Characterization and Properties of an Inhibitor of Factor VIII in the Plasma of Patients with Hemophilia A Following Repeated Transfusions

By HERBERT S. STRAUSS AND EZIO MERLER

A CIRCULATING ANTICOAGULANT, which specifically inhibits Factor VIII (antihemophilic factor or AHF), has been found in patients with hemophilia A (classic hemophilia), in some women within a year postpartum, and in elderly individuals. The inhibitor has been shown to be a gamma globulin and to be stable when heated for 30 minutes at temperatures as high as 65°C. When the anticoagulant reacts with Factor VIII, its interaction is optimal at pH 7.5, increases with increasing temperature, and is dependent on the relative concentration of reactants. The anticoagulant, whether obtained from hemophiliacs or nonhemophiliacs, appears functionally similar.

Based on reaction kinetics and the apparent failure to demonstrate its combination with Factor VIII, the anticoagulant has been called an enzyme. Contrasting data have shown that the inhibitor is utilized in the reaction with Factor VIII and that it agglutinates tanned red cells previously coated with plasma containing Factor VIII. In addition, the inhibitor has been shown to be a γG globulin, which was removed by precipitation with a rabbit anti-γG antibody. On the basis of these observations the anticoagulant has been considered an antibody. With rare exceptions the anticoagulant appears only after exposure of hemophilic individuals to repeated transfusions of blood or blood containing Factor VIII. Furthermore, this inhibitor increases with subsequent transfusions. As previously noted, however, the anticoagulant does not develop in all hemophiliacs. A preliminary report has appeared presenting evidence that Factor VIII inhibitors are γK₂ monotypic antibody molecules.

In the present communication, data are presented which support the concept that the inhibitor of Factor VIII is a γG globulin antibody. This conclusion is based on the following considerations:

1. The inhibitory activity resides in the Fab fragment, obtained by digestion of the γG globulin with papain.

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2. The serum level of inhibitor increases sharply following blood transfusion and then decreases slowly to the preinfusion level.

3. Small amounts of the anticoagulant were inactivated by normal plasma.

4. Clearance of inhibitor from the normal circulation is rapid, suggesting an immune disappearance of an antigen-antibody complex.

MATERIALS AND METHODS

Qualitative Determination of Inhibitor of Factor VIII

The material to be tested (0.2 ml.) was incubated at 37°C for 1 hour with 0.2 ml. of normal citrated plasma. The normal citrated plasma had been adsorbed with aluminum hydroxide and diluted ten times with an imidazole buffer of pH 7.4. A thromboplastin generation test was then performed on this mixture by the addition of 0.2 ml. of normal serum, which had been diluted 20 times with imidazole buffer, 0.2 ml. of a 0.015 per cent suspension of crude cephalin as a substitute for platelets and 0.2 ml. of 0.025 M CaCl₂. Any prolongation of the minimum substrate clotting time compared to that of a control of normal saline indicated the presence of inhibitor of Factor VIII. Clotting time of the control was 11 ± 1 seconds.

Quantitative Determination of Inhibitor of Factor VIII

Quantitative estimation of inhibitor was performed by a modification of the method of Biggs. The material to be tested (0.2 ml.) was incubated at 37°C with an equal volume of diluted adsorbed normal plasma for 1 hour. Citrated plasma from 10 to 12 healthy donors was pooled, adsorbed with aluminum hydroxide gel, and kept in 0.2 aliquots at −20°C for not more than 4 weeks. Before use, it was thawed and diluted twofold with imidazole buffer, pH 7.4. Following incubation the mixture was assayed for residual Factor VIII by a method based on the thromboplastin generation test. The adsorbed plasma used as the standard for the assay of Factor VIII was derived from the same pool as the one used for incubation with the inhibitor and it was assigned a level of Factor VIII of 100 per cent. Controls of imidazole buffer, normal saline or adsorbed plasma from a hemophiliac lacking inhibitor consistently yielded a residual activity of Factor VIII of 21 ± 2 per cent. This is lower than the expected 25 per cent since Factor VIII is partially destroyed during incubation. One unit of inhibitor was defined as the quantity contained in 1 ml. that inactivated 50 per cent of the Factor VIII present in the test system; in other words, a 1 ml. volume which contained 1 unit of inhibitor yielded a residual activity of Factor VIII of 10.5 per cent after incubation with the normal pooled plasma. The quantity of inhibitor in a sample was calculated from Figure 1. When residual activity of Factor VIII was below 5 per cent, the unknown sample was sufficiently diluted with imidazole buffer to obtain residual Factor VIII activity between 5 and 15 per cent, which is equivalent to an inhibitor concentration between approximately 0.5 and 2.0 U./ml. Units of inhibitor were determined by multiplying the value read from the graph by the dilution factor. For example, if a sample which was diluted 20 times yielded a residual activity of Factor VIII of 6.3 per cent (from Fig. 1), the diluted sample contained 54/34 = 1.6 U./ml.; the undiluted sample contained 1.6 × 20 or 32 U./ml. When samples from different patients were assayed by this procedure, comparable inactivation curves were obtained. Similarly, various pools of normal plasma reacted with the same inhibitor to give an identical number of units.

The use of small inhibitor concentrations in the incubation mixture precluded the possibility of an inhibitory effect on Factor VIII taking place during the brief period in which residual Factor VIII was measured. An inhibitor operative against any of the serum factors (Factors IX, X, XI, XII) in the thromboplastin generating mixture was ruled out by the following control: The test material and the normal adsorbed plasma were separately incubated for 1 hour; then 0.2 ml. amounts of each were mixed, and Factor VIII
Fig. 1.—Calibration curve for quantitation of Factor VIII inhibitor obtained by diluting a sample of inhibitor, arbitrarily assigned 100 per cent activity. The logarithm of residual Factor VIII activity is plotted against concentration of inhibitor.

of this mixture was measured. Control values of Factor VIII were 21 ± 2 per cent.

When plasma was quantitated, the value obtained was multiplied by 7/6 to correct for the dilution with sodium citrate. Serum was either obtained by spontaneous clotting of blood or by clotting of native plasma with bovine thrombin. When used undiluted, serum was citrated (one part of 3.2 per cent sodium citrate to 6 parts of serum) to prevent clotting in the incubation mixture. Addition of citrate was not necessary when serum was diluted at least twice with imidazole buffer. On 20 duplicate determinations the standard error was 13.2 per cent. Six and four-tenths units correspond to 1 unit of Biggs.10 *

In Vitro Binding of Inhibitor by Normal Plasma

Diluted serum possessing inhibitor activity (0.2 ml.) was incubated at 37 C. for 1 hour with 0.8 ml. normal adsorbed plasma and with 0.8 ml. normal saline as a control. If Factor VIII activity was absent at the end of incubation, inhibitory activity of the mixture was then quantitated by incubation with diluted normal plasma as described above.

Separation of Protein

Zone electrophoresis in starch was done using a 0.1 M borate buffer of pH 8.6 at a potential of 2 volts/cm. for 21 hours. Segments of the starch block were eluted with 0.15 M NaCl. Concentration of protein in the eluates was determined by measuring the ultraviolet absorption at 280 m\(\mu\).

Zone ultracentrifugation was done in gradients of sucrose (10 to 40 per cent). The protein in each of 15 fractions was measured and the activity of inhibitor determined.

* Determined on a standard sample kindly supplied by Dr. R. Biggs, Oxford, England.
γG globulin possessing inhibitory activity was separated from other serum proteins by chromatography on columns of DEAE cellulose in a 0.007 M phosphate buffer, of pH 6.3. The γG globulin was dialyzed against distilled water, lyophilized, and redissolved in 0.15 M NaCl. Protein concentration was determined by measuring the optical density at 280 mµ (E1%49=14.9). All residual protein on the DEAE cellulose column was eluted with a 0.4 M phosphate buffer of pH 5.2.

γG globulin was digested with 2X crystalized papain (Worthington) in the pH stat at pH 7.0 and 37 C. The substrate to enzyme ratio was 100:1. Digestion took place in the presence of 0.1 M cysteine. The digested gamma globulin was filtered through a column of Sephadex G-75 resin in 0.15 M NaCl. The material included in the Sephadex resin was dialyzed versus 0.001 M tris buffer, pH 7.6, and applied to a column of CM cellulose. Unadsorbed material (Fab fragment) was dialyzed versus distilled water and freeze dried. Residual protein was eluted from the CM column with the tris buffer of pH 7.6 made 0.3 M with NaCl. Fc fragment was separated from this material by zone electrophoresis at pH 8.6.

γG globulin was digested with crystalline pepsin (Worthington) in the pH stat at pH 4.5 and 37 C. for 90 hours. F(ab') fragments were separated from the digested gamma globulin by filtration through Sephadex G-75 resin in 0.15 M NaCl.

γG globulin in serum was measured by comparison with standard samples, in an immunologic plate assay with an antiserum to γG globulin.

Double diffusion experiments and immunoelectrophoretic analyses were done with antisera prepared by injection of (1) horses with normal serum and (2) rabbits with purified γG globulin.

Sera were obtained from 7 patients with severe hemophilia A, who had developed an inhibitor of Factor VIII following repeated transfusions.

RESULTS

Starch Block Electrophoresis

Two ml. of human serum containing 1350 units of inhibitor was separated by starch block electrophoresis (Fig. 2). Inhibitory activity was found only in association with gamma globulin. Maximum inhibitory activity prolonged the substrate clotting time to 33 seconds.

Density Gradient Ultracentrifugation

When protein contained in the gamma globulins separated by starch block electrophoresis was subjected to density gradient ultracentrifugation, the inhibitory activity was found associated with protein contained in the top layers of the sucrose gradient, indicating that the inhibitor was not a macroglobulin. Again maximum inhibitory activity prolonged the substrate clotting time to 32 seconds. This suggested that the inhibitor was a γG globulin (Fig. 3). Fractions containing inhibitory activity (Nos. 10–14) were pooled, dialyzed against distilled water and freeze-dried. The protein was subsequently dissolved in 0.15 M NaCl.

Antigenic Analysis

When the fraction obtained from the sucrose gradient separation was reacted upon immunoelectrophoresis with both the horse and the rabbit antisera, only one line of precipitation was noted. On Ouchterlony analysis, this line of precipitation gave a reaction of bending and complete fusion with that formed by normal human gamma globulin: the protein was therefore γG globulin.
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Fig. 2.—Separation by starch block electrophoresis (pH 8.6) of serum containing an inhibitor of Factor VIII.

Fig. 3.—Separation by sucrose gradient ultracentrifugation of the gamma globulins containing an inhibitor of Factor VIII.

Preparation of Inhibitor by Column Chromatography

γG globulins possessing inhibitory activity were prepared by chromatography on DEAE cellulose. Table 1 shows the inhibitory activity of these gamma globulins. The conditions for eluting the γG globulin from the DEAE cellulose were so chosen that only 53 per cent of the gamma globulins of serum were eluted by the buffer. The material eluted, however, comprised only γG globu-
Table 1.—Yield of Inhibitor of Factor VIII in γG Globulins Separated by DEAE Cellulose Chromatography of Serum from One Patient

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of γG globulin (mg. %)</th>
<th>Inhibitory activity (U./ml.)</th>
<th>Specific inhibitory activity † (U./μM γ-G)</th>
<th>Percentage of γG globulin recovered</th>
<th>Specific inhibitory activity (U./μM γ-G)</th>
<th>Yield of specific inhibitory activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>990</td>
<td>420</td>
<td>6770</td>
<td>53% *</td>
<td>6880</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>1300</td>
<td>678</td>
<td>7560</td>
<td>26% †</td>
<td>4850</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>990</td>
<td>420</td>
<td>6150</td>
<td>16% †</td>
<td>3960</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>990</td>
<td>420</td>
<td>6150</td>
<td>—</td>
<td>3580</td>
<td>58</td>
</tr>
</tbody>
</table>

* Before lyophilization.
† After lyophilization.
‡ Assuming a molecular weight for γG globulin = 145,000.

Table 2.—Specific Inhibitory Activity of Fractions of γG Globulin Obtained by Papain Digestion

<table>
<thead>
<tr>
<th></th>
<th>Specific inhibitor U./μM</th>
<th>Per cent of specific activity present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γG globulin</td>
<td>518 *</td>
<td>100</td>
</tr>
<tr>
<td>Fab fragment</td>
<td>92 †</td>
<td>18</td>
</tr>
<tr>
<td>Fc fragment</td>
<td>17 ‡</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γG globulin</td>
<td>3580 *</td>
<td>100</td>
</tr>
<tr>
<td>Fab fragment</td>
<td>780 *</td>
<td>22</td>
</tr>
<tr>
<td>Fc fragment</td>
<td>15 ‡</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Assuming molecular weight for γG globulin = 145,000.
† Assuming molecular weight for Fab fragment = 52,000.
‡ Assuming molecular weight for Fc fragment = 48,000.
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The remainder of the serum proteins were eluted from the column by a phosphate buffer, 0.4 M at pH 5.2. Quantitative recovery of the specific inhibitory activity in the eluates was obtained prior to dialysis and lyophilization. Twenty-five per cent of the total inhibitory activity of serum was recovered in the \( \gamma \)G globulin eluted from the DEAE cellulose following lyophilization.

Filtration of the \( \gamma \)G globulins through a column of Sephadex G-75 resin in 0.5 M NaCl yielded a symmetrical pattern of elution. Specific inhibitory activity of the various fractions comprised in this pattern were uniform, supporting the conclusion that the inhibitor had the attributes of normal \( \gamma \)G globulin.

Papain Digestion

Table 2 shows the inhibitory activity of fragments obtained by digesting two different samples of \( \gamma \)G globulin with papain. Fab fragments possessed 18 per cent and 22 per cent, respectively, of the original specific inhibitory activity of lyophilized gamma globulin. Analysis of these fragments by double diffusion in agar against an antiserum to \( \gamma \)G globulin showed that all undigested \( \gamma \)G globulin had been removed during filtration through Sephadex. It also showed that the Fab and Fc fragments were pure. Fc fragments possessed 3 per cent and 0.4 per cent, respectively, of the original inhibitory activity.

Digestion with Pepsin

The inhibitory activity of the \( \mathcal{F}(ab')_2 \) fragments obtained by digestion of \( \gamma \)G globulin with pepsin is shown in Table 3. The \( \mathcal{F}(ab')_2 \) fraction had an asymmetrical elution pattern, with different specific inhibitory activity in the ascending and the descending portions of the chromatographic effluent (36 per cent and 61 per cent, respectively). A component appearing late in the elution diagram had mild inhibitory activity but did not react with an antiserum to \( \gamma \)G globulin. Fc related fragments obtained after digestion of the \( \gamma \)G globulin with pepsin possessed no inhibitory activity.

Reduction with Mercaptoethanol and Recombination

\( \gamma \)G globulin containing inhibitor was reduced with mercaptoethanol (0.2 M) in 4 M urea for 2 hours at room temperature; it was subsequently dialyzed against 0.15 M NaCl. Before reduction the specific inhibitory activity was 17,800 U./\( \mu \)M; after reduction and dialysis, 6 per cent of the original activity was recovered.

In Vitro Binding of Inhibitor by Normal Plasma

A low inhibitory activity of serum could be diminished by incubation with normal plasma. However, when excess of inhibitor was added to normal plasma, the unbound inhibitor possessed activity equal to that of an unreacted sample of serum diluted to the same extent. For example, serum containing 500 units of inhibitor per ml. was diluted 4 times and 10 times and then incubated with normal adsorbed plasma, as described above. The serum diluted 4 times had, after incubation, a residual inhibitory activity of 22 U./ml., compared to that of
Table 3.—Specific Inhibitory Activity of Fractions of \( \gamma \)G Globulin Obtained by Digestion with Pepsin

<table>
<thead>
<tr>
<th>Protein Substance</th>
<th>Concentration of Protein in Test Substance (mg.%)</th>
<th>Inhibitory Activity (U./ml)</th>
<th>Specific Inhibitory Activity (U./µM)</th>
<th>Per cent of Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \gamma )G globulin</td>
<td>5.8</td>
<td>1.63</td>
<td>4080</td>
<td>100</td>
</tr>
<tr>
<td>Fraction I*</td>
<td>12.6</td>
<td>1.83</td>
<td>1450</td>
<td>36</td>
</tr>
<tr>
<td>Fraction II†</td>
<td>5.3</td>
<td>1.31</td>
<td>2470†</td>
<td>61</td>
</tr>
<tr>
<td>“Late” fraction</td>
<td>9.7</td>
<td>0.68</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Proteins obtained from the ascending portion of the chromatographic effluent.
† Proteins obtained from the descending portion of the chromatographic effluent.
‡ Assuming a molecular weight for F(ab')2 fragments = 100,000.

the control, which had an activity of 24 U./ml. From the dilution, which the serum was subjected to, the expected value for the control was \( \frac{500 \times 0.2}{1.0 \times 10^4} = 24.0 \) U./ml. The serum diluted 10 times had, after incubation with normal plasma, a residual activity of 2.2 U./ml., compared to that of the control, which had an activity of 7.8 U./ml. The expected value for the control was \( \frac{500 \times 0.2}{1.0 \times 10^7} = 10.0 \) U./ml. Binding of inhibitor by normal plasma was demonstrable only when the inhibitor was not present in excess in the incubation mixture.

In Vivo Binding of Inhibitor by Factor VIII

Binding of inhibitor by Factor VIII introduced into the circulation by transfusion of fresh frozen plasma or Fraction I (Merck, Sharp and Dohme) was observed repeatedly in a patient with severe hemophilia A. The level of Factor VIII in his plasma was less than 0.5 per cent of normal. The serum level of inhibitor on repeated examinations was found to be between 0.7 and 1.0 units per ml. At the time of these determinations he had not been transfused for at least 2 months. The effect of repeated infusions on the plasma concentration of Factor VIII, the half-disappearance time of Factor VIII, and the serum level of inhibitor is shown in Figure 4. Five days after infusion of Factor VIII was begun, the inhibitory activity of serum was reduced to zero; the half-disappearance time of Factor VIII was increased to 12 hours, a normal value for hemophiliacs not possessing the anticoagulant. By day 12 the plasma levels of Factor VIII decreased, the half-disappearance time decreased, and the concentration of inhibitor increased to reach nearly 2 U./ml. by day 17. This increase in the inhibitory activity of gamma globulin must have been due to increased synthesis of inhibitor produced by antigenic stimulation with Factor VIII.

Effect of Blood Transfusions on the Inhibitor of Factor VIII in Hemophiliacs Resistant to Transfusion Therapy

The concentration of inhibitor of Factor VIII in 3 patients with severe hemophilia A, and known to possess the inhibitor of Factor VIII, was measured for 3 months following an exchange transfusion (Fig. 5). In 2 patients (P.P. and N.TR.), whose serum levels of inhibitor were considerable prior to the
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Fig. 4.—Effects of transfusions in a patient who has severe hemophilia A with mild Factor VIII inhibitor. Attained levels of Factor VIII, “half disappearance times” of Factor VIII, and serum levels of inhibitor are shown. Dots represent Factor VIII levels prior to infusions; arrows indicate Factor VIII levels 5 minutes after completion of infusions. The Fraction I used was “Human Fibrinogen, AHF-rich,” Merck, Sharp and Dohme.

exchange, a gradual rise of inhibitor concentration occurred soon after the exchange. In one patient (C.S.), whose serum level of inhibitor prior to the exchange was only approximately 1 unit, a rise of inhibitor concentration occurred after 3 days. The two patients who did not receive multiple transfusions following the exchange reached maximal levels within 2 weeks. The concentration of inhibitor gradually decreased over a period of several months to preinfusion levels.

A similar rise in inhibitor level was observed in the sera of 3 other severe hemophiliacs after the presence of inhibitor was suspected because bleeding failed to stop despite repeated transfusions of fresh-frozen plasma (Fig. 6).

Disappearance of Inhibitor from Normal Plasma

Figure 7 shows the concentration of the inhibitor of Factor VIII and the concentration of Factor VIII in the plasma of a normal recipient of 100 ml of plasma containing inhibitor of Factor VIII. Immediate disappearance of Factor VIII activity from the plasma was noted. The half-disappearance time of inhibitor was 7½ hours. When the inhibitor reached a low level, Factor VIII reappeared and its level doubled in 4½ hours. This indicated that the turnover of Factor VIII in a nonhemophiliac is rapid.
Fig. 5.—Serum levels of inhibitor in three known refractory patients with severe hemophilia A before and after exchange transfusion. Maximum levels after exchange transfusion were 490 U./ml. (P.P.), 880 U./ml. (N.TR.), and 830 U./ml. (C.S.). The thin arrows indicate the days on which blood transfusions were given to P.P.

DISCUSSION

Two views are generally held to explain the biological activity of the inhibitor of Factor VIII: that it is either an enzyme or an antibody. The data presented in this communication favor the latter.

The inhibitory activity was found in the yG globulin fraction of serum. It was furthermore associated with the bivalent F(ab')2 fragment obtained by peptic digestion of the gamma globulin and with the monovalent Fab fragment obtained by digestion of the gamma globulin with papain. Considerable significance has been ascribed to the inability of the inhibitor to precipitate specifically with Factor VIII, either in the tube or in an agar gel. Lines of precipitation formed by the inhibitor with animal preparations of Factor VIII have, presumably quite correctly, been ascribed to the interaction of animal proteins with the human yG globulin. In fact, the inability to form specific precipitates following interaction of Factor VIII with inhibitor prompted the suggestion that the inhibitor was not antibody. Since, however, monovalent Fab fragments possess inhibitory activity, the failure to precipitate loses some of its significance. Present data do not preclude the possibility that the inhibitor is an “incomplete” antibody. Nor is it necessary to assume that the antibody be “incomplete” since antibody excess could just as easily prevent precipitation.

Since the inhibitory activity appeared distributed equally among the various portions of the elution pattern of protein from the chromatographic separa-
Fig. 6.—Serum levels of inhibitor in three patients with severe hemophilia who received repeated transfusions of fresh-frozen plasma. Maximum levels were 480 U./ml. (TH.H.), 880 U./ml. (J.CR.), and 460 U./ml. (R. FL.). The arrows indicate the days on which transfusions were given.

Inhibitions and, moreover, since antigen-binding subfractions of the yG globulin contained the inhibitory activity, the possibility that a contaminating protein species could elute together with the gamma globulins was excluded.

At present it is not clear why 6 per cent of the inhibitory activity should be reconstituted on reoxidation of the reduced and unfolded globulin. It is interesting that this amount is similar to that found on reoxidation of reduced and unfolded Fab fragments of yG antibody. 

It is noteworthy that the inhibitor appears in the circulation of hemophilic patients only after transfusion of blood has provided a stimulus. The amount of inhibitor in the circulation increases sharply following stimulation, only to decrease slowly over extended periods of time to preinfusion levels. This behavior is consistent with a model in which synthesis of protein increases, as in antibody synthesis, following antigenic stimulation, but precludes the possibility that the inhibitor is an enzyme, reacting in a simple enzyme substrate model.

After the injection of inhibitor into a normal circulation, its disappearance is rapid and is not in keeping with the rate of catabolism of isologous gamma globulin from the circulation. T½ is decreased from an average value of 17 ± 3 days to 7½ hours. It is noteworthy that Factor VIII disappeared from the circulation while the inhibitory globulin was cleared. Again this behavior is suggestive of the rapid clearance from the living system of antigen-antibody aggregates.
Although all these experiments clearly suggest that inhibitor is an antibody, we do not understand why this antibody develops only in certain hemophiliacs. Factor VIII inhibitor was encountered in 12 of 67 (18 per cent) patients with severe hemophilia A (Factor VIII less than 0.5 per cent), who were repeatedly observed at the Children's Hospital over the past 6 years. Discussion of the clinical significance of this complication of hemophilia will be presented in a subsequent paper. Our studies have indicated, however, that the level of inhibitor varies considerably in different patients and at various times in the same patient, depending on recent exposure to Factor VIII by transfusions. The choice of therapy (use of Factor VIII concentrates, exchange transfusion) for effective control of life-threatening hemorrhage depends on the actual inhibitor-level present at the time.

**Summary**

A circulating anticoagulant, which specifically inhibits Factor VIII (AHF), has been detected in some patients with hemophilia A who had received multiple transfusions. The inhibitor was quantitated by measurement of the degree of inactivation of Factor VIII. The data presented provide strong
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evidence for the antibody nature of the Factor VIII inhibitor in hemophilia:
(1) All specific inhibitory activity of serum was detected in the γG globulin obtained by chromatography of the sera on DEAE cellulose. (2) Fab fragments obtained by digestion of the γG globulin with papain, contained 18–22 per cent of the specific inhibitory activity, while Fc fragments contained 0.4–3 per cent. F(ab′)2 fragments obtained by digestion with pepsin contained 36–61 per cent of the specific inhibitory activity of γG globulin. (3) The level of the inhibitor of Factor VIII increased sharply following transfusions of blood and decreased slowly to its preinfusion level. (4) When small amounts of inhibitor were incubated in vitro with excess Factor VIII, the inhibitor activity was decreased. Infusion of Factor VIII into a patient with a low level of inhibitor decreased the inhibitor activity. (5) Clearance of the isologous inhibitor from the circulation of a normal subject was rapid.

SUMMARIO IN INTERLINGUA

Un anticoagulante circulante que inhibi specificamente le factor VIII eseva trovate in certe patientes con hemophilia A qui habeva recipite multiplice transfusiones. Le inhibitor eseva quantificate per mesurar le grado de inactivation de factor VIII. Le datos presentate provide un forte evidentia pro le natura anticorporee del inhibitor de factor VIII in hemophilia: (1) Omne le specif activitate inhibitori del sero eseva detegite in le globulina γG obtenite per chromatographia del seros super cellulosa DEAE. (2) Fragmentos Fab obtenite per digestion del globulina γG con papaina contineva inter 18 e 22 pro cento del specific activitate inhibitori, durante que fragmentos Fc contineva 0,4 a 3 pro cento. Fragmentos F(ab′)1, obtenite per digestion con pepsina contineva 36 a 61 pro cento del specific activitate inhibitori de globulina γG. (3) Le nivello del inhibitor de factor VIII accresceva marcatamente post transfusiones de sanguine e postea decresceva lentemente al valor pre-transfusional. (4) Quando micre quantitates de inhibitor eseva incubate in vitro con un excesso de factor VIII, le activitate del inhibitor eseva reducete. Le infusion de factor VIII ad in un paciente con un basse nivello de inhibitor decresceva le activitate de inhibitor. (5) Le clearance del inhibitor ab le circulation de un subjecto normal sub conditiones isologe eseva rapida.

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