Inhibition of Fibrin Monomer Polymerization by Lambda Myeloma Globulins

By Morton Coleman, Edward M. Vigliano, Marc E. Weksler, and Ralph L. Nachman

Blood obtained from seven patients with lambda type myeloma proteins showed evidence of gelatinous bulky clots, impaired clot retraction, and circulating anticoagulant activity associated with interference of fibrin monomer polymerization. Five patients had \( \gamma G \), and two had \( \gamma A \), myeloma proteins. The pathologic plasmas and isolated myeloma proteins had anticoagulant activity that prolonged both thrombin and reptilase times, that was not absorbed by BaSO\(_4\), or neutralized by protamine sulfate, and that resisted heating to 56\(^\circ\)C for 10 min. Addition of excess calcium partially corrected the anticoagulant effect. The anticoagulant activity of the isolated whole myeloma proteins, the enzymatic fragments, and polypeptide chains was measured by a thrombin time assay and a spectrophotometric system with fibrin monomer. Low concentrations of the isolated IgGL proteins inhibited fibrin polymerization. The IgAL proteins did not demonstrate this activity at low concentrations but were active at concentrations comparable to in vivo levels. F(ab')\(_2\), and Fab fragments produced from the IgG proteins by enzymatic digestion possessed full inhibitory activity of the native intact proteins. Fc fragments and isolated polypeptide chains did not display significant anticoagulant activity. The results suggest that the Fab sites of certain lambda myeloma proteins may bind to fibrin during clotting and fibrin polymerization.

The "gelation" phenomenon has been observed in patients with plasma cell myeloma.\(^1\)\(^2\) This phenomenon is characterized by gelatinous, bulky, friable clots; poor clot retraction; and prolonged thrombin times resulting from inhibition of fibrinogen-fibrin conversion.\(^3\)\(^6\) This latter finding has been related to anticoagulant activity interfering with polymerization of fibrin monomer.\(^7\)\(^10\) Such anticoagulant activity has been associated with the isolated myeloma proteins.\(^2\)\(^11\)\(^12\)

The present report describes seven patients with lambda type myeloma proteins that inhibited fibrin monomer polymerization. Studies are presented that delineate further the manner in which these myeloma proteins and their structural subunits affect fibrin formation.

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INHIBITION OF FIBRIN MONOMER POLYMERIZATION

MATERIALS AND METHODS

Myeloma Typing

Immunoelectrophoresis was performed according to the technique of Scheidegger.\textsuperscript{13} Lambda and kappa specific light chain antisera were prepared in rabbits as previously described.\textsuperscript{14} TM, TA, and TG specific antisera were obtained from a commercial source (Behring Diagnostics, Woodbury, N.Y.) The typing sera were checked for specificity using myeloma proteins and Bence Jones proteins of known type. Subgroup heavy chain analysis and Gm typing, when performed, were determined by methods described previously by Kunkel and associates.\textsuperscript{15,16} These studies were kindly performed by Dr. Stephen D. Litwin, Division of Genetics, Department of Medicine, Cornell University Medical College, New York.

Coagulation Studies

Whole blood was obtained by clean venepuncture and added to one-tenth volume 3.8% trisodium citrate. Methods of measurement of platelets, fibrinogens, and Factor V and VIII have been previously described.\textsuperscript{17} Plasminogen levels were assayed by the method of Alkjaersig et al.\textsuperscript{18} Immunologic determination of fibrin split products by latex flocculation (F1-Test R, Hyland Div., Travenol Lab., Costa Mesa, Calif.) was performed on serum obtained from fresh whole blood incubated for 30 min with 100 NIH units bovine thrombin (Parke, Davis, Detroit, Mich.). Thrombin clotting times, unless otherwise noted, were performed by the addition of 0.2 ml diluted bovine thrombin to 0.2 ml citrated plasma so that normal clotting times were 18–20 sec. Reptilase clotting times were determined by adding 0.1 ml of reptilase (kindly provided by A. G. Pentapharm, Basel, Switzerland), an extract of Bothrops jararaca venom, to 0.2 ml of citrated plasma prewarmed to 37°C. Protamine titration was performed by adding serial saline dilutions of 0.02 ml of 0.01%–0.00001% protamine sulfate solution (Protamine sulfate injection, USP. Eli Lilly & Co., Indianapolis, Ind.) to 0.18 ml citrated plasma and clotting the mixture with 0.2 ml thrombin. Plasma was absorbed by Bentonite, Al(OH)\textsubscript{3} and Ba(SO\textsubscript{4}) as previously described.\textsuperscript{11}

Viscosity Studies

Plasma viscosity measurements were determined by a modification of the method of Capra and Kunkel\textsuperscript{19} utilizing a Cannon-Manning Micro-Viscometer. Values were reported relative to H\textsubscript{2}O at 37°C.

Protein Isolation

Myeloma proteins were isolated primarily by zone electrophoresis on polyvinyl copolymer (Pevikon, Mercer Chemical, New York). The isolated globulins showed only one precipitin arc on immunoelectrophoresis against rabbit antihuman serum.

Calcium Correction Studies

The effect of calcium upon the thrombin time was measured by mixing 0.15 ml plasma and 0.1 ml of a 15 mg/ml globulin solution with 0.1 ml of thrombin and 0.05 ml of 0.025 M calcium chloride solution or phosphate buffered saline, so that control times were 20–25 sec. In the alternate system using bovine fibrinogen (kindly provided by Warner-Chilcott, Morris Plains, N.J.) as a substrate, 0.2 ml of bovine fibrinogen (3 mg/ml) was substituted for plasma. The effect of varying globulin or calcium concentrations on the thrombin time was determined by substituting the test reagents in mixtures similar to that described for the plasma substrate.

Spectrophotometric Studies

Fibrinogen to fibrin conversion was measured by adding 0.1 ml bovine thrombin (5.0 NIH units/ml) to a mixture containing 0.9 ml bovine fibrinogen and 0.1 ml test gamma globulin solution. The reaction was monitored every 30 sec as increasing OD at 350 mm on a Beckman DU spectrophotometer.
Table 1. Myeloma Typing and Coagulation Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Group Type</th>
<th>Gm Typing</th>
<th>Globulin Conc. (g/100 ml&lt;sup&gt;+&lt;/sup&gt;)</th>
<th>Relative Plasma Viscosity&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Bulky Clot (Gelification)</th>
<th>Clot Retraction</th>
<th>Platelet Count&lt;sup&gt;‡&lt;/sup&gt; (x 10&lt;sup&gt;4&lt;/sup&gt;/cu mm)</th>
<th>Thrombin Time§ Without Mixing (sec)</th>
<th>Thrombin Time§ With Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.Q.</td>
<td>λκ1A</td>
<td>—</td>
<td>7.9</td>
<td>2.5</td>
<td>Present</td>
<td>Poor</td>
<td>290</td>
<td>58</td>
<td>23</td>
</tr>
<tr>
<td>S.T.</td>
<td>λκ1A</td>
<td>—</td>
<td>9.0</td>
<td>3.7</td>
<td>Present</td>
<td>Absent</td>
<td>162</td>
<td>90</td>
<td>38</td>
</tr>
<tr>
<td>A.K.</td>
<td>λκκ1</td>
<td>Gm(f)</td>
<td>4.5</td>
<td>1.8</td>
<td>Absent</td>
<td>Good</td>
<td>160</td>
<td>41</td>
<td>24</td>
</tr>
<tr>
<td>E.J.</td>
<td>λκκ1</td>
<td>Gm(f)</td>
<td>3.8</td>
<td>1.7</td>
<td>Absent</td>
<td>Fair</td>
<td>179</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td>L.B.</td>
<td>λκκ1</td>
<td>Gm(a)</td>
<td>10.7</td>
<td>5.1</td>
<td>Present</td>
<td>Poor</td>
<td>290</td>
<td>66</td>
<td>30</td>
</tr>
<tr>
<td>H.L.</td>
<td>λκκ1</td>
<td>Gm(f)</td>
<td>6.8</td>
<td>2.6</td>
<td>Present</td>
<td>Absent</td>
<td>175</td>
<td>120</td>
<td>39</td>
</tr>
<tr>
<td>G.C.</td>
<td>λκκ1</td>
<td>Gm(f)</td>
<td>2.2</td>
<td>1.6</td>
<td>Present</td>
<td>Absent</td>
<td>147</td>
<td>120</td>
<td>58</td>
</tr>
</tbody>
</table>

<sup>+</sup>Normal 1.8-2.8 g/100 ml.

<sup>†</sup>At 37°C, normal 1.6-1.9.

<sup‡</sup>Normal 180-400 x 10<sup>4</sup>/cu mm.

<sup§</sup>Control times 18-20 sec. Without mixing represents determinations on undiluted myeloma plasma; with mixing represents mixture of one part myeloma plasma diluted with four parts normal plasma.
INHIBITION OF FIBRIN MONOMER POLYMERIZATION

To prepare fibrin monomer, bovine fibrinogen was heated to 40°C for 1 hr, then clotted with thrombin (5 U/100 mg fibrinogen) for 30 min at 37°C. The fibrin was removed by glass rod, washed in saline, dried, and redissolved in 5 M urea. After three cycles of dissolution, polymerization, and washing, the fibrin was dissolved in 5 M urea to which an equal volume of 0.05 M acetate buffer, pH 4.8 was added. The solution was dialyzed overnight against the acetate buffer. Fibrin monomer and probably dissolved polymers remain soluble at this pH and clot only when the pH is brought to neutrality. A mixture consisting of 0.9 ml imidazole-buffered saline, pH 7.2, and test globulin was added to 0.1 ml monomer solution. Polymerization was then measured every 30 sec as increasing OD at 350 nm on a Beckman DU spectrophotometer.

Preparation of Myeloma Subunits

Myeloma subunits were obtained by modifications of methods previously described. Fab and Fc fragments were prepared from IgG myeloma proteins by mercuripapain digestion using a protein/enzyme ratio of 100:1. The proteins were enzymatically digested for 16 hr at 37°C in 0.1 M phosphate buffer, pH 7.4 with 0.01 M cysteine and 0.002 M EDTA. Digestion was terminated by dialysis against cold saline. The fragments were separated by zone electrophoresis on Pevikon and concentrated by dialysis against polyethylene glycol (Matheson, Coleman and Bell Division Matheson Co., Norwood, O.) Purity of the enzymatic fragments was ascertained by immunoelectrophoresis and immunodiffusion analysis with appropriate antisera. F(ab')2 fragments of myeloma proteins were prepared by pepsin digestion using a protein/enzyme ratio of 100:1. The proteins were subjected to enzymatic digestion for 24 hr at 37°C in 0.1 M acetate buffer, pH 4.1. Digestion was terminated by dialysis at 4°C against phosphate-buffered saline, pH 7.2. F(ab')2 fragments were then further isolated by G-200 Sephadex (Pharmacia, Uppsala, Sweden) chromatography. Purity was ascertained by immunoelectrophoresis and immunodiffusion analysis with appropriate antisera.

Heavy (H) and light (L) chain polypeptides were obtained by reducing isolated myeloma proteins at 20 mg/ml with a solution of 0.1 M β-mercaptoethanol and normal saline brought to pH 8.0 by small amounts of 1 M Tris. The reaction was allowed to proceed for 4 hr at room temperature. The solution was then made 0.2 M in iodoacetamine. The pH was maintained at 8.0 by the further addition of 1 M Tris. After 10 min, the solution was dialyzed against 1 M propionic acid at 4°C. Dialysis was continued at 4°C for 72 hr. H and L chains were isolated by gel filtration on a G-100 Sephadex column in 1 M propionic acid and 0.001 M iodoacetamide. The separated fractions were dialyzed in order against 0.5 M, 0.1 M, 0.001 M propionic acid and water. Polypeptide chains were concentrated by dialysis against polyethylene glycol.

Radioiodinated Binding Studies

Binding of fibrin monomer by the myeloma proteins was ascertained by iodinating myeloma proteins with 125I by the chloramine T method of McConahey and Dixon. Radioactivity was measured on a dual channel scintillation counter. Bovine fibrinogen (3 mg/ml) was incubated with 6 mg/2 ml of the radioactive material for 30 min and then clotted with thrombin (5.0 NIH units) for 60 min. The clots were collected on a glass rod, washed, and counted. A 1% protamine sulfate solution (0.5 cc) was added to the remaining supernatant to precipitate fibrin monomer from soluble fibrin monomer-gamma globulin complexes. The resultant precipitate was graded optically with 0 constituting clear solution and 4 representing dense, cloudy precipitation.

RESULTS

Myeloma Typing

The results of serologic typing on the seven myeloma patients are listed in Table 1. Every myeloma protein was of the lambda light chain type. Five
IgG myeloma proteins were of the γG1 subgroup. On Gm typing, four were Gm(f) and one was Gm(a). Two IgA myeloma proteins were both of the γA1 subgroup.

Coagulation Data

There was no evidence of intravascular coagulation; determinations of platelets, fibrinogen, Factor V, Factor VIII, and plasminogen were normal or elevated. There was no immunologic evidence of fibrin or fibrinogen breakdown products in serum.

Pertinent coagulation data are shown in Table 1. Gelification of the clot and absent or poor clot retraction, despite relatively normal platelet counts, were present with five of seven myeloma plasmas.

Prolongation of the thrombin time did not correlate with the concentration of the globulin or relative plasma viscosity. The thrombin times remained elevated in several instances despite dilution of one part myeloma plasma with four parts normal plasma.

Reptilase clotting times were prolonged using mixtures of normal and myeloma plasma similar to those used with the thrombin times. Myeloma plasma, heat-defibrinated at 56°C for 10 min, effectively prolonged the thrombin time in mixing experiments. Protamine titrations of the myeloma plasmas failed to correct the elevated thrombin times. Anticoagulant activity was not removed by absorption of plasma with Bentonite, Al(OH)₃ or BaSO₄.

Antithrombin Activity of Isolated Myeloma Proteins

The isolated myeloma proteins when added to plasma or fibrinogen resulted in prolongation of the thrombin times (Table 2). Such anticoagulant activity was present to a significant degree with all of the isolated IgG myeloma proteins in a relative concentration of 10 mg myeloma protein/ml plasma substrate. The anticoagulant IgA myeloma proteins (patients A. Q. and S. T.) were considerably less active but did produce moderate elevations of the thrombin times when compared to control kappa IgA myeloma (patient A. B.). One IgA myeloma (patient A. Q.) required concentrations more closely corresponding to in vivo myeloma levels, i.e., 80 mg, to produce significant prolongation (doubling) of the thrombin time.

Effect of Calcium on Thrombin Times

The addition of a fixed concentration of 0.025 M calcium improved the thrombin time of mixtures containing plasma or fibrinogen substrate with the isolated myeloma proteins (Table 2). The additional calcium, however, only partially ameliorated the anticoagulant effect, since in most instances the thrombin times with the inhibitory myeloma proteins did not shorten to those of Cohn fraction II (CF2) or control, noninhibitory IgA myeloma protein (patient A. B.) at the same calcium concentration.

The effect of varying concentrations of calcium on the inhibitory role of the myeloma proteins on the thrombin time was determined. In this study, plasma and globulin mixtures were clotted with thrombin in the presence of
Fig. 1. Plasma thrombin time with variable myeloma and calcium concentrations. Increasing concentrations of anticoagulant myeloma protein G.C. produced correspondingly longer thrombin times compared to control Cohn fraction II (CF2). Addition of calcium aliquots to 0.05 M only partially ameliorated inhibitory effect of myeloma protein.

varying amounts of calcium. A typical study with the IgG protein from patient G. C. is shown in Fig. 1. Increased amounts of calcium to 0.05 M appeared to lessen the effect of the inhibitory myeloma protein with resultant shorter thrombin times. As expected the thrombin time prolongation was greater at higher protein concentrations. At very high calcium concentrations an additional inhibitory effect was noted even in the presence of control Cohn fraction II (patient CF2).

Table 2. Thrombin Clotting Times With Isolated Myeloma Proteins and Calcium

<table>
<thead>
<tr>
<th>Myeloma Proteins</th>
<th>Plasma Thrombin Time (sec)</th>
<th>Fibrinogen Thrombin Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Ca++</td>
<td>With Ca++</td>
</tr>
<tr>
<td>Control—without myeloma protein</td>
<td>21.2</td>
<td>15.7</td>
</tr>
<tr>
<td>CF2—control IgG (Cohn Fraction II)</td>
<td>21.8</td>
<td>16.0</td>
</tr>
<tr>
<td>A. B.—control IgA (no gelatinizing)</td>
<td>20.0</td>
<td>14.2</td>
</tr>
<tr>
<td>A. Q. (IgA)</td>
<td>25.1</td>
<td>16.8</td>
</tr>
<tr>
<td>S. T. (IgA)</td>
<td>27.2</td>
<td>19.7</td>
</tr>
<tr>
<td>A. K. (IgG)</td>
<td>34.7</td>
<td>22.1</td>
</tr>
<tr>
<td>E. J. (IgG)</td>
<td>38.3</td>
<td>31.7</td>
</tr>
<tr>
<td>L. B. (IgG)</td>
<td>42.6</td>
<td>32.2</td>
</tr>
<tr>
<td>H. L. (IgG)</td>
<td>44.2</td>
<td>34.2</td>
</tr>
<tr>
<td>G. C. (IgG)</td>
<td>60.0</td>
<td>43.0</td>
</tr>
</tbody>
</table>
Effect of Myeloma Proteins on Fibrinogen-Fibrin Conversion

The magnitude of fibrinogen-fibrin conversion was measured spectrophotometrically after the addition of a fixed amount of thrombin (Fig. 2). Significant inhibition of fibrinogen-fibrin conversion was observed at protein concentrations of 3 mg/ml using the IgG myeloma proteins. The IgA myeloma proteins with anticoagulant properties were inactive in this system at this protein concentration.

Effect of Myeloma Proteins on Fibrin Polymerization

The latter stage of fibrinogen-fibrin conversion, that of fibrin monomer polymerization, was measured spectrophotometrically (Fig. 3). Polymerization was inhibited by all of the isolated IgG myeloma proteins at 3 mg/ml concentrations similar to that used to inhibit fibrinogen-fibrin conversion. With representative IgG myeloma (patient G. C.) the minimal concentration of protein needed to inhibit polymerization was 1.5 mg/ml. Progressively higher concentrations of protein produced correspondingly greater inhibition. At
INHIBITION OF FIBRIN MONOMER POLYMERIZATION

Fig. 3. Effect of fibrin polymerization by isolated anticoagulant TA myeloma, patient G.C. Increasing concentrations of myeloma protein produced progressively greater inhibition. At 6 mg/ml and higher no significant clot formation occurred; optical density at these concentrations essentially reflected added protein. Control polymerization is represented by Cohn fraction II (CF2) at 6 mg/ml.

Concentrations of 6.0 mg/ml and higher, fibrin clot formation was completely inhibited.

The IgA myeloma proteins (patients S. T. and A. Q.) displayed no significant inhibition of polymerization at low concentrations (Fig. 4). At concentrations more comparable to in vivo levels (S. T., 30 mg/ml; A. Q., 80 mg/ml), the IgA myeloma proteins were inhibitory.

Effect of Myeloma Subunits on Fibrin Polymerization

For these studies, the IgG from patient G. C. is presented as a representative sample. At concentrations of 3 mg/ml, the Fab and F(ab')2 fragments inhibited polymerization of fibrin monomer to the same degree as 6 mg/ml of the intact anticoagulant myeloma protein (Fig. 5).

Polymerization of fibrin monomer was not inhibited by Fc fragments (3
mg/ml) produced from the anticoagulant myeloma protein, since the magnitude of polymerization was comparable to that of 6 mg/ml control Cohn fraction II (patient CF2, 6 mg/ml). No significant inhibition was produced by the isolated polypeptide light (L) and heavy (H) chains at similar concentrations of 3 mg/ml.

**Binding of Fibrin Monomer to Myeloma Protein**

Washed clots produced in the presence of labeled globulin revealed a sixfold increase in percentage radioactivity of clots made in the presence of inhibitory IgG myeloma protein, compared to noninhibitory myeloma proteins (Table 3). The addition of protamine sulfate to the supernatants produced a significant cloudy precipitate in only those solutions containing inhibitory proteins, suggesting the presence in solution of significant quantities of fibrin monomer.

**DISCUSSION**

In the presence of normal fibrinogen concentrations, the thrombin clotting time is essentially a measurement of the enzymatic action of thrombin on the fibrinogen molecule, cleaving off fibrinopeptides A and B thereby producing fibrin monomer; and the speed of fibrin monomer polymerization. The plasmas of the myeloma patients in the present study demonstrated prolonged thrombin times. Anticoagulant activity was present, since mixtures of small amounts of these myeloma plasmas with normal plasmas produced prolongations of the thrombin time.

Heparin or heparinlike anticoagulants directly inhibit the enzymatic activity of thrombin. A few instances of circulating "heparinoid" anticoagulants have been described in myeloma. In these instances, protamine sulfate, usually in high concentration, abolished the antithrombin activity. In this study, protamine sulfate titration, in the concentrations normally used to neutralize heparin, did not correct the anticoagulant activity.
The anticoagulant in the myeloma plasmas instead exhibited properties identical to the inhibitor described by Loeliger and Hers in a patient with a hemorrhagic diathesis and hypergammaglobulinemia. The inhibitor, which was present in plasma and serum, was neither neutralized by protamine sulfate nor absorbed by BaSO₄. It was stable to 56°C for 10 min and decreased in activity with a corresponding diminution of gamma globulin concentration. Verstraete and Vermyleen carefully characterized an anticoagulant with a similar group of properties in three of six patients with multiple myeloma. The anticoagulant was termed “anti-thrombin V.”

Reptilase converts fibrinogen to fibrin by splitting off fibrinopeptide A. Direct antithrombins, such as heparin, do not significantly inhibit the reptilase time. In this study, reptilase times were prolonged using mixtures of normal and patients’ plasmas similar to those used with the thrombin times, suggesting that the anticoagulant activity might be directed at a later, “nonthrombin” dependent stage, i.e., fibrin monomer polymerization. Uehlinger, and Lüscher and Labhart had earlier associated the prolonged thrombin times in myeloma with an impairment of fibrinogen conversion. They hypothesized that the myeloma proteins act as a protective colloid to fibrinogen intermediates, precluding in some way the formation of fibrin strands. Studies by a number of other investigators and the data obtained in our kinetic systems essentially corroborate this concept. The isolated myeloma proteins inhibited the fibrin polymerization phase of fibrinogen-fibrin conversion in concentrations correlating with the demonstrable anticoagulant activity of the diluted pathologic plasmas as noted in Table 1.

The exact relationship of gelification of the clot to the prolonged thrombin times has not been completely clarified. Previous authors have implied that these gelatinous clots represented the morphologic counterpart of defective fibrin polymerization. Gelification has been described in most prior reports describing antithrombin V activity. Prolongation of the thrombin time, however, is considerably more common in myeloma than is gelation. Our clinical data suggest that the phenomenon of gelation occurred in those pa-
tients with the highest concentration of antithrombin activity resulting either from a very high concentration of inhibitor myeloma protein or from myeloma proteins with the greatest specific anticoagulant activity. Gelation probably thus represents a “bulk” expression of the same phenomenon seen with more sensitive thrombin time.

In the study of binding, increased radiolabeled inhibitor myeloma globulin was found in clots, suggesting that such clots may be a complex of fibrin and gamma globulin. Some radioactivity, however, was found in the clots formed with control protein. This is not surprising since Regoezzi, using trace-labeled plasma proteins, had demonstrated trapping of gamma globulin and Bence Jones protein, particularly kappa types, into fibrin clots. Cohen et al, using ultracentrifugation analysis, were unable to demonstrate complexing of fibrinogen and myeloma proteins from a patient demonstrating impairment of fibrin polymerization. It is possible though that complexing may occur solely with fibrin monomer, since hidden reactive sites may become available only after fibrinopeptides are split off the native fibrinogen molecule.

Absent clot retraction is an accompaniment of the gelatinizing process. Vigliano and Horowitz studied the platelets in patients A. Q. They concluded that platelet function including clot retraction was normal if the platelets were washed and resuspended in normal platelet poor plasma. Clot retraction by normal platelets was abolished if used in the patient’s platelet poor plasma. Similar findings have also been reported by Cohen et al. and Lackner et al. in other myeloma patients demonstrating impairment of fibrin monomer polymerization. They have postulated that the abnormal proteins interfered with clot retraction either by coating the platelets or by interaction with fibrin, thus precluding the action of platelets on the fibrin strands. It is tempting to conclude the latter, i.e., that absent clot retraction is but another manifestation of fibrin-gamma globulin complexing.

The exact nature of the complex between fibrin monomer and the inhibitor myeloma proteins has not been elucidated. Since the myeloma proteins in this study were all of the lambda light chain type, it is possible that inhibition or complex formation in some fashion required the presence of lambda light chains. Two additional myeloma proteins previously reported to inhibit fibrin polymerization were also typed by our laboratory. Both were found to be lambda TC1 proteins, one patient, B. N., on Gm typing was found to be Gm(f) and the other, E. J., Gm(a). Craddock et al. reported a myeloma patient whose serum and urinary proteins inhibited fibrin polymerization. The urinary proteins exhibited the heat and chemical characteristics of a Bence Jones protein. Both serum and urinary proteins, however, had similar migratory patterns on electrophoresis. Since appropriate antisera at that time were unavailable, it is not certain whether inhibition resulted from light chains or their dimers, i.e., Bence Jones proteins, or an intact myeloma protein.

All the lambda IgG inhibitory myeloma proteins were of the major TC1 heavy chain subgroup that comprises approximately 60% of all IgG pro-
teins. Although this sample of proteins is small, the possibility should be considered that myeloma proteins of the restricted type and subgroup specificity lambda \( \gamma \) may be particularly prone to inhibitor activity. The \( \text{Gm} \) determinants followed essentially a normal distribution pattern. The possibility that other type IgG myeloma proteins may act as anticoagulants is of course not precluded by our findings. In fact, several reported myeloma proteins inhibiting fibrin monomer have been typed by others. These proteins were not found to be lambda \( \gamma \) myeloma proteins.

The isolated light and heavy polypeptide chains did not significantly inhibit polymerization; however, the F(ab) as well as the F(ab') fragments were markedly inhibitory. At concentrations of 3 mg/ml the F(ab) and F(ab') fragments of representative protein G. C. produced the same degree of inhibition as twice the amount of native undigested protein (Fig. 5). These observations, noted also with our other IgG inhibitor proteins, raise the interesting possibility that the inhibition or complexing of fibrin monomer by these myeloma proteins might represent antibody activity directed against hidden determinants of an autologous plasma protein—fibrinogen.

The data presented in this study do not prove that these IgG myeloma inhibitor proteins are true antibodies. More rigid criteria must be satisfactorily fulfilled before the anticoagulant activity could be described in terms of an immunologic reaction. Stoichiometric inhibition of fibrin monomer by the myeloma proteins should be demonstrated. Specifically, one Fab combining site of myeloma protein should be required to inactivate one molecule (or one polymerization site) of fibrin monomer. It would be important to identify the site or sites on the fibrin monomer with which the myeloma protein interacts. Fractionation of fibrin monomer into subunit polypeptide chains may help identify these regions in the molecule. Other immunologic assay systems, such as the tanned red cell technique, have not yet proven useful in demonstrating fibrin monomer-myeloma protein interaction (personal observation).

The inhibition of fibrin polymerization by the IgG myeloma proteins probably cannot be ascribed to a nonspecific effect of increased quantities of protein. Prolongation of the thrombin time was produced by some of the isolated IgG myeloma proteins in concentrations approximately one-fifth to one-tenth normal IgG concentrations. The IgA myeloma proteins, however, were considerably less active and required concentrations comparable to in vivo levels, suggesting that these proteins may have produced nonspecific protein interference. Protein-protein interaction of IgA myeloma with albumin has been described. The high degree of activity of the IgG proteins suggests perhaps a somewhat different mechanism. Nilehn and Nilsson reported 16 of 39 patients with IgG myeloma who had prolongation of the thrombin time, although none of the 14 IgA myeloma patients demonstrated this abnormality. Uehlinger and Lüscher and Labhart originally emphasized that hyperglobulinemia or hyperproteinemia did not necessarily impair clotting. Similarly, Ratnoff in a survey of 11 patients with myeloma was unable to correlate the concentration of globulin with elevations of clotting
or thrombin times, although eight demonstrated abnormalities in coagulation. Frick surveyed 45 patients with myeloma and found prolongation of the thrombin time in 12. No direct correlation was found between the prolongation of the thrombin time and the level of total serum globulins. These observations are confirmed by our study, where no consistent correlation could be demonstrated between thrombin times and either protein concentration or viscosity.

Another possible mechanism by which myeloma proteins could interfere with fibrin polymerization involves the binding of bivalent cations. A number of investigators studying gelation have noted that additional calcium ions could shorten the prolonged thrombin times and/or convert the gelatinous clots to a more normal, opaque fibrous consistency. Glueck et al. investigating this phenomenon in two patients found extremely high concentrations of calcium in the abnormal globulin fractions. In vivo infusion of ionized calcium improved the abnormal thrombin times. A myeloma patient with faulty fibrin polymerization was recently studied by Cohen et al. with electron microscopic observations and a spectrophotometric techniques similar to our own. They demonstrated correction of the myeloma induced clotting defects by the addition of excess calcium. Using equilibrium dialysis though they were unable to show binding of calcium by the myeloma proteins. Perkins et al. noted that while calcium has some countering effect to inhibition, no amount of calcium would completely correct the thrombin time. Shortening of the thrombin time, after calcium addition, occurs in normal plasma, an effect attributed to enhancement of fibrin polymerization. In our study, regardless of the amount of excess calcium, the thrombin times of our inhibitory proteins could not be shortened to those times found with control proteins using a like amount of calcium.

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