The Effects of Injection of Human Factor VIII Antibody Into Rabbits

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Rabbits were injected with an immunoglobulin fraction of human serum containing a factor VIII antibody. Factor VIII levels fell abruptly, persisted below 10% of a rabbit plasma standard for 12 hr, and returned to normal by 120-168 hr. The factor VIII antigen-antibody reaction did not result in intravascular clotting as evaluated by kinetic studies with $^{125}$I-fibrinogen. However, small falls in factor V and factor VII levels were observed over a 6-hr period after the injection. Platelets fell to about one-half of initial values within 15 min, rose to 80% of initial levels over 2 hr, and subsequently declined to 65%-70% of initial levels. WBC levels fell to below 20% of initial values 2 hr after the injection but returned to about 75% of initial values by 6 hr. Total hemolytic complement activity was unaffected. Animals made granulocytopenic with nitrogen mustard and animals with hereditary C4 deficiency behaved similarly to normal animals. One may conclude that the injection of human factor VIII antibody into rabbits produces a rabbit model with impaired intrinsic coagulation suitable for studies of the mechanism of endotoxin-induced intravascular clotting.

Although it is known that human factor VIII antibodies can neutralize rabbit factor VIII in vitro, data are not available on the effects of injecting human factor VIII antibody into rabbits. In the experiments reported herein, an immunoglobulin fraction of human serum containing factor VIII antibody was injected into rabbits, and its effects upon factor VIII levels, fibrinogen kinetics, factor V and factor VII levels, WBC and platelet counts, and total hemolytic complement activity were evaluated. The data to be presented establish that human factor VIII antibody can be used to produce a rabbit model with selective inhibition of the intrinsic clotting reactions. In a second paper, we will describe the effects of the administration of Gram-negative endotoxin in such a rabbit model.

MATERIALS AND METHODS

New Zealand male rabbits weighing 2.0 to 3.0 kg and male rabbits with a hereditary deficiency of the sixth component of complement (purchased from Rancho de Conejo, Vista, Calif.) weighing 1.8 to 2.6 kg were fed Purina rabbit chow and allowed free access to water. During an experiment and for at least 3 days before, KI (200 mg/liter) and NaCl (1.8 g/liter) were added to the drinking water of rabbits receiving radioactive fibrinogen.

Animals were bled for fibrinogen assays and radioactivity measurements by inserting a disposable 20-gauge needle into a marginal ear vein and allowing nine parts of blood to drip directly
from the hub of the needle into marked plastic tubes containing one part of 2\(^{-}\) \(\text{Na}_2\text{EDTA.0.26\degree}\), EACA anticoagulant. Blood for other clotting factor assays was obtained similarly except that a balanced citrate anticoagulant\(^2\) was used. Blood was also taken from the hub of the needle into heparinized capillary tubes to determine the microhematocrit and into calibrated plastic capillary tubes (Unopette, Becton-Dickinson) for platelet and WBC counts. The blood was centrifuged at 10,000 rpm for 10 min and the supernatant plasma was removed, centrifuged again at 10,000 rpm for 10 min and stored at \(-20\degree\text{C}\).

Nitrogen mustard (Mustargen, Merck, Sharp and Dohme, Westpoint, Pa.) was dissolved in sterile water just prior to use.

Preparation of Human Factor VIII Antibody and Normal Human Immunoglobulin Control Material

Plasma from a hemophiliac with a very high titer factor VIII inhibitor was processed as described previously.\(^4\) Briefly, the plasma was clotted with calcium and thrombin; 1 part of citrate anticoagulant was added to 5 parts of serum; the serum was adsorbed with one-tenth volume of \(\text{Al(OH)}_3\) gel diluted 1:4 with distilled water; and the adsorbed serum was heated to \(56\degree\text{C}\) for 2 hr. After such treatment the serum was devoid of measurable clotting factor activities. The factor VIII antibody activity was then isolated by precipitation with 50\(^\circ\) ammonium sulfate followed by starch zone electrophoresis. The fractions containing maximum antibody activity were then eluted from the starch, pooled, dialyzed against 0.15 \(M\) NaCl, concentrated, and stored in capped plastic vials at \(-20\degree\text{C}\). Normal human plasma was processed similarly to prepare human immunoglobulin control material. All preparations were sterilized just prior to use by passage through a filter (0.45 \(\mu\) Swinnex-25, Millipore Corp., Bedford, Mass.).

The two batches of factor VIII antibody fraction used for our experiments had protein concentrations of 14.3 mg/ml and 15.5 mg/ml, respectively. When 1 part of material from the first batch was added to 59 parts of normal rabbit plasma and incubated at room temperature for 1 hr, the mixture had a residual factor VIII activity of 9\(^\circ\), of the standard reference plasma (see below).

Both batches of factor VIII antibody were cultured prior to sterilization by filtration, and both batches contained the following organisms: \(\text{Bacillus subtilis. diphtheroids, and hemolytic Staphylococcus aureus. coagulase negative. In addition, batch one contained two unidentified species of Pseudomonas and batch two contained alpha Streptococcus. One of three batches of control material was also cultured prior to filtration. It contained hemolytic S. albus coagulase negative and two unidentified species of Pseudomonas. All batches of antibody and control material were negative on culture after filtration.}

Preparation of \(^{125}\text{I}-\text{Fibrinogen}

Rabbit fibrinogen was purified by a slight modification of McFarlane's technique.\(^5\)\(^6\) Saturated neutral ammonium sulfate solution was added to plasma. The precipitate at 18\(^\circ\) saturation was discarded. The supernate was increased to 25\(^\circ\) saturation. The resultant precipitate was dissolved in 0.005 \(M\) sodium citrate. Precipitation with 25\(^\circ\) ammonium sulfate was repeated two more times, and the final precipitate was dissolved in 0.005 \(M\) sodium citrate to one-tenth the original plasma volume. Clottability was greater than 95\(^\circ\).

The purified fibrinogen was labeled according to the techniques of McFarlane\(^3\) and of Atencio et al.\(^7\) utilizing carrier-free Na \(^{125}\text{I}\) without reducing agents (New England Nuclear, Boston, Mass.). Unbound radioactivity was removed by dialysis. Labeled material contained less than 0.5 atoms of iodine per molecule of fibrinogen and initial specific activities of 15.8 to 39.5 \(\mu\)C/mg. When clotted with thrombin, a mean of 93\(^\circ\)–95\(^\circ\) of the radioactivity was found in the fibrin clot. About 0.9\(^\circ\) of the radioactivity was present as free iodide, i.e., remained in the supernatant of a precipitate formed by adding 1 volume of 40\(^\circ\) trichloracetic acid (TCA) to 1 volume of the radioactive fibrinogen preparation. The labeled material was sterilized by Millipore filtration, stored at \(-20\degree\text{C}\), and used within 4 wk of preparation.

Measurement of Fibrinogen and Radioactivity

Plasma fibrinogen concentration, whole plasma radioactivity, radioactivity of the fibrin clot, radioactivity remaining after removal of the fibrin clot (serum radioactivity), and residual radio-
activity of the supernatant after precipitating serum or plasma proteins with TCA were measured as described previously. The only modification was the use of an equal volume of 40% TCA as described above to precipitate proteins. Radioactivity was measured in an autogamma scintillation counter (Nuclear Chicago, Model 1085). Values for radioactivity were corrected as described by Atencio for cumulative loss of radioactivity due to prior sampling.

The rabbits were kept in metabolic cages which permitted quantitative collection of urine at 24-30-hr intervals. Cages were washed daily with 1% KI solution and the wash water added to the urine. Total volume was measured and an aliquot was removed for counting of radioactivity. Feces were also collected daily and suspended in 200-500 ml of a KI solution for 3-6 hr. The suspension was then filtered through a plastic screen. The volume of filtrate was measured and an aliquot was removed for counting of radioactivity. Total fecal counts were added to total urine counts. These data were also corrected for loss of radioactivity due to removal by blood sampling.

Other Assay Methods

Platelets were counted by phase microscopy according to the technique of Brecher et al. Total WBC were counted on the same sample. One hundred or 200 cells were counted on a Wright-stained blood smear for differential counts. Factor VIII was measured in a one-stage assay using an activated partial thromboplastin time technique. Factor VII and Factor V were measured in one-stage assays utilizing human brain thromboplastin and hereditary specific deficiency plasmas as substrates.

Clotting factor activities were expressed as per cent of a standard prepared by pooling plasma from five normal rabbits. From 18 to 36 ml of blood was drawn from each animal by inserting a needle into the central artery of the ear and allowing the blood to flow directly into a plastic tube containing one-ninth volume of balanced citrate anticoagulant. The resultant plasma was checked for nascent clotting by use of a plasma protamine paracoagulation test, and only plasma with a negative test was incorporated in the pool. It was stored at -20°C.

Hemolytic Complement Determination

Total hemolytic complement was estimated by a modification of the method of Osler et al. Three per cent sheep cells were sensitized with an equal volume of a 1/250 dilution of glycerinated rabbit antisheep cell hemolysin (Hyland Laboratories). Five-hundredths milliliter of this sensitized cell suspension were mixed in a microtiter plate with 0.025 ml of increasing dilutions of the test serum diluted in a gelatin-barbital buffered saline solution containing calcium and magnesium. Mixtures were incubated for 60 min at 37°C, and the amount of hemolysis was estimated visually. The end point was expressed as that dilution of test serum producing hemolysis equivalent to that obtained by complete hemolysis of a 50% control red cell suspension (CH50).

Correction for Dilution of Plasma With Anticoagulant and for Change in Hematocrit

Whole plasma radioactivity and fibrinogen radioactivity were always corrected for dilution of plasma by anticoagulant and for change in hematocrit during the experiment. The following formula was used for these corrections: 

\[ C_{cm} = \frac{PH_i}{PH_0} \],

where \( C_{cm} \) is a combined correction factor for anticoagulant dilution and hematocrit change, \( PH_0 \) is the initial plasma hematocrit value, and \( PH_i \) is the hematocrit value of an individual sample. The derivation of this formula has been described elsewhere.

These corrections were not applied to clotting factor data expressed as per cent of the reference standard. They were applied, however, whenever such data were converted into per cent of an animal's initial clotting factor activity before injection of test material.

Experimental Design

Eleven normal rabbits (mean weight, 2.6 kg) were given 27.5 to 37.5 mg/kg (mean, 34.5 mg/kg) of a factor VIII antibody preparation in a volume of 2.0 to 2.5 ml/kg. Six additional rabbits (mean weight, 2.3 kg) were injected with 1.75 mg/kg of HN2 4 days before the administration of 33.0 to 36.0 mg/kg of a factor VIII antibody preparation. Three rabbits with hereditary C6
deficiency (mean weight, 2.3 kg) were also injected with 36.0 mg/kg of a factor VIII antibody preparation. Six normal rabbits (mean weight, 2.5 kg) were given 23.0 to 33.0 mg/kg (mean, 29.0 mg/kg) of a human immunoglobulin control preparation in a volume of 1.5 to 4.0 ml/kg.

Blood samples were drawn in all rabbits at the following times: immediately before the injection of the test material, and at 1, 3, 6, and 24 hr after the injection. Additional blood samples were drawn in many animals at the following times after the injection: 15 min, 2, 12, 48, 72, 96, 120, and 168 hr. Fibrinogen, factor VIII, factor V, and factor VII were measured on all blood samples. Platelets and WBC were counted on all blood samples drawn up to 48 hr.

Fibrinogen catabolism was evaluated with 125I-fibrinogen in six normal rabbits given factor VIII antibody preparation and in two normal rabbits given control immunoglobulin preparation. These rabbits received 17.0-43.5 μC of 125I-fibrinogen 48 hr before the test material. Blood was drawn 5 min, 12 hr (three animals), either 24 or 30 hr, and 48 hr after the injection of the 125I-fibrinogen. Then, the test material was injected and repeated blood samples were drawn at intervals as cited above. For the control animals the data before and after injection of the test material were combined to obtain single values for the intercept and slope of the terminal portion of the plasma fibrinogen radioactivity curve. For the animals given factor VIII antibody, the data before and after the injection were treated separately to calculate separate values for the intercept and slope before and after the injection. These were then compared to evaluate the effect of the antibody upon plasma fibrinogen catabolism. The linear least-squares method was used for all calculations.

Plasma nonclottable protein radioactivity (counts after removing fibrin clot minus counts after precipitating residual protein with TCA) was calculated for each sample and expressed as percent of the injected dose of radioactivity. Total body 125I not bound to protein was calculated as described by Atencio et al., utilizing whole plasma radioactivity of the 5-min sample to calculate plasma volume. Urinary radioactivity data were expressed at \( u(t) \) where \( u(t) \) represented cumulative excretion of 125I in the urine to time \( t \).

RESULTS

Behavior of Animals

The normal rabbits and the C6-deficient rabbits injected with factor VIII antibody appeared to become ill 10 to 20 min after the injection. For about the next 3 hr it was difficult to obtain blood samples from marginal ear veins. The animals were listless and consumed little food or water for about 10–24 hr. (One animal drank very little water for 5 days.) In most animals urinary output fell markedly for 24 hr. Nevertheless, no animal given factor VIII antibody died, and after about 24 hr the rabbits gradually became more active.

Animals given nitrogen mustard (HN2) were less active than normal and ate less during the 4-day period between injection of HN2 and injection of factor VIII antibody. Nevertheless, these animals also appeared to recover within 48 hr after injection of factor VIII antibody. The one exception was a rabbit which remained obviously ill, developed a nasal discharge, and at autopsy had multiple lung abscesses from which a species of Pasteurella was cultured.

The animals injected with human immunoglobulin control material also appeared to become ill after the injection but to a lesser degree. Their food and water intake was reduced for the first hours after the injection, but they appeared to recover fully within 24 hr.

Factor VIII

Factor VIII levels fell immediately after injecting human factor VIII antibody into normal rabbits, into granulocytopenic rabbits, and into C6 deficient rabbits (see Fig. 1). Six hours after the injection mean values were as follows: for the
normal rabbit group, 7.5% ± SE 0.8%; for the granulocytopenic group, 9.0% ± 0.9%; and for the C6-deficient group, 13.0% ± 2.1%.

Mean levels below 10% of the reference standard persisted for more than 12 hr in the normal and granulocytopenic group. Factor VIII levels were still markedly depressed at 24 hr and returned gradually to initial levels by 120–168 hr. The control immunoglobulin fraction produced no significant change in factor VIII levels.

**Fibrinogen Concentration**

Serial plasma fibrinogen levels after the administration of factor VIII antibody or of control immunoglobulin fraction are plotted in Fig. 2. The elevated mean initial fibrinogen level for the HN2 animals of 575 mg/100 ml contrasts with a mean value of 300 mg/100 ml for these animals before injection with HN2 and agrees with earlier data from this laboratory of increased fibrinogen levels in rabbits given HN2. In all of the groups of animals, measured fibrinogen levels decreased slightly during the 6 hr after injection of the test material, but differences between initial levels and 6 hr levels were not significant ($p > 0.05$). When the 6-hr values were corrected for change in hematocrit and dilu-
tion with anticoagulant and expressed as a per cent of the initial fibrinogen value, the following mean percentages were obtained: control rabbits, 102\% ± 5.5\%; normal rabbits given factor VIII antibody, 91.0\% ± 5.0\%; granulocytopenic rabbits given factor VIII antibody, 103\% ± 7.5\%; C\(_6\) deficient rabbits given factor VIII antibody, 87.5\% ± 5.0\%. A rise in mean fibrinogen level was observed in all groups of animals beginning with 12-hr sample.

**Fibrinogen Radioactivity**

The second portion of the mean plasma fibrinogen radioactivity curve (beginning 26 hr after injecting \(^{125}\)I-fibrinogen and ending 144 hr after injecting the fibrinogen) for two rabbits given a control human immunoglobulin preparation is plotted in Fig. 3A. The equation for this line is: \(q(t) = 65.4 e^{-0.022t}\). From the slope of the line and the cluster of data points obtained during the 6 hr after injection of the control material, one can see that the control material produced neither an acute nor a late effect upon plasma fibrinogen radioactivity. Excretion of radioactivity in the urine decreased for 24 hr following the injection. The total body pool of iodide not bound to protein increased from a value of 5.6\% of the injected dose, just prior to giving the control material, to a value of 9.7\% 24 hr later.

As explained in the Materials and Methods section, the second portion of the mean plasma fibrinogen radioactivity curve for six rabbits injected with a factor VIII antibody preparation was plotted as two lines, one line before and one line after the injection of the antibody (see Fig. 3B). Data from 16 samples

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**Fig. 3.** (A) Left-hand panel: The line identified by the symbol \(q(t)\) is the second portion of the mean plasma fibrinogen radioactivity curve for two rabbits injected with control immunoglobulin material. The line identified by the symbol \(1-e(t)\) is a plot of the mean difference between the injected dose of radioactivity (which has been called 1) and the cumulative urinary excretion of radioactivity at time \(t\) for the two rabbits. (B) Right-hand panel: The second portion of the mean plasma fibrinogen radioactivity curve for six rabbits injected with factor VIII antibody has been calculated as two lines. The first line was obtained from data before injection of antibody and the second line was obtained from data beginning 24 hr after the injection of antibody. Multiple samples drawn during the first 12 hr after the injection are connected by a dotted line and shown in greater detail in the insert. Mean excretion of urinary radioactivity for these animals is plotted as described above.
representing four time points between 12 and 50 hr after injection of $^{125}$I-fibrinogen were used to obtain the equation for the line before injection of antibody (first solid segment of the line shown in Fig. 3B). Its equation is $q(t) = 81.6 e^{-0.0176t}$. The SE of the exponential constant is ± 0.0020. Data from 19 samples representing six time points from 72 to 216 hr were used to obtain the equation for the line after injection of antibody (second solid segment of the line shown in Fig. 3B). Its equation is $q(t) = 81.5 e^{-0.0154t}$. The SE of the exponential constant is ± 0.0015. Thus, these two equations have identical coefficients, and their exponential constants do not differ significantly ($p > 0.10$).

If the injection of factor VIII antibody had resulted in a permanent excess disappearance of plasma fibrinogen radioactivity, such as occurs with intravascular clotting, then the coefficient of the equation (which defines the intercept of the line) would have been significantly lower for the equation after the injection.

Nevertheless, multiple samples taken over the first 12 hr after injection of factor VIII antibody did reveal a transient, slight fall in plasma fibrinogen radioactivity in five of the six rabbits (see insert of Fig. 3B). However, the radioactivity appeared subsequently to return to the plasma compartment. As in the controls, decreased urinary excretion of radioactivity was noted in the 24 hr after injection of the antibody. This was associated with an increase in the mean total body pool of iodide not bound to protein from a value of 6.1%, before injection of antibody, to a value of 10.0% 24 hr later.

Mean radioactivity curves for plasma nonclottable protein are shown in Fig. 4. The injection of factor VIII antibody was not followed by a significant increase in the level of nonclottable protein radioactivity as might occur with activation of fibrinolysis.

**Factor V and Factor VII**

Figure 5 is a plot of mean values for factor V levels in the different groups of animals. Note that the initial mean values for all groups (obtained from ear vein plasma) exceeded 100% of the activity of the standard reference plasma (pooled plasma from arterial blood). Factor V activity in plasma from ear vein blood has been shown previously to exceed factor V activity in plasma from arterial blood drawn by cardiac puncture from the same animals. For an unknown reason, mean factor V activity was lower in the initial sample from the granulocytopenic animals than in the subsequent samples from these animals. Plasma factor V activity fell modestly over 6 hr in most animals injected with factor VIII antibody. When measured values for the 6-hr samples were cor-
Fig. 5. Effect of injection of factor VIII antibody upon mean factor V activity (numbers of rabbits as in legend to Fig. 1).

Fig. 6. Effect of injection of factor VIII antibody upon mean factor VII activity (numbers of rabbits as in legend to Fig. 1).

Fig. 7. Effect of injection of factor VIII antibody upon mean platelet count (numbers of rabbits as in legend to Fig. 1).
reduced and expressed as a per cent of initial mean values, the percentages were as follows: control group, 103% ± SE 13.3%; normal rabbits given factor VIII antibody, 72% ± 5.7%; granulocytopenic rabbits given factor VIII antibody, 142% ± 37.8%; C6-deficient rabbits given factor VIII antibody, 55% ± 6.7%.

When the measured value at 6 hr for the granulocytopenic animals was expressed as a per cent of the mean value of the second sample (drawn 15 min after injection of antibody) the percentage was 89% ± 2.3%.

Mean values for measured factor VII levels are plotted in Fig. 6. One can see that mean factor VII levels fell in all groups of animals given factor VIII antibody but did not fall in the control group. At 6 hr the corrected values expressed as percent of initial values were as follows: control group, 97% ± SE 3.3%; normal rabbits given factor VIII antibody, 73% ± 2.8%; granulocytopenic rabbits given factor VIII antibody, 82% ± 3.8%; and C6-deficient rabbits given factor VIII antibody, 71% ± 4.4%. These differences were statistically significant (p < 0.05) for all groups given antibody. Interestingly, a transient rise in factor VII levels was noted in every group, including the control group, beginning between 24 and 48 hr after injection of the test material.

**Platelets**

Mean values for platelet counts are plotted in Fig. 7. In the animals given control immunoglobulin material, the mean platelet count fell gradually over the first 2 hr from an initial level of 663,800 ± SE 78,100 per cu mm to a level of 528,700 ± 67,200 per cu mm. Although this single difference is not statistically significant (p > 0.10), the shape of the curve suggests that the injection of control immunoglobulin material was followed by a gradual, temporary real fall in platelet levels.

Platelet levels fell strikingly within 15 min of the injection of factor VIII antibody into normal rabbits and into C6-deficient rabbits. The 15-min values were about one-half of the values before injection. Initial counts were lower in the animals made granulocytopenic with HN2 and their platelets showed less of a fall within the first 15 min. In all groups receiving antibody, the platelet count rapidly rose after this initial fall and by 2 hr had returned to about 80% of the level before injection. This was then followed by a gradual second small decline in platelet levels. However, one should note that at 6 hr mean platelet counts were 70%–80% of mean initial levels. At 24 hr mean platelet counts were between about 55% and 70% of initial levels.

**WBC**

The injection of either factor VIII antibody or control immunoglobulin material caused the total WBC to fall. However, the fall was much greater after injection of antibody (see Fig 8). Differential counts, carried out in four rabbits given control immunoglobulin material and three normal rabbits given factor VIII antibody, established that the fall in total WBC count stemmed primarily from a loss of granulocytes. The mean granulocyte count before injection of control immunoglobulin material was 2733 per cu mm; 1 hr after the injection the mean granulocyte count was 461 per cu mm, and 6 hr after the injection the mean granulocyte count had returned to 7860 per cu mm. The effect of
antibody upon the granulocyte count in a normal rabbit may be summarized as follows: count before injection, 3685 per cu mm; 15 min after injection, 162 per cu mm; 1 hr after injection, 181 per cu mm; 2 hr after injection, 139 per cu mm; 3 hr after injection, 1236 per cu mm; and 6 hr after injection, 3870 per cu mm. Note that the granulocyte count returned to normal by 6 hr in animals given either control immunoglobulin material or factor VIII antibody.

No granulocytes were seen in a 100 cell differential count carried out before the injection of antibody in four of six rabbits treated with HN2. The granulocyte counts of the other two rabbits were 23 and 56 per cu mm.

Hematocrit

Mean hematocrit levels for rabbits given control immunoglobulin material and for normal rabbits given factor VIII antibody are summarized in Fig. 9. Changes similar to the latter were observed in the C6 deficient animals and in the granulocytopenic animals. The fall in hematocrit in the control animals reflects the removal of a mean volume of 26 ml of blood by sampling during the first 24 hr. The animals given factor VIII antibody developed a bleeding tendency which resulted in extra loss of blood at the time of sampling. The difference after 24 hr between the hematocrit of the control animals and the animals given antibody could have resulted from this extra blood loss.
Total Hemolytic Complement Activity

A 1-to-8 dilution of serum from a normal rabbit produced 50% hemolysis in the complement assay. This same level of complement activity was found in multiple samples taken after the injection of factor VIII antibody (15 min, 1, 2, 6, 24, and 48 hr). Similar results were obtained in another rabbit given factor VIII antibody but not included in the study group because a smaller amount of antibody was injected. No hemolytic complement activity could be detected in the serum of rabbits with hereditary C6 deficiency.

DISCUSSION

The data presented above establish that the injection into rabbits of a partially purified human immunoglobulin fraction containing factor VIII antibody activity produced a severe, specific factor VIII deficiency in these animals. Factor VIII levels fell to less than 10% of the activity of a pooled rabbit reference standard within 15 min, and these low levels of activity persisted for a minimum of 12 hr. This was associated with a transient, slight fall in fibrinogen level (Fig. 2), which was not statistically significant, and with a transient excess loss of labeled fibrinogen radioactivity from the plasma (see insert of Fig. 3B). However, plasma fibrinogen radioactivity then returned to values expected from the slope of the plasma fibrinogen radioactivity curve before the injection (see Fig. 3B), i.e., no excess permanent loss of radioactive fibrinogen from the intravascular compartment could be demonstrated. Moreover, plasma non-clottable protein radioactivity did not increase significantly. Thus, the reaction between the animal’s factor VIII antigen and the antibody did not appear to cause either intravascular clotting or fibrinolysis of sufficient degree to alter fibrinogen kinetics significantly.

The cause for the temporary slight fall in plasma fibrinogen radioactivity following the injection of antibody is unknown. Urinary excretion of radioactivity was reduced during the 24 hr after injection of either antibody or control immunoglobulin fraction. This reduced excretion was associated with a decreased urinary output during this period, and an increase in the total body pool of radioactive iodide not bound to protein.

Granulocyte and platelet counts also fell after injection of the antibody fraction (see Figs. 7 and 8). Since the falls were observed in C6-deficient rabbits, the mechanism was independent of activation of late complement components. Conceivably, the mechanism involved activation of early complement components, but, if so, the degree of activation failed to reduce the level of total hemolytic complement activity in two rabbits in which these levels were followed serially. The initial fall in platelet count observed after injection of antibody into normal or C6-deficient rabbits was greater than the initial fall in platelet count observed after injection of antibody into granulocytopenic animals. The reason is unknown but could be related to recent observations that some forms of platelet injury induced by antigen-antibody complexes are mediated by leukocytes.15

Interestingly, granulocyte counts also fell significantly after the injection of control immunoglobulin fraction. This raises the possibility that the fraction
contained nonantibody activities directly affecting granulocytes, e.g., kallikrein which is reportedly chemotactic for granulocytes.\textsuperscript{16} We should also point out that cultures taken before sterilization of both our control and our antibody fractions yielded evidence of bacterial contamination, including Gram-negative bacteria in three preparations. Since sterilization by passage through a filter of 0.45-\(\mu\)m pore size would not remove endotoxin, we cannot eliminate the possibility that small amounts of endotoxin in our fractions could have directly affected granulocytes and platelets. However, if endotoxin was injected the amount was insufficient, as discussed above, to increase plasma fibrinogen consumption.

The failure to demonstrate increased fibrinogen consumption does not rule out the possibility that a factor VIII antigen-antibody reaction resulted in minimal activation of early steps in clotting. Damage to platelets could have made available platelet factor 3 activity, and damage to granulocytes could have been associated with generation of granulocyte tissue thromboplastic activity.\textsuperscript{17} Activation of factor VII during clotting initiated by granulocyte tissue thromboplastic activity could conceivably account for the small falls in factor VII activity observed after injection of antibody into normal and C6-deficient rabbits (see Fig. 6). However, the fall in factor VII also observed in granulocytopenic rabbits injected with antibody could not have stemmed from this mechanism. The fall in factor V activity after injection of antibody (see Fig. 5) could have resulted from consumption of factor V during minimal intravascular clotting. However, the initial levels of factor V activity in ear vein plasma from our animals were elevated. This observation is consistent with earlier evidence of increased factor V activity in ear vein plasma from normal rabbits, presumably due to its activation by minute amounts of thrombin generated during collection of the blood.\textsuperscript{14} Consequently, a fall in factor V activity in the samples collected after injection of the antibody could also have resulted from prevention of such activation during sampling by the induced factor VIII deficiency. In a further attempt to evaluate activation in vivo of intrinsic clotting we measured factor XII levels in animals injected with antibody fractions. Reliable data could not be obtained because of the effect of factor VIII antibody in the test sample upon the assay system.

A major goal of these experiments was to develop a rabbit model with selective impairment of the intrinsic clotting reactions for studies of the mechanism of endotoxin-induced clotting. There were three requirements for this model. First, the process producing the selective impairment should not, in itself, cause significant fibrinogen consumption. Second, platelet, WBC, and complement levels should be adequate at the time of the injection of endotoxin. Finally, the impairment of intrinsic clotting should persist for at least 6 hr after injection of endotoxin, i.e., during the interval in which endotoxin-induced clotting occurs.\textsuperscript{10} The injection of human factor VIII antibody into rabbits was found to provide an animal model that meets these requirements.

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