A Study of Fibrinogen Turnover in Classical Hemophilia and Congenital Afibrinogenemia

By Aaron R. Rausen, Andre Crucaud, Campbell W. McMillan and David Gitlin

It has been postulated that in the normal individual a continuous clotting process occurs which utilizes fibrinogen to maintain normal vascular integrity. This thesis has been used to account for the shorter half-life of fibrinogen as compared to other plasma proteins. According to this theory, individuals with severe impairment of clotting not attributable to abnormalities involving fibrinogen should not utilize available fibrinogen as readily as the normal person. The turnover of circulating fibrinogen in such a patient would be slower than in the normal person. If, however, fibrinogen turnover is not due primarily to its utilization in clotting, then such individuals should have normal fibrinogen turnover rates.

In order to evaluate the role of clotting in the turnover of fibrinogen, the metabolism of $^{131}$I-labeled fibrinogen was determined in six patients with classical hemophilia of varying severity. A concurrent turnover study was performed in a boy with congenital afibrinogenemia.

The data indicate that at least the major part of the turnover of fibrinogen is probably not attributable to in vivo clotting.

Methods

Patients

Six boys with classical hemophilia and one boy with congenital afibrinogenemia, who previously had been studied by immunochemical technics, were selected for study. Pertinent data are summarized in table 1. Patients D.L., J.W., P.N. and J.M. were free of any overt hemorrhage during the study period. Patient R.M. developed a moderate hemorrhosis of the right knee on the fifth day of study that responded well to elevation, immobilization and cold compresses for five days. Patient C.S. had a mild hemorrhosis of the left wrist at the onset of study and bled into the right ankle at the end of the first week of study; both areas responded slowly to local measures. Patient C.M. had a large area of subcutaneous hemorrhage involving the entire middle third of his left arm, of one day's duration, that was successfully treated with an intravenous infusion of six Gm. of fibrinogen concentrate (Cutter Laboratories) 15 minutes after having received a tracer dose of radioiodinated fibrinogen.

Beginning two days before administration of radioiodinated fibrinogen, each patient was given by mouth 150 to 250 mg. of potassium iodide daily throughout the study. Coagula-
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Labeling of Fibrinogen with $^{131}$I

Irradiated, Seitz filtered fraction I, prepared by the Blomback and Blomback modification of the low temperature, ethanol, water method of Cohn and his colleagues (Method 6), was used as the starting material for the preparation of purified fibrinogen. The latter was prepared by the method of Morrison, Edsall and Miller. The purified fibrinogen was labeled with $^{131}$I by a method described elsewhere, and the final preparation was dialyzed against several changes of 0.15M NaCl containing 0.1 per cent disodium ethylene diamine tetraacetate for 24 hours and then sterilized by filtration through a sintered glass filter. Upon adding thrombin to the final preparation of radioiodinated fibrinogen, it was found that at least 85 per cent of the radioactivity was present in the fibrin. It was calculated that the labeled fibrinogen had an average of less than one atom of iodine per molecule of fibrinogen. At least 99 per cent of the total radioactivity could be precipitated in 10 per cent trichloracetic acid, indicating that less than one per cent of the total radioactivity was not protein bound.

Method of Study

Approximately 0.4 $\mu$C/Kg. body weight of radioiodinated fibrinogen was injected intravenously into each patient via an antecubital vein. Ten ml. of venous blood was obtained from the opposite arm at irregular intervals beginning 10 to 15 minutes after injection and then at 48 to 72 hour intervals for the next 13 days. Each sample was obtained in a syringe minimally wetted with a 10,000 U/ml heparin solution; the plasma was separated by centrifugation and a 3 ml. aliquot of the plasma was diluted with 10 ml. of saline in standard sized screw cap vials. All samples were counted in a well measuring 1 inch in diameter and 2 inches in depth in a 3 inch by 3 inch NaI crystal scintillator. To each sample, 500U of thrombin were added to convert the fibrinogen to fibrin. The clotted material was separated from the supernatant with wooden applicator sticks and then by centrifugation of the supernatant for 10 minutes at 4,000 rpm to remove any fine fibrin flecks. The radioactivity remaining in the supernatant was assayed and subtracted from the radioactivity of the plasma sample to determine the radioactivity in fibrinogen. The same needle and syringe used to inject each patient was used to prepare counting standards to eliminate the problem of adjusting for decay when assaying each sample.

The rate of disappearance of radioactivity in the fibrinogen from the fourth to sixth day to the end of the study period was used to compute the half-life of the injected fibrinogen in the six patients with hemophilia. In the patient with congenital afibrinogenemia, the plasma radioactivity values were used to calculate the disappearance curve of injected fibrinogen since it was difficult to completely separate from the plasma the small quantity of fibrinogen in the samples four or more days after fibrinogen infusion. In all instances, less than 10 per cent of plasma radioactivity remained in a sample after addition of thrombin and the expression and removal of the resulting fibrin clot.

Immunologic Studies

Immunoelectrophoresis in agar as described by Scheidegger was performed using the serum of each of the seven patients studied in the antiserum wells. The antigen was a 1 Gm. per cent solution of modified fraction I of Cohn electrophoresed for 3½ hours at 70v. A control immunoelectrophoresis was run using antiserum produced by multiple injections of a rabbit with modified human fraction I of Cohn. The gels were observed every 12 hours for 72 hours for the presence of precipitin bands.
Table 1.—Data on Patients Studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age in years</th>
<th>Weight in Kg.</th>
<th>Whole blood clotting time in minutes*</th>
<th>Plasma AHF activity % normal*</th>
<th>Plasma fibrinogen in mg. %</th>
<th>Half-life of radioiodinated fibrinogen in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. L.</td>
<td>10</td>
<td>32.4</td>
<td>14</td>
<td>22</td>
<td>1</td>
<td>222</td>
</tr>
<tr>
<td>J. W.</td>
<td>17</td>
<td>52.6</td>
<td>13</td>
<td>23</td>
<td>2</td>
<td>222</td>
</tr>
<tr>
<td>P. N.</td>
<td>18</td>
<td>74.4</td>
<td>16</td>
<td>21</td>
<td>3</td>
<td>234</td>
</tr>
<tr>
<td>J. M.</td>
<td>14</td>
<td>55.1</td>
<td>18</td>
<td>22</td>
<td>4</td>
<td>246</td>
</tr>
<tr>
<td>R. M.</td>
<td>10</td>
<td>31.8</td>
<td>65</td>
<td>89</td>
<td>&lt;1*</td>
<td>187</td>
</tr>
<tr>
<td>C. S.</td>
<td>11</td>
<td>27.7</td>
<td>74</td>
<td>119</td>
<td>&lt;1*</td>
<td>258</td>
</tr>
<tr>
<td>C. M.</td>
<td>14</td>
<td>58.0</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>75</td>
<td>2</td>
</tr>
</tbody>
</table>

Normal range: 5–15 60–180 175–415 1.5–6.0

*+ inhibitor to AHF.

RESULTS

1. Disappearance of Radioiodinated Fibrinogen from the Plasma in Patients with Classical Hemophilia

The survival of radioiodinated fibrinogen in the plasma of all six patients with classical hemophilia was quite comparable (table 1, fig. 1). After the first four days all the patients had essentially similar plasma fractional turnover rates, the calculated half-life of plasma radioiodinated fibrinogen ranging from 2.8 to 3.6 days.

2. Disappearance of Radioiodinated Fibrinogen from the Plasma in a Patient with Congenital Afibrinogenemia

The disappearance curve of radioiodinated fibrinogen in the patient with congenital afibrinogenemia is illustrated in figure 1. The half-life of radioiodinated fibrinogen was calculated to be 3.0 days.

3. Immunochemical Studies

No antifibrinogen antibodies were detected in the sera of the six patients with hemophilia or the boy with congenital afibrinogenemia.

DISCUSSION

It will be noted that the patients studied who suffered from classical hemophilia had AHF activity levels in their plasma from undetectable amounts to 4 per cent of normal. Clinically, patients D.L., J.W. and R.M. had relatively mild disease without evidence of arthropathy and having had but few transfusions of blood or plasma during their lives. Patient P.N., while having experienced several severe episodes of hemorrhage, was asymptomatic when studied. Patients R.M. and C.S., who were first cousins, were severely affected, rarely being free of either hemorrhoses or extensive subcutaneous hemorrhages. In addition, patient C.S. had developed laboratory evidence of plasma AHF inhibitor activity and had failed to gain benefit from fresh frozen plasma or fraction I infusions for several years. Notwithstanding the varied clinical courses, the plasma disappearance curves of intravenously administered radioiodinated fibrinogen in these six patients, as well as in the patient with congenital afibrinogenemia, were strikingly similar. Nine years...
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Fig. 1.—The disappearance of radioiodinated plasma fibrinogen from the circulation after intravenous injection. The values for fraction of total dose of radioiodinated fibrinogen in plasma in each sample were determined by comparing the radioactivity in each sample with the radioactivity in the sample obtained 10 to 15 minutes after injection of radioiodinated fibrinogen. This latter sample was considered to represent the total dose of radioactivity injected (i.e., 1.000 fraction of total dose at zero time). The following symbols apply: ○ D. L.; △ J. W.; ■ F. N.; □ J. M.; ○ R. M.; ▲ C. S.; - - - C. M.

ago, immunochemical studies in patient C.M. showed a normal half-life of fibrinogen of slightly less than four days; the half-life of radioiodinated fibrinogen in the present study was 3.0 days or essentially the same, indicating that the preparation of radioiodinated fibrinogen used was adequate for the determination of the turnover of this protein. Realizing that differences in labeling technics, variation in the stability and purity of the resultant product, and differences in method of analysis of data exist between various studies, the fibrinogen half-life range of 2.8 to 3.6 days obtained in this study is quite similar to the range reported in normal adults by others. In normal adults, Christensen obtained a range for the half-life of radioiodinated fibrinogen of 4.0 to 4.7 days; Hammond and Verel found a range of 4.1 to 6.0 days; and Adelson et al. obtained a range of 1.5 to 3 days. Using intravenously administered S-labeled fibrinogen, Volwiler et al. obtained a fibrinogen half-life ranging from 3.4 to 4.2 days. Since the turnover of fibrinogen in these patients was the same as in normal persons, or at the least slightly faster than the 4 to 6 day half-lives found by some investigators, this is against in vivo clotting being significantly involved in the utilization of fibrinogen in these patients.
An initial phase of rapid plasma disappearance of injected fibrinogen observed in this study is similar to the observations of others using radioactive tracer techniques in patients with normal fibrinogen levels or immunochemical methods in patients with congenital afibrinogenemia. The initial phase of rapid disappearance of injected fibrinogen from the plasma has been ascribed to its distribution and equilibration between the plasma and extravascular space. This phenomenon has been shown to occur when turnover rates of other plasma proteins have been similarly studied.

In view of the recent demonstration of antifibrinogen antibodies causing the rapid disappearance of fibrinogen injected into a patient with congenital afibrinogenemia, it is of interest that examination of patient C.M.'s serum, as well as the sera of all six patients with hemophilia, was negative for antifibrinogen antibodies. All seven patients had received multiple infusions of blood, plasma and/or fraction I during the past 6 to 17 years.

This study suggests that in vivo clotting is not a major factor in the turnover of fibrinogen.

**Summary**

The rate of disappearance from the plasma of intravenously administered I\(^{131}\)-labeled fibrinogen was studied in six patients with classical hemophilia and in one patient with congenital afibrinogenemia.

The six patients with hemophilia had radioiodinated fibrinogen half-lives ranging from 2.8 to 3.6 days, while the patient with congenital afibrinogenemia had a labeled fibrinogen half-life of 3.0 days. These results compare favorably with fibrinogen turnover rates measured in normal adults by others and were similar to the normal fibrinogen turnover rate determined in the patient with congenital afibrinogenemia in a previous study. This failure to demonstrate a prolongation of survival of fibrinogen in patients with hemophilia suggests that in vivo clotting, if it occurs at all normally, is not a major factor in the turnover of fibrinogen.

**Summario in Interlingua**

Le rapiditate del disparition ab le plasma de intravenosemente administrate fibrinogeno a marcation con I\(^{131}\) eseva studiate in sex patientes con classic hemophilia e in un paciente con congenite afibrinogenemia.

In le patientes con hemophilia le periodos de medie valor del radio-iodate fibrinogeno variava inter 2,8 e 3,6 dies, durante que in le paciente con afibrinogenemia le correspondente periodo eseva 3,0 dies. Iste resultatos es favorabemente comparabile con le valores del metabolismo de fibrinogeno determinate per altere recercatores in adultos normal. Illos eseva simile al normal rapiditate del metabolismo de fibrinogeno determinate in le paciente con congenite afibrinogenemia in un previe studio. Iste absentia de un prolongation del superviventia de fibrinogeno in patientes con hemophilia suggere que le ecagulation in vivo, si illo occurre del toto de manie ra normal, non es un factor major in le metabolismo de fibrinogeno.
ACKNOWLEDGMENT

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REFERENCES

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