 0.01 \) increased the total deposition of radioactive material in the organs. The greatest changes occurred in the kidney and spleen (Table 3).

(3) HN2—In the animals pretreated with HN2, relatively little deposition of radioactive material was found, the counts not being significantly different from those in the fibrinogen group of animals (Table 3).

(4) SK—The infusion of SK for 4 1/2 hr resulted in low organ counts, with the radioactivity being comparable to that found in the fibrinogen group of animals (Table 3).

Radioactivity in the Urine After 5 1/2 hr

The total amount of radioactivity excreted in the urine in 5 1/2 hr was least in the fibrinogen, fibrinogen + EACA, and in the FM + HN2 groups of animals. These three groups were not significantly different from each other and were, respectively, 6.9\(^{a}\) (2.0\(^{a}\) – 10.9\(^{a}\)), 9.4\(^{a}\) (5.6\(^{a}\) – 14.2\(^{a}\)), and 7.8\(^{a}\) (3.7\(^{a}\) – 21.3\(^{a}\)). The FM + EACA and FM + SK groups had the greatest radioactive excretion, being 17.7\(^{a}\) (6.9\(^{a}\) – 24.1\(^{a}\)) and 16.8\(^{a}\) (8.1\(^{a}\) – 29.6\(^{a}\)), respectively. These two values were significantly \( p < 0.05 \) higher than the former three. The untreated FM group values [15.0\(^{a}\) (4.4\(^{a}\) – 26.4\(^{a}\))] were significantly \( p < 0.05 \) greater than the fibrinogen group.
DISCUSSION

Fibrinogen turnover under normal conditions has been reported not to be altered by either anticoagulants or EACA. In the \( ^{125} \text{I-} \text{fibrinogen-infused rabbits}, \) we found relatively little radioactivity in the organs. Most of the radioactive material found could be extracted with either saline or acetic acid and therefore represented precipitated fibrinogen or unstabilized fibrin. In a previous study, we found that the radioactive material remaining after acetic acid can be extracted only by breaking the disulfide bonds with mercaptoethanol and therefore has the characteristics of stabilized fibrin. The infusion of EACA did not alter either the concentration of the radioactivity found in the blood of these animals or the amount present in the organs. The positive EGT and SDPS test found in some of these rabbits at the end of 5\( \frac{1}{2} \) hr is probably attributable to the effect of prolonged anesthesia and blood removal. The findings in the fibrinogen animals therefore support the view that the major physiologic pathway of fibrinogen catabolism is not by consumption due to intravascular fibrin formation.

The findings in the rabbits infused with soluble fibrin differed sharply from those of the fibrinogen animals. Fibrin has been shown to be monomeric when it is dissolved in 1.0 \( M \) NaBr at an acidic pH. Infusion of this FM solution resulted in substantial fibrin deposition. Fibrin could have formed as a result of the polymerization of FM as it reached a physiologic pH. However, a dynamic process of fibrin formation was indicated by the sequential changes in the blood and organ radioactivity following the infusion.

Most of the radioactive material found in the organs of the FM rabbits had the characteristics of stabilized fibrin, since it could not be extracted from homogenized tissue with acetic acid. The deposition in the organs was progressive over 5\( \frac{1}{2} \) hr and was accompanied by a gradual loss of radioactivity from the blood and its appearance in the urine. By 24 hr, little radioactivity was left in either the blood or the organs and had presumably been excreted, although a 24-hr urine collection was not obtained.

Among the organs examined, most of the fibrin was in the liver, but the greatest concentration was in the spleen. A similar finding was recently obtained by us in rabbits in which intravascular fibrin deposition was induced by two doses of endotoxin administered 24 hr apart. In the latter study, \( ^{51} \text{Cr-labeled rabbit leukocytes} \) were shown also to be concentrated in the spleen, and a significant correlation was observed between the quantity of radioactivity in the organs and the fall in leukocyte count after the second dose of endotoxin. It was postulated that leukocytes were actively involved in fibrin formation and that the concentration of fibrin in the spleen was related to the property of this organ to remove decaying leukocytes from the circulation. The predominant concentration of fibrin deposits in the spleen found in the present study suggests that a similar mechanism of fibrin formation from soluble FM complexes may be involved. Rabbits rendered leukopenic but not thrombocytopenic by pretreatment with \( \text{HN}_2 \) had significantly less fibrin deposited in the organs, significantly slower loss of radioactivity from the blood, and little radioactivity excreted in the urine. These findings are consistent with the hypothesis that leukocytes may trigger intravascular fibrin formation and deposition from solu-
ble FM complexes. Horn has suggested that leukocytes' cationic proteins and acid muco-substance may induce nonenzymatic fibrin formation from circulating FM, and this property of granulocytes has been demonstrated in vitro by Hawiger et al. The concept of nonenzymatic fibrin formation from circulating FM was originally proposed by Lipinski et al.

The distribution of fibrin in these models is in sharp contrast to that obtained following the infusion of massive doses of thrombin plus EACA. Regoeczi and Brain found that this results in most of the fibrin being found in the lungs and kidneys. They concluded that this distribution was related to a process of a passive filtration of microclots by the organs. In our studies, the findings indicate that fibrin deposition is due to an active process, the nature of which probably determines the organ distribution. The formation of FM, its interaction with leukocyte constituents, and the inhibition of fibrinolytic activity all appear to be involved in this process.

In the FM animals given EACA, significantly more fibrin was deposited in the organs, particularly the kidneys and spleen. These findings are in accordance with those of Müller-Berghaus et al., who found that EACA increased glomerular microclot formation in rabbits infused with soluble fibrin made up in acetic acid. The loss of blood radioactivity was also significantly accelerated by EACA, presumably reflecting more rapid organ deposition. Surprisingly, these animals excreted a large amount of radioactivity in the urine, suggesting that fibrin degradation was taking place despite the EACA. Blood fibrinolytic activity was effectively inhibited since no plasminogen activation could be induced by SK in the blood from these animals. The mechanism by which fibrin degradation occurred in the presence of EACA is not known but may involve intracellular degradation as postulated by Regoeczi.

In the FM animals treated with SK, fibrin deposits were effectively lysed, since little radioactivity remained in the organs at 5 hr., and the excretion of radioactivity in the urine was increased. Blood radioactivity was not significantly altered, and the SDPS test and EGT remained strongly positive. These findings indicate that soluble FM complexes, in contrast to fibrin deposits, are insensitive to fibrinolytic activity. Although a positive SDPS test may be due to fibrin degradation products rather than FM, the EGT is insensitive to these products and therefore indicates the presence of FM.

It may be concluded that the biologic properties of FM in vivo differ from those of fibrinogen. A major pathway for the clearance of FM from the blood involves a process of intravascular fibrin formation and selective deposition with subsequent degradation. The reticuloendothelial system and leukocytes appear to be involved in this process perhaps by inducing nonenzymatic polymerization of soluble FM complexes. This hypothesis is supported by recent observations that heparin does not inhibit fibrin deposition in the present experimental model. A comparable finding has also been reported by Müller-Berghaus et al., indicating that although thrombin is necessary for the formation of FM from fibrinogen, this enzyme is not required for the subsequent conversion to fibrin in vivo. These experimental observations may contribute to our understanding of the pathophysiology of disseminate intravascular coagulation, a condition in which excessive FM formation is known to occur.
REFERENCES

1. Shainoff JR, Page IH: Significance of cryo-
fibrin in fibrinogen-fibrin conversion. J Exp 

2. Lipinski B, Wegrzynowicz Z, Budzynski 
A2, Kopec M, Lattalo ZS, Kowalski E: Soluble 
unclottable complexes formed in the presence 
of fibrinogen degradation products (FDP) dur-
ing fibrinogen-fibrin conversion and their 
potential significance in pathology. Thromb Diath 
Haemorrh 17:65, 1967

3. Hyde E, Joyce D, Gurewich V, Flute P, 
Barrera S: Intravascular coagulation during 
pregnancy and the puerperium. J Obstet 
Gynaecol Br Commonw 80:1059, 1973

4. Pindyck L, Lichtman HC, Kohl SG: Cryo-
fibrinogenaemia in women using oral contra-
ceptive. Lancet 1:51, 1970

5. Alkjaersig N, Fletcher AP, Burstein R: 
Thromboembolism and oral contraceptive med-
ication. Sexual hormone and Blutgerinnung. 
XIV Hamburger Symposium über Blutgerin-
nung. Stuttgart, F. K. Schattauer Verlag, 1971

6. Godal HC, Abildgaard U: Gelation of 
soluble fibrin in plasma by ethanol. Scand J 
Haematol 3:342, 1966

7. Seaman AJ: The recognition of intravas-
cular clotting. The plasma protamine para-
coagulation test. Arch Intern Med 125:1016, 
1970

8. Gurewich V, Hutchinson E: Detection of 
intravascular coagulation by a serial dilution 
protamine sulfate test. Ann Intern Med 75:895, 
1971

9. Slaastad RA, Jeremic M: The laboratory 
diagnosis of low-grade disseminated intra-
vascular coagulation. A study in rabbits. Scand J 
Haematol 11:50, 1973

10. Niewiarowski S, Stewart GD, Marder 
VJ: Non-enzymatic formation of highly ordered 
polymers from fibrinogen and fibrin degrada-
tion products. Biochim Biophys Acta 221:326, 
1970

11. Niewiarowski S, Gurewich V: Labora-
tory identification of intravascular coagulation. 
J Lab Clin Med 77:665, 1971

12. Arnesen H: Studies on the thrombin clot-
ting time II. The influence of fibrin degradation 

13. Niewiarowski S, Ream VJ, Thomas DP: 
Effect of fibrinogen derivatives on platelet ag-
gregation. Thromb Diath Haemorrh (suppl) 
42:49, 1970

14. Lipinska I, Lipinski B, Gurewich V: 
Fibrinogen heterogeneity in human plasma. 
Electrophoretic demonstration and character-
ization of two major fibrinogen components. 
J Lab Clin Med (in press)

15. McFarlane AS: Efficient trace-labelling 

16. Swain WR, Feders MB: Fibrinogen 

17. Kowalski E, Kopec M, Niewiarowski S: 
An evaluation of the euglobulin method for the 
determination of fibrinolysis. J Clin Pathol 
12:215, 1959

18. Lewis JH, Fergsen EE, Schoenfeld C: 
Studies concerning the turnover of fibrinogen 
131 in the dog. J Lab Clin Med 58:247, 1961

19. Davies JWL, Forsberg K, Liljedahl SO, 
Martenson O, Reizerasten P: The effect of anti-
coagulants on post-operative fibrinogen metab-

20. Gajewski J, Alexander B: Effect of ep-
silonaminocaproic acid on the turnover of 
labelled fibrinogen in rabbits. Circ Res 13:432, 
1963

21. Lipinski B, Nowak A, Gurewich V: The 
organ distribution of 131-fibrin in the general-
ized Shwartzman reaction and its relation to 

22. Endres GF, Scheraga HA: Equilibrin in 
the fibrinogen-fibrin conversion. IX. Effects of 
calcium ions on the reversible polymerization 
of fibrin monomer. Arch Biochem Biophys 
153:266, 1972

23. Horn GR, Collins RD: Studies on the 
pathogenesis of the generalized Shwartz-
man reaction. The role of granulocytes. Lab Invest 
18:101, 1968

24. Hawiger J, Collins RD, Horn GR: Pre-
cipitation of soluble fibrin monomer complexes 
by lysosomal protein fraction of polymorpho-
131:349, 1969

25. Lipinski B, Jeljaszewicz J: A hypothesis 
for the pathogenesis of the generalized Shwartz-

26. Regoecci E, Brain MD: Organ distribu-
tion of fibrin in disseminated intravascular 

27. Muller-Berghaus G, Roka L, Lasch HG: 
Induction of glomerular micro clot formation 
by fibrin monomer infusion. Thromb Diath 
Haemorrh 29:375, 1973

28. Regoecci E: in Plasma Protein Metabo-
29. Gurewich V, Lipinski B, Lipinska I: Comparative study of precipitation and para-
30. Lipinski B, Gurewich V, Nowak A, Wetmore R: The effect of heparin and dipyrida-
mole on the deposition of fibrin-like material in rabbits infused with soluble fibrin, monomer
or fibrinogen. Thromb Res (in press)
The Fate of Soluble Fibrin Monomer in Relation to Intravascular Fibrin Formation and Degradation in Rabbits

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