Relationships between Spleen, Platelets and Factor VIII Levels

By EUGENE P. LIBRE, DALE H. COWAN, STANLEY P. WATKINS, JR. AND N. RAPHAEL SHULMAN

Physiologic elevations of Factor VIII occur in health and disease and are either transient or sustained (Table 1).1-17 The same stressful stimuli that produce transient increases in Factor VIII, notably adrenalin or exercise, also produce transient elevations of circulating platelets.18-20 Since the platelet response is abolished by splenectomy, in the present work it was of interest to compare changes in platelet and Factor VIII levels during adrenalin infusions in individuals with and without spleens. These studies showed that Factor VIII elevations in response to adrenalin are dependent on the presence of the spleen.

Although there seems to be no common basis for chronic Factor VIII elevation in the diseases listed in Table 1, a number of the disorders are associated with thrombocytopenia. Studies on a group of patients with chronic thrombocytopenia or thrombocytosis showed that an inverse relationship frequently is present between platelet and Factor VIII levels.

In vitro attempts to demonstrate either an inverse or direct relationship between platelet and Factor VIII levels in the presence and absence of adrenalin were not successful, and transfusion of platelets into thrombocytopenic recipients did not significantly alter their Factor VIII levels.

Materials and Methods

Adrenalin Infusion

Adrenalin (hydrochloride), at a concentration of 6.6 µg./ml. in physiologic saline was administered intravenously at a constant rate by means of an infusion pump* or by intravenous drip over a 30-minute period to deliver a total dose varying from .0014 mg./kg. to .014 mg./kg. (i.e., 0.1 mg.–1 mg. in a 70 kg. individual). Since similar changes in levels of

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*Peristaltic Action Pump, American Instrument Co, Silver Spring, Md.

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Table 1.—Elevated Levels of Factor VIII

<table>
<thead>
<tr>
<th>Stressful Stimuli</th>
<th>Disease Associated</th>
<th>Sustained</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalin (1)</td>
<td>Diabetes (5)</td>
<td></td>
<td>Pregnancy (14)</td>
</tr>
<tr>
<td>Exercise (2)</td>
<td>Malignancies (4)</td>
<td></td>
<td>Progestational drugs (15)</td>
</tr>
<tr>
<td>Fever (3)</td>
<td>Thyrotoxicosis (7,8)</td>
<td>Long-term anticoagulants (6)</td>
<td></td>
</tr>
<tr>
<td>Surgery (4)</td>
<td>Coronary artery disease (9)</td>
<td>Australian Aborigines (16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cushing's syndrome (11)</td>
<td>African Bantu (17)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X-irradiation disease (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemolytic anemia (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sickle-cell anemia (13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Factor VIII and platelets were produced over the entire dose range, .0042 mg./kg. was selected as the standard dose to obtain a typical hematologic response with minimum side effects. During the infusions, the pulse rate increased an average of 40 ± 10 beats per minute. The systolic pressure increased 30 ± 15 mm Hg. and diastolic pressure decreased 25 ± 10 mm. Hg.

Assays of Clotting Factors

All blood samples were drawn and stored in plain acid-cleaned glassware. As an anticoagulant, 1 part of 1.36 M (40 percent) trisodium citrate was used to 100 parts of blood. Plasma samples were stored at −20 C. before assaying.

The Factor VIII content of plasma was determined by Pitney’s modification of the thromboplastin generation test (TGT),21 using human brain cephalin instead of platelets.22 Factor VIII concentration in units/ml. was based on a Factor VIII standard prepared by pooling plasma from 20 normal individuals. One unit of Factor VIII is the amount present in 1 ml. of normal human plasma.23

In vivo levels of Factor VIII below 3 percent were measured using a two-stage prothrombin consumption technique.24

The Factor V content of plasma samples was determined by their ability to correct the one-stage prothrombin time of Factor V-deficient aged oxalated plasma.25 The Factor VII content of plasma samples was determined by their ability to correct the one-stage prothrombin time of plasma from a patient congenitally deficient in Factor VII.26,27 The Factor IX content of serum was determined by its activity in the TGT using serum congenitally deficient in Factor IX as a diluent.28

Plasma fibrinogen was determined by measuring the Kjeldahl nitrogen content of clots,29 using a factor of 6.25 to convert nitrogen to protein. Platelets were counted by the method of Brecher and Cronkite.30

RESULTS

Effects of Adrenalin Infusion on Factor VIII and Platelet Levels

Measurements of platelet and Factor VIII levels during and after adrenalin infusion in normal and asplenic individuals are shown in Figure 1. Platelets and Factor VIII levels increased markedly in normal subjects, but only slightly or not at all in asplenic individuals. The platelet rise reached a maximum earlier (approximately 20 minutes) than did the rise of Factor VIII (approximately 30 minutes). The platelet levels returned to baseline approximately 30 minutes after the infusion ended, whereas the Factor VIII levels returned more slowly.
Fig. 1.—Effect of adrenalin on Factor VIII and platelet levels. The mean percent changes of Factor VIII and platelets observed in ten normal individuals during and after adrenalin infusions are indicated by the upper line (solid circles) in each portion of the figure. The vertical bars represent one standard deviation. The two lower lines (open circles) in the upper portion of the figure indicate the changes in Factor VIII levels in two asplenic subjects. The lower line (open circles) in the bottom portion of the figure represents the mean percent change in platelet level observed in five asplenic individuals.

Plasma levels of fibrinogen prothrombin, Factors V, VII and IX remained unchanged throughout the adrenalin infusions in both normal and asplenic individuals.

In Figure 2 are plotted the maximum absolute and percent changes from baseline of platelets and Factor VIII levels in normal individuals, patients with ITP, patients with splenomegaly and asplenic individuals given adrenalin infusions. All individuals with spleens had increases in Factor VIII and platelet levels; however, there was no correlation between the degree of increase in Factor VIII and platelets, whether on an absolute or percentage basis. For example, although three thrombocytopenic patients (open triangles) had small increases in platelets (up to 25,000/mm.³), their Factor VIII elevations were
Fig. 2.—Comparison of increases in platelets and Factor VIII. Results of adrenalin infusion in 13 normal individuals ●; three patients with ITP △; one patient with hereditary elliptocytosis □; one patient with hereditary spherocytosis ■; and six asplenic individuals ○. The single plotted point was that obtained approximately 30 minutes after the start of the infusion when increases in Factor VIII and platelets were maximum.

Similar to those of individuals whose platelets were greatly increased (75,000–150,000/mm$^3$). Asplenic individuals (open circles) showed relatively little increase in Factor VIII, compared to individuals with spleens, and frequently showed a decrease in platelets.

Strenuous exercise in a normal individual produced an absolute platelet rise of 56,800/mm$^3$ and a Factor VIII increment of 289 units/100 ml., but the same exercise in an asplenic individual did not produce a rise in platelets or Factor VIII.

Plasma obtained from two normal individuals at 8 A.M. and 8 P.M. for seven days showed no significant variation in Factor VIII levels indicating no diurnal or circadian variation.

Correlation of In Vitro Assay with In Vivo Activity

To determine whether the increased Factor VIII assayed in plasma after an adrenalin infusion had activity in vivo like normal Factor VIII, plasma collected from a normal individual before and after an adrenalin infusion was administered to a hemophilic. The increased level of Factor VIII produced by adrenalin was reflected by a proportional increase of Factor VIII in the hemophilic recipient (Fig. 3). The survival curves of Factor VIII in both instances were similar and characteristic of that factor.$^{23}$

Attempt to Reproduce In Vivo Adrenalin Effect In Vitro

The possibility that Factor VIII elevation during an adrenalin infusion might
Fig. 3.—In vivo survival in a hemophiliac of pre- and post-adrenalin Factor VIII obtained from a normal individual. The lower curve indicates the survival of Factor VIII in a hemophiliac following infusion of 10 ml. of normal plasma. The concentration of Factor VIII in this normal plasma was 95 percent by in vitro assay. The upper curve represents the survival of Factor VIII in the same hemophiliac after infusion of 10 ml. of plasma obtained from the same normal donor 30 minutes after the start of an adrenalin infusion. The concentration of Factor VIII in this post-adrenalin normal plasma was 300 percent by in vitro assay. Both curves show a 4–5 hour half-life for the first phase, which lasts approximately 8 hours, and a 10-hour half-life thereafter. These curves are typical of those obtained in other hemophiliacs by Shulman, Marder, and Hiller.24

in part represent release or elution of Factor VIII from platelets was evaluated by adding adrenalin to platelet-rich plasma in vitro, as described in Table 2. Under these experimental conditions, adrenalin did not cause significant change in the plasma Factor VIII level. Incubation at 37 C. for 60 minutes rather than 15 minutes resulted only in decrease of Factor VIII activity both in control and test mixtures.

Association of Abnormal Factor VIII Levels with Certain Disease States

Sise and co-workers reported increased Factor VIII levels in five patients with radiation-induced thrombocytopenia and also in eight patients having idiopathic or secondary thrombocytopenia. As shown in Figure 4, we have also found increased levels of Factor VIII in patients with thrombocytopenia associated with various diseases: 12 patients with chronic ITP, one with aplastic anemia, two with leukemia, and one with lymphoma. Moreover, decreased
Table 2.—Incubation of Normal Platelets with Adrenalin at 37 C. for 15 Minutes

<table>
<thead>
<tr>
<th>Adrenalin Conc. µg/ml.</th>
<th>Factor VIII Conc. units/100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>.00012</td>
<td>37</td>
</tr>
<tr>
<td>.0012</td>
<td>37</td>
</tr>
<tr>
<td>.012</td>
<td>45</td>
</tr>
<tr>
<td>.12</td>
<td>38</td>
</tr>
<tr>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>.0012 (PPP)</td>
<td>36</td>
</tr>
</tbody>
</table>

Equal parts of adrenalin in .147 M NaCl were mixed with platelet-rich plasma (PRP) prepared from citrated blood to give a final concentration of platelets in the incubation mixture of 287,500/mm.³, and final concentrations of adrenalin ranging from .00012 to 1.2 µg/ml. These concentrations of adrenalin represent 1/1000 to ten-fold the maximum in vivo concentration that would have been obtained during the standard adrenalin infusion of 4.2 µg/kg. assuming simple dilution of adrenalin in the plasma without loss. The actual concentration of adrenalin in vivo was undoubtedly much lower than that calculated on the basis of simple dilution because of its rapid disappearance from the circulation. Adrenalin at a final concentration of .0012 µg/ml. was also added to platelet-poor plasma (PPP) containing <10,000/mm.³. All tubes were incubated at 37 C. for 15 minutes after which they were centrifuged at 1000 g. for 15 minutes. The supernatant fluid was removed and frozen at −20 C. until assayed for Factor VIII. The tabulated value for Factor VIII concentration at each concentration of adrenalin is the mean of three determinations. The differences between the various incubation mixtures are within the experimental error of the assay method.

levels of Factor VIII in patients with high platelet counts were observed in two cases of idiopathic thrombocytosis and four cases of thrombocytosis secondary to chronic myelogenous leukemia (Fig. 4). This reciprocal relationship between platelet and Factor VIII levels was evident only when platelet counts were less than 30,000/mm.³, or more than 500,000/mm.³, and was not invariable. Of 20 patients with platelet counts below 30,000/mm.³, 16 had Factor VIII levels greater than 150 percent; whereas nine patients with platelet counts above 40,000, yet in the thrombocytopenic range, had normal levels of Factor VIII. Of eight patients with platelet counts above 500,000/mm.³, six had Factor VIII levels below 75 percent.

**Experimental Observations on Effects of Platelets on Factor VIII Levels**

In view of the apparent reciprocal relationship between Factor VIII and platelets, the possibility that Factor VIII might be adsorbed or its stability affected by platelets in vitro was evaluated. Normal plasma was incubated for one hour at room temperature with various concentrations of washed and unwashed normal and hemophilic platelets, as described in Table 3. There was no significant change in Factor VIII level when plasma was incubated with unwashed normal or hemophilic platelets. However, both normal and hemophilic washed platelets lowered Factor VIII. The degree of decrease in Factor VIII produced by washed platelets was the same with normal and hemophilic...
platelets and similar decreases occurred over a tenfold range in platelet concentration.

Platelet concentrates prepared from four units of whole blood in three instances and from 15 units in one instance did not significantly change the Factor VIII level when transfused into four thrombocytopenic patients. In all instances the initial platelet level was in the range of 10,000-25,000/mm³ and the initial Factor VIII level in the normal range. Factor VIII measurements were made immediately, and 1, 2, 4, and 16 hours after transfusion.

**DISCUSSION**

The transient elevation of Factor VIII level produced in vivo by adrenalin was found to be dependent on the presence of the spleen as is the transient elevation of circulating platelets produced by the same stimulus. The increases in Factor VIII and platelets were not proportional to each other on an absolute or percentage basis (Fig. 2), in vitro elution experiments gave no evidence of Factor VIII release from platelets with or without adrenalin (Table 3), and asplenic individuals with normal or increased numbers of circulating platelets did not show a Factor VIII rise in response to adrenalin. These various findings suggest that Factor VIII and platelet elevations following adrenalin are separate phenomena, although both require the presence of the spleen.

Since individuals without spleens have normal or may even have elevated levels of Factor VIII, it is evident that Factor VIII is not made exclusively in the spleen, although recently Webster et al. have presented evidence that
the spleen may be capable of synthesizing Factor VIII. Ingram found that Factor VIII elevation produced by adrenalin could be blocked by pronethalol, a drug that blocks the β adrenergic effect of adrenalin,33 and McClure et al. found that platelet elevations could be prevented by the same drug.34 Alpha adrenergic blockade with phentolamine did not prevent elevation of Factor VIII or platelets following adrenalin.33,34 These findings suggest that altered circulatory dynamics may be the basis for platelet and Factor VIII elevation following adrenalin.35 The initial suggestion by Weaver and co-workers,36 on the basis of experiments utilizing the technique of cross-circulation between normal and hemophilic dogs, that Factor VIII may be stored in the spleen remains a likely possibility; but the additional possibility that the spleen plays an intermediate role by releasing a humoral substance that acts elsewhere has not been ruled out.

Although most clotting factors are known to be made in the liver, there are a number of reports stating that Factor VIII is not made in the liver.37-39 In severe liver disease, Factors I, II, V, VII, IX and X are substantially reduced but there is no concomitant deficiency of Factor VIII. The production of hepatocellular damage in dogs by chloroform,40 and in rats by carbon tetrachloride,41 also has not been associated with decreased levels of Factor VIII, but these observations do not exclude the possibility that a small amount of functioning hepatic tissue may be capable of supporting nearly normal levels of Factor VIII. Organ removal experiments have not been conclusive because of intravascular coagulation and fibrinolysis that attends heptectomy. On the other hand, Gardikas and co-workers42 have observed that, in normal persons, the level of Factor VIII in hepatic venous blood is substantially higher than in peripheral blood. Additional studies are required for definitive identification of the site of Factor VIII production.

In view of the variety of diseases and miscellaneous conditions associated with Factor VIII elevation, a number of different mechanisms may be responsible. However, patients with several of the diseases associated with Factor VIII elevation also had thrombocytopenia, a circumstance noted previously by Sise and co-workers in radiation disease.6 In addition, we found decreased Factor VIII levels in patients with thrombocytosis. Efforts to reproduce in vitro the apparent inverse relationship between platelets and Factor VIII levels were unsuccessful (Table 3) as were those of Sise. In these experiments, the Factor VIII concentration after incubation with washed platelets tended to be lower than with unwashed platelets. Since the Factor VIII decrease with washed platelets was not dependent on platelet concentration, it does not appear to be an adsorption phenomenon but may be related in some way to platelet injury that occurs during washing.43 There was no evidence from observations in four platelet transfusions with up to 15 units of platelets that exogenous platelets are capable of lowering Factor VIII in thrombocytopenic individuals.

**SUMMARY**

(1) The spleen is essential for the Factor VIII elevations observed after adrenalin infusion and other acutely stressful stimuli. The spleen appears to be
Table 3.—Effect of Incubating Plasma with Platelets on the Level of Factor VIII

<table>
<thead>
<tr>
<th>Platelet Prep.</th>
<th>Platelet Conc./mm.³</th>
<th>Number of Determinations</th>
<th>Mean % Factor VIII Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed</td>
<td>$2 \times 10^5$</td>
<td>10</td>
<td>-27.9</td>
</tr>
<tr>
<td>Normal</td>
<td>$1.5 \times 10^6$</td>
<td>9</td>
<td>-10.6</td>
</tr>
<tr>
<td>Washed</td>
<td>$2 \times 10^5$</td>
<td>13</td>
<td>-27.6</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>$1.5 \times 10^6$</td>
<td>8</td>
<td>-33</td>
</tr>
<tr>
<td>Unwashed</td>
<td>$2.5 \times 10^6$</td>
<td>4</td>
<td>+5.7</td>
</tr>
<tr>
<td>Normal</td>
<td>$1 \times 10^5$</td>
<td>4</td>
<td>-12.2</td>
</tr>
<tr>
<td>Unwashed</td>
<td>$2 \times 10^5$</td>
<td>4</td>
<td>+10.5</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>$4.5 \times 10^5$</td>
<td>4</td>
<td>-2.7</td>
</tr>
<tr>
<td>Unwashed</td>
<td>$2.5 \times 10^4$</td>
<td>4</td>
<td>-5.2</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>$1 \times 10^4$</td>
<td>4</td>
<td>-8</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^5$</td>
<td>4</td>
<td>-18</td>
</tr>
<tr>
<td></td>
<td>$4 \times 10^5$</td>
<td>2</td>
<td>+17.5</td>
</tr>
</tbody>
</table>

To prepare washed platelets, blood from fasting normal and hemophilic individuals was collected by gravity drip into a plastic tube, using 1 part of 10% EDTA as anticoagulant for 50 parts of blood. Blood was centrifuged for 3 minutes at room temperature at 1000 g. to obtain platelet-rich plasma and for 15 minutes at 1000 g. to obtain platelet buttons. Platelets were washed three times in .147 M NaCl to remove plasma and EDTA, were resuspended in .147 M NaCl and counted. Platelet buttons obtained from saline suspensions were suspended in platelet-poor plasma with known Factor VIII content to give final platelet concentrations of $2 \times 10^5$/mm³, and $1.5 \times 10^6$/mm³. Reaction tubes were incubated for one hour at room temperature, spun for 15 minutes at 1000 g., and the supernatant was stored at -20 C. until assayed for Factor VIII. The preparation of unwashed platelets consisted of platelet-rich plasma obtained from fasting normal and hemophilic individuals, using 1.36 M (40%) trisodium citrate as anticoagulant. Platelet-rich plasma was mixed with platelet-poor plasma obtained from the same blood sample to produce final platelet concentrations of $2.5 \times 10^4$, $1 \times 10^4$, $2 \times 10^4$, $4 \times 10^4$, and $4.5 \times 10^4$/mm³. The incubation and remainder of the procedure were the same as described above for washed platelets.

(2) The Factor VIII elevations do not appear to be dependent on the concomitant platelet rises that occur after adrenalin.

(3) An inverse relationship between platelet and Factor VIII levels is frequently found in pathological states associated with chronic thrombocytopenia or thrombocytosis.

(4) Platelet transfusions do not appear to influence Factor VIII levels.

(5) In vitro attempts to demonstrate adsorption of Factor VIII by platelets or elution of Factor VIII from platelets in the presence and absence of adrenalin were not successful.

SUMMARIO IN INTERLINGUA

1. Le splen es indispensabile pro le elevationes de Factor VIII observate post le infusion de adrenalina e le application de altere acutemente stressose stimulos. Il para que le splen
es capace a thesaurisar e liberar Factor VIII o a affectuar le liberation de Factor VIII ab un altere sito.

2. Le elevationes de Factor VIII non pare depender del concomitante augmento del numeramation plachettal notate post infusiones de adrenalina.

3. Un relation inverse inter le plachettas e le nivellos de Factor VIII es frequentemente incontrate in statos pathologic associate con chronic thrombocytopenia o thrombocytosis.

4. Il pare que infusiones de plachettas exerce nulle influentia super le nivello de Factor VIII.

5. Essayos de demonstrar in vitro le adsorption de Factor VIII per plachettas o le elution de Factor VIII ab plachettas in le presentia e etiam in le absentia de adrenalina non esseva successose.

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