Heckathorn's Disease: Variable Functional Deficiency of Antihemophilic Factor (Factor VIII)

By Oscar D. Ratnoff and Jessica H. Lewis

A family is described in which a syndrome resembling moderately severe classic hemophilia was apparently inherited as an X chromosome-linked trait. In two affected individuals, the titer of functional antihemophilic factor varied dramatically from time to time, while the conversion of prothrombin to thrombin was impaired in no apparent relationship to AHF functional activity. A transfusion of 200 ml of fresh-frozen plasma did not correct the serum prothrombin times in either patient. In vitro, the additions of 10% of normal plasma or serum or washed plain or frozen platelets also did not normalize the serum prothrombin times. No inhibitor could be demonstrated in the blood of either patient. In one patient, RH, dissipation of infused cryoprecipitate AHF was abnormally slow, and, after an intensive course of transfusion of cryoprecipitate and whole blood, the titer of functional AHF remained at normal levels for at least 1 wk. The plasma of RH inhibited a human antibody against AHF in proportion to its titer of functional AHF (i.e., the defect was CRM-−) despite the presence of relatively greater amounts of antigenic material recognized by heterologous antiserum. No qualitative abnormality of the AHF-like material in RH's plasma was identified. Inheritance of the abnormality appears superficially to be X chromosome-linked; on this assumption, three of four obligate carriers of the disorder were recognized by the presence of excess amounts of AHF-like antigens relative to AHF functional activity. This coagulation disorder has been designated Heckathorn's disease and may presage the discovery of other examples of hemophilia-related syndromes.

In classic hemophilia, the functional deficiency of antihemophilic factor (factor VIII) varies from family to family, but it is relatively constant among affected individuals in any one family. Recently, we have encountered a family in which a bleeding tendency appeared to be inherited as an

From the Department of Medicine, Case Western Reserve University School of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106; the Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15261; and the Central Blood Bank of Pittsburgh, Pittsburgh, Pa. 15219.

Submitted December 13, 1974; accepted March 6, 1975.

Supported in part by NIH USPHS Grants HL 01661 and HL 02254 from the National Heart and Lung Institute and in part by grants from the American Heart Association.

Dr. Ratnoff is a Career Investigator of the American Heart Association.

Address for reprint requests: Oscar D. Ratnoff, M.D., Department of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106.

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X chromosome-linked characteristic. Unexpectedly, in two affected male members of this family, the titer of functional AHF varied dramatically from time to time. The present report describes some characteristics of this unusual disorder which, with the surviving patient's permission, we have designated by his surname as Heckathorn’s disease.

CASE REPORTS

RH (V-14),* the Cleveland proband, 34 yr old in 1974, was born with a hematoma on his scalp that required incision and drainage. He has always bruised readily after minor trauma, and sometimes spontaneously. After the loss of deciduous teeth, he bled so severely that on at least one occasion he was transfused. Three weeks after tonsillectomy, at the age of 6, he bled suddenly and required transfusion. He did not bleed during the eruption of teeth, but he has had significant bleeding after some, but not all, dental extractions. He has not bled spontaneously into joint spaces, but after jumping a fence when he was 13 yr old, he bled in and around his left hip, leading to permanent limitation of motion of this joint. At no time has the patient had epistaxis nor, until the last few months, unusual bleeding from cuts, a symptom now vexing him.

Physical examination in September 1973 demonstrated a marked flexion contracture of the left hip and a fading bruise over the right ankle.

In February 1974, the patient underwent left hip arthroplasty, under cover of cryoprecipitate and whole blood and packed red blood cell transfusions. The postoperative course was uneventful except for elevation of temperature for 5 days and a momentary episode of lower anterior midsternal chest pain on postoperative day 2. Arterial P\textsubscript{a}O\textsubscript{2}, immediately thereafter was 61 mm Hg, and the heart rate was 130/min, but no other evidences of pulmonary embolism were obtained. Cryoprecipitate therapy was interrupted for 2 days but then continued through postoperative day 10. The patient has regained satisfactory motion in the affected hip.

CL (IV-3), the Pittsburgh proband, one of the patient's three maternal uncles, exsanguinated at the age of 52, 8 days after partial gastrectomy accompanied by intractable hiccups. He bled severely after neonatal circumcision and, at age 25, after tonsillectomy; the latter episode was treated by transfusion. He had also bled excessively after cholecystectomy and after traumatic avulsion of teeth and once required transfusion after a massive epistaxis. He had a lifelong bleeding tendency after trivial injury, not dissimilar to that of RH, although he never bruised easily and underwent an operation on a leg when he was 48 without difficulty.

FAMILY HISTORY (see Fig. 2)

RW (II-2), RH’s maternal great-grandfather, was said to have had a severe bleeding tendency but died of “heart failure and dropsy.”

FL (III-7), RW’s daughter, was 75 yr old in 1974. She has always bruised easily and spontaneously and as an adult had frequent nosebleeds, often requiring packing. She had menorrhagia until hysterectomy was performed at the age of 50 because of “tumor.” She bled excessively during this operation and after each of four deliveries, after dental extractions, and after four rectal operations and an appendectomy, but was never transfused. A cholecystectomy was not followed by hemorrhage.

NH (IV-5), FL’s daughter and RH’s mother, 56 yr old in 1974, has always bruised readily and often spontaneously. She required transfusion after each of two ectopic pregnancies.

CP (V-12), RH’s older sister, 36 yr old in 1974, has always bruised readily and spontaneously. She had had menorrhagia, necessitating dilatation and curettage in 1960 and underwent hysterectomy at the age of 32, apparently because of local problems; she bled repeatedly in the weeks after surgery, requiring reoperation and transfusion. Eight hours after the delivery of her older child in 1960 the patient went into hemorrhagic shock, requiring transfusion of 5 U of blood. The patient has also had gingival bleeding, melena (attributed to

*The numbers in parentheses refer to the family tree depicted in Fig. 2.
"colitis"), and excessive bleeding from cuts, but she underwent tonsillectomy and appendectomy without difficulty.

ChP (VI-4), CP's daughter, 14 yr old in 1974, bruises easily and has had frequent nosebleeds.

RhH (VI-7), RH's older daughter, 10 yr old in 1974, bruises easily and bled excessively after she broke her arm.

TH (V1-8), RH's younger daughter 5 yr old in 1974, bruises easily.

None of the other members of RH's family is known to have a bleeding tendency.

MATERIALS AND METHODS

I. Pittsburgh Studies

Coagulation studies of CL and RH employed previously described methods35 or standard procedures.

II. Cleveland Studies

All studies conducted were performed with the blood or plasma of patient RH and his surviving relatives.

Citrated plasma of normal subjects and of RH and his relatives and a standard plasma pool of 25 normal human male subjects was prepared and stored as previously reported.6'7

The techniques used for studies of hemostatic mechanisms were described earlier,8-16 with the following modifications and additions: The clotting time of whole blood was measured at 25°C in disposable glass and polystyrene tubes (internal diameter, 11 mm). Permaplastin (Alban Scientific Co., St. Louis, Mo.), a suspension of rabbit brain thromboplastin, was used to measure the one-stage prothrombin time and serum prothrombic activity. A suspension of 50 mg of kaolin (acid-washed, NF, Fisher Scientific Co., Fairlawn, N.J.) in 5 ml of 0.1% soybean phosphatides (Centrolex “O”, the gift of Central Soya Co., Chicago, Ill.) in 0.15 M sodium chloride solution was used to measure the partial thromboplastin time and for functional assays of Hageman factor (factor XII), Fletcher factor, plasma thromboplastin antecedent (PTA, factor XI), Christmas factor (factor IX), and AHF. The presence of fibrin-stabilizing factor (factor XIII) was determined grossly by absence of dissolution of a recalcified plasma clot in 1% monochloroacetic acid within 24 hr at room temperature. Platelet aggregation was tested by the addition of adenosine diphosphate (ADP), bovine collagen (the gift of Dr. H. Bensusan, Case Western Reserve University), or ristocetin (the gift of Abbott Laboratories, N. Chicago, Ill.) at final concentrations, respectively, of 5 μg, 78 μg and 1 mg/ml of plasma that contained approximately 250,000 platelets/cu mm.17

The titer of functional AHF18 and serum prothrombic activity19 were measured as described earlier. Antigenic material related to AHF in plasma was measured by a modification20 of an earlier quantitative immunoelectrophoretic technique,7 using monospecific antihuman AHF rabbit antiserum. One unit of AHF functional activity and 1 U of AHF-like antigenic material are the amounts found in 1 ml of standard pooled human plasma; the unitages of functional and antigenic AHF should not be thought of as representing identical amounts of the agents tested.7

The effect of rabbit antiserum against AHF on AHF functional activity in plasma was studied by incubating 0.05 ml of plasma and 0.05 ml of either rabbit antiserum7 or normal rabbit serum for 1 hr at 37°C in 10 × 75-mm polystyrene tubes. Thereafter, the mixture was diluted to 1.0 ml with barbital-saline buffer and the titer of functional AHF determined immediately. Both the rabbit antiserum and normal rabbit serum had been absorbed with a fraction of normal human plasma, soluble at 3% and insoluble at 8% ethanol, adsorbed with calcium phosphate (10 mg/ml) and heated at 60°C for 1 hr.7

The presence in the patient's plasma of antigens blocking human antibodies to AHF was tested in the same manner, using untreated plasma from a patient with a circulating anticoagulant, apparently of spontaneous origin, diluted 80- or 160-fold with barbital-saline buffer; buffer, alone, was used as a control.

The presence in the patient's plasma of antigens blocking human antibodies to AHF was tested in comparison to pooled normal plasma by the method of Hoyer and Breckenridge,21 modified by diluting the normal plasma serially in hemophilic plasma devoid of cross-reacting material. The
plasma of several patients with severe hemophilia did not inhibit the circulating anticoagulant in this assay.

Gel filtration of pooled normal plasma or the patient's plasma was performed at room temperature in 0.9 x 28-cm columns of agarose (Biogel A-15, 4% agarose, Bio-Rad Laboratories, Richmond, Ca.) equilibrated with imidazole-saline buffer (0.02 M imidazole, 0.14 M sodium chloride, pH 6.5). Briefly, the 3% ethanol-insoluble precipitate of 10 ml of plasma was dissolved in 2 ml of imidazole-saline buffer and mixed with 2 ml of 20% (w/v) polyethylene glycol (average molecular weight, 6000-7500, Matheson, Coleman and Bell, Norwood, Ohio) in the same buffer. The precipitate which formed was dissolved in 1 ml of imidazole-saline buffer; a wash in 6% ethanol, used earlier, was omitted. The dissolved precipitate was applied to the column, which was eluted with the same buffer, collecting 1-ml fractions. All fractions were tested for AHF functional activity, and those possessing this activity were further examined for AHF-like antigenic material by quantitative immunoelectrophoresis.

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulfate was performed as described earlier, using 4% or 5% acrylamide gel and 2.7% cross linking. One hundred micrograms of either unreduced or reduced samples of AHF, separated by gel filtration and concentrated approximately fourfold, were applied to the gel. In one such run, the protein concentration of a normal fraction was 26 µg/ml and that of RH, 20 µg/ml. In addition to markers used earlier, human fibrinogen (fraction I-2) was tested, assuming its molecular weight to be 340,000.

Immunoelectrophoresis of 3% ethanol precipitates of plasma against rabbit anti-AHF antiserum was performed as described earlier, applying 250 V for 75 min at pH 8.4 in 0.05 M sodium barbital and at pH 6.0 in 0.04 M phosphate buffer. Immunodiffusion studies were also conducted as reported earlier.

Barbital-saline buffer was 0.025 M barbital sodium in 0.125 M sodium chloride (pH 7.5).

Protein determinations were performed by the method of Lowry, Rosebrough, Farr, and Randall.

RESULTS

Studies of Hemostatic Mechanisms in CL and RH

In 1968, CL and RH were examined at the University of Pittsburgh. Remarkably, the titer of functional AHF activity in CL's plasma varied, on four occasions, from 0.10 to 1.20 U/ml (Table 1). The prothrombin time of serum and serum prothrombic activity, which measure the rate of conversion of prothrombin to thrombin during clotting, were abnormal on all occasions, in no apparent relationship to AHF functional activity. No evidence was obtained suggesting a quantitative or qualitative defect in CL's platelets, although platelet retention by a glass-bead filter was decreased in one of three tests. The bleeding time was normal. All other tests of hemostatic function gave normal results. These included clotting time and clot retraction in glass and siliconized tubes, tourniquet test, prothrombin time, partial thromboplastin time, thrombin time, thromboplastin generation test, assays for fibrinogen (factor I), prothrombin (factor II), proaccelerin (factor V), factors VII and X, Christmas factor (factor IX), plasma thromboplastin antecedent (PTA, factor XI), Hageman factor (factor XII), fibrin-stabilizing factor (factor XIII), platelet count, and platelet aggregation with ADP and collagen.

Similar data were obtained in studies of RH (Table 1). Again, the titer of functional AHF varied dramatically, from 0.10 to 0.75 U/ml, but the prothrombin time of serum and serum prothrombic activity were consistently abnormal. The serum prothrombin time of the patient's whole blood was not lengthened by adding normal plasma, serum, or platelets (Table 2). No inhibitor could be found in either blood. The results of other tests of hemostatic function on RH were also within normal limits.
Two milliliter whole blood were added to material in glass tubes, gently mixed, and the serum prothrombin time (SPT) determined after 2 hr at 37°C.

Table 1. Titers of Functional AHF, Antigens Related to AHF, and Serum Prothrombic Activity in CL and RH

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>AHF Coagulant Activity (U/ml)</th>
<th>AHF-like Antigen Activity (U/ml)</th>
<th>Serum Prothrombic Activity (%)</th>
<th>Serum Prothrombin Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>Jan. 18, 1968</td>
<td>1.20</td>
<td>—</td>
<td>54</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Jan. 22, 1968</td>
<td>0.25</td>
<td>—</td>
<td>48</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Feb. 7, 1968</td>
<td>0.10</td>
<td>—</td>
<td>36</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>March 11, 1968</td>
<td>0.80</td>
<td>—</td>
<td>35</td>
<td>14.2</td>
</tr>
<tr>
<td>RH</td>
<td>Feb. 7, 1968</td>
<td>0.10</td>
<td>—</td>
<td>34</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>March 11, 1968</td>
<td>0.75</td>
<td>—</td>
<td>—</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>Dec. 1, 1969</td>
<td>0.50</td>
<td>—</td>
<td>—</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>Sept. 25, 1973</td>
<td>0.54</td>
<td>0.91</td>
<td>30</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Oct. 25, 1973</td>
<td>0.28</td>
<td>0.59</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Feb. 5, 1974</td>
<td>0.30</td>
<td>0.41</td>
<td>&lt; 10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Feb. 6, 1974</td>
<td>0.24</td>
<td>0.73</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>April 22, 1974</td>
<td>0.51</td>
<td>2.31</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>May 14, 1974</td>
<td>0.50</td>
<td>1.78</td>
<td>&lt; 10</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>1968–69 (Pittsburgh)</td>
<td>1.00 (0.55–1.60)</td>
<td>—</td>
<td>&lt; 10</td>
<td>&gt; 20</td>
</tr>
<tr>
<td></td>
<td>1973–74 (Cleveland)*</td>
<td>≥0.97 (0.40–2.38)</td>
<td>0.92 (0.39–2.20)</td>
<td>&lt; 25</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥0.97 (0.43–2.22)</td>
<td>0.90 (0.40–2.05)</td>
<td>&lt; 25</td>
<td>—</td>
</tr>
</tbody>
</table>

*Geometric mean and 99% confidence limits.

RH was reexamined at Case Western Reserve University in 1973 and 1974. Again, the titer of functional AHF varied widely from time to time. The concentration of antigens related to AHF was significantly higher than that of AHF functional activity on some, but not all, occasions (Table 1, Fig. 1). Serum prothrombic activity was abnormally high on one of three occasions, without correlation to the titer of functional AHF (Table 1). The clotting time of whole blood in polystyrene tubes was 300 min (normal, 164 ± 39 (SD) min). Other tests of hemostatic function, including the clotting time in glass tubes, bleeding time, thrombin time, prothrombin time, partial thromboplastin time, platelet count, clot retraction, and specific assays for prothrombin, Christmas factor, PTA, Hageman factor, and fibrin-stabilizing factor, gave normal results. Notably, platelet function as measured in a thromboplastin generation test, platelet adhesiveness to glass, and aggregation of platelets by ristocetin, ADP,

Table 2. The Effects of Added Materials on Serum Prothrombin Time of RH's Whole Blood*

<table>
<thead>
<tr>
<th>Material</th>
<th>SPT (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ml saline</td>
<td>16.2</td>
</tr>
<tr>
<td>0.2 ml normal serum</td>
<td>17.0</td>
</tr>
<tr>
<td>0.2 ml normal platelet-poor plasma</td>
<td>16.4</td>
</tr>
<tr>
<td>0.2 ml washed normal platelets, subject I</td>
<td>16.0</td>
</tr>
<tr>
<td>0.1 ml washed normal platelets, subject II</td>
<td>16.0</td>
</tr>
<tr>
<td>0.1 ml washed RH platelets</td>
<td>15.8</td>
</tr>
<tr>
<td>0.1 ml washed normal platelets (subject III), frozen and thawed 3 times</td>
<td>14.4</td>
</tr>
<tr>
<td>0.1 ml washed RH platelets, frozen and thawed 3 times</td>
<td>15.4</td>
</tr>
</tbody>
</table>

*Two milliliters whole blood were added to material in glass tubes, gently mixed, and the serum prothrombin time (SPT) determined after 2 hr at 37°C.
Fig. 1. The titer of functional AHF and the concentration of AHF-like antigens in the plasma of normal adult males (+) and in RH's plasma (O) on six occasions, none less than 2 mo after the transfusion of cryoprecipitate. The center line is the regression line for normal males, the outer two lines are the 99% confidence belt, and the other two lines are the 95% confidence belt.

and collagen was normal. A circulating anticoagulant against AHF could not be demonstrated in the patient's plasma.

Studies of Hemostatic Mechanisms in Relatives of CL and RH

The titer of functional AHF, the concentration of antigens related to AHF, and, in some cases, the bleeding time were determined in available family members by techniques in use at Case Western Reserve University (Fig. 2, Table 3). Four individuals were obligate carriers, assuming that the patients' disorder was inherited as an X chromosome-linked trait. Of these, RH's maternal grandmother, FL (III-7), was normal by laboratory test; NH (IV-5) and RhH (VI-8) had significantly more antigenic material related to AHF than AHF functional activity in plasma, data typical of carriers of classic hemophilia, and TH (VI-7) was normal, but her data fell between the 95% and 99% confidence belts for normal women by regression analysis. By discriminant analysis, TH was recognized to be a carrier. RH's father (IV-6) and his nephew (VI-4) were normal, while his sister (V-12) and her daughter (VI-5) appeared to be carriers of the trait. In all individuals tested, the bleeding time was normal.

The Effect of Transfusion on AHF Activity and Antigens Related to AHF in CL and RH

CL and RH were each transfused with 200 ml of fresh-frozen plasma in 1968. Notably, the titer of functional AHF did not rise within a 24-hr period after transfusion, but in both patients the titer of AHF was normal before the pro-
HECKATHORN'S DISEASE

Fig. 2. Pedigree of Heckathorn's disease. The carrier state in V-12 and VI-5 was demonstrated only by laboratory studies.

procedure (Table 4). Transfusion did not correct the abnormal serum prothrombin time present in either of the patients.

In anticipation of surgery, RH was transfused with 21 bags of cryoprecipitated AHF on February 6, 1974, to determine his response (Fig. 3). Immediately after transfusion, the titer of AHF activity rose from 0.24 to 0.80 U/ml, the anticipated result. During the next 14 hr, the titer of functional AHF fell to 0.45 U/ml, as if the half-disappearance time of the infused material were approximately 12 hr. Twenty-four hours after transfusion, however, the titer of functional AHF was essentially unchanged. Antigens related to AHF remained at high levels throughout the period of observation.

On February 8, 1974, RH underwent arthroplasty. Between this date and February 18, 1974, he was transfused with a total of 165 bags of cryoprecipitated AHF, 2 liters of whole blood, and approximately 750 ml of red blood

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Table 3. Studies of Hemostatic Mechanisms in Family Members

<table>
<thead>
<tr>
<th>Patient</th>
<th>Carrier by Test</th>
<th>AHF Coagulant Activity (U/ml)</th>
<th>AHF-like Antigen (U/ml)</th>
<th>Bleeding Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL*(III-7)♀</td>
<td>-</td>
<td>0.78</td>
<td>1.00</td>
<td>5.5</td>
</tr>
<tr>
<td>NH*(IV-5)♀</td>
<td>+</td>
<td>0.66</td>
<td>1.52</td>
<td>4</td>
</tr>
<tr>
<td>WH(IV-6)♂</td>
<td></td>
<td>1.48</td>
<td>1.10</td>
<td>-</td>
</tr>
<tr>
<td>CP(V-12)♀</td>
<td>+</td>
<td>0.72</td>
<td>1.23</td>
<td>5</td>
</tr>
<tr>
<td>RP(VI-4)♂</td>
<td></td>
<td>1.22</td>
<td>1.11</td>
<td>4.5</td>
</tr>
<tr>
<td>ChP(VI-5)♀</td>
<td>+</td>
<td>0.84</td>
<td>1.44</td>
<td>-</td>
</tr>
<tr>
<td>TH*(VI-7)♀</td>
<td>-†</td>
<td>0.75</td>
<td>1.19</td>
<td>-</td>
</tr>
<tr>
<td>RhH*(VI-8)♀</td>
<td>+</td>
<td>0.44</td>
<td>0.80</td>
<td>-</td>
</tr>
</tbody>
</table>

*Carriers by history.
†Positive at 95% level of certainty, negative at 99% level.
Table 4. The Effect of Transfusion of Fresh-frozen Plasma on the Titer of Functional and AHF*

<table>
<thead>
<tr>
<th>AHF Functional Activity</th>
<th>CL (U/ml)</th>
<th>RH (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before transfusion</td>
<td>0.80</td>
<td>0.75</td>
</tr>
<tr>
<td>1 hr after transfusion</td>
<td>0.75</td>
<td>0.90</td>
</tr>
<tr>
<td>4 hr after transfusion</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>24 hr after transfusion</td>
<td>0.70</td>
<td>0.65</td>
</tr>
</tbody>
</table>

*One unit (200 ml) of fresh-frozen plasma was transfused intravenously 90 min after the pretransfusion assay.

cells. No studies of functional AHF activity or AHF-like antigen were performed during this period, but beginning 1 day thereafter daily measurements were obtained (Fig. 4). Surprisingly, during the succeeding week, the titer of functional AHF remained at normal levels. Indeed, 3 and 4 mo later the titer of functional AHF was still 0.51 and 0.50 U/ml, and of antigenic material related to AHF, 2.31 and 1.78 U/ml, respectively (Table 1).

Some Properties of the Antihemophilic Factor-like Agent in RH's Plasma

Studies of the AHF-like agent in RH's plasma, performed on samples obtained in April and May 1974, did not demonstrate any qualitative difference compared to similar material in hemophilic plasma. Like normal AHF, functional AHF in RH's plasma was inactivated both by human and rabbit antibodies directed against this factor. RH's plasma also blocked the inhibitory properties of human antibodies against AHF, using the technique of Hoyer and Breckenridge, in rough proportion to his titer of functional AHF (Table 5). Thus, in all probability, RH is CRM-.

Functional AHF was excluded from agarose columns in a manner similar to normal functional AHF, and antigenic material related to AHF was present in maximal concentration in the same fractions as functional AHF. AHF-like material, prepared in this way, was subjected to polyacrylamide-gel electrophoresis in 4% acrylamide in the presence of sodium dodecyl sulfate. Upon staining, a dark band was visible at the top of the gel column at the same point as a some-
what lighter band in columns to which a similar fraction of normal AHF was applied; the molecular weight of this band was estimated to be greater than approximately 350,000, the upper limit of resolution of the gel. This large-molecular-weight material contained antigens related to AHF, as determined by quantitative immunoelectrophoresis. Upon reduction with β-mercaptoethanol and electrophoresis upon 5% acrylamide gels, a major band of stainable material was observed at a location estimated to represent agents with a molecular weight of approximately 240,000, not significantly different from the reduced fragment of normal or hemophilic individuals.22 A single line of identity formed upon immunodiffusion of 3% ethanol precipitates of normal plasma, that of a patient with classic hemophilia, and that of RH, tested against rabbit antiserum against human AHF. An antiserum to normal AHF also detected antigens of similar anodal mobility upon immunoelectrophoresis of 3% ethanol precipitates of normal and RH plasmas, both at pH 8.4 and pH 6.0.

In short, except that the RH plasmas tested contained relatively more anti-

Table 5. Cross-reacting Material in RH's Plasma*

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Plasma AHF</th>
<th>Neutralization Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Functional Activity (U/ml)</td>
<td>Antigen (U/ml)</td>
</tr>
<tr>
<td>Normal, undiluted</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Normal, 50%</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Normal, 12.5%</td>
<td>0.125</td>
<td>—</td>
</tr>
<tr>
<td>RH, undiluted</td>
<td>0.50</td>
<td>1.78</td>
</tr>
</tbody>
</table>

*In 12 × 75-mm polystyrene tubes, 0.025 ml plasma from a patient with a circulating anticoagulant (diluted 80-fold with barbital-saline buffer) was incubated at 37°C for 30 min with 0.3 ml of either the patient's plasma or pooled normal plasma (diluted serially with hemophilic plasma). The hemophilic plasma had no detectable AHF functional activity nor antigenic material cross-reacting with the circulating anticoagulant. Thereafter, 0.1 ml of pooled normal plasma was added to each tube, and incubation was continued for an additional 60 min. The contents of each tube were diluted to 1.0 ml (i.e., 20-fold with respect to the second addition of normal plasma) and assayed for residual functional AHF in comparison to the functional AHF titer of a mixture of 0.525 ml of buffer and 0.1 ml pooled normal plasma, incubated at 37°C for 1 hr and then diluted serially with buffer before testing. The relative concentration of neutralizing antigen (cross-reacting material) in RH's plasma was determined by interpolation of its residual AHF functional activity into a semilogarithmic plot of the residual AHF functional activity of normal plasma.
genic material than functional AHF, no qualitative abnormalities of AHF were demonstrable.

DISCUSSION

At least three hereditary disorders have been discerned in which functional AHF activity in plasma is decreased below normal. Probably the most common is classic hemophilia, in which plasma appears to be deficient in functional AHF but contains normal amounts of antigenic material detected in functional assays\(^7,26\) and by immunologic\(^7,27\) and physicochemical\(^28\) techniques. In von Willebrand's disease, recognized less frequently than classic hemophilia, the titer of functional AHF is often decreased below normal, and the concentration of antigens detected by an antibody directed against purified preparations of AHF is decreased in proportion to functional activity; in some instances, relatively less antigenic material appears to be present, but assays for antigen are unreliable when this material is present in low concentrations.\(^7\) Additionally, von Willebrand's disease is distinguished from classic hemophilia by its autosomal recessive inheritance, the presence of an abnormally long bleeding time, decreased retention of platelets by columns of glass beads, impaired aggregation of platelets by ristocetin, and a paradoxical increase in functional AHF activity maximal about 6–8 hr after transfusion of normal or hemophilic plasma or plasma fractions. In a third, rare form of functional AHF deficiency, concomitant functional deficiency of proaccelerin (factor V) is detected; in one such patient, the concentration of antigenic material related to AHF was normal.\(^7\)

Classic hemophilia encompasses a heterogeneous group of disorders. In different families, the concentration of functional AHF differs in rough proportion to the clinical severity of the disease;\(^1,12\) within a given family, the degree of the defect measured in the laboratory is usually similar among different affected individuals. We have observed no deviations from this rule within families with severe classic hemophilia. In nine families with mild or moderately severe hemophilia in whom more than one affected individual was tested, the titer of functional AHF was closely similar within the same family. In a tenth family, however, the titer of functional AHF varied among six affected individuals from 0.16 to 0.48 U/ml in rough proportion to the subjects' ages; in each case, the diagnosis of classic hemophilia was confirmed by the presence of excessive amounts of antigenic material related to AHF relative to functional activity, and the bleeding time was normal, while obligate carriers were readily identified in the laboratory.

Hoyer and Breckenridge\(^29\) and Denson\(^30\) described a second type of heterogeneity. The plasma of most patients with hemophilia, said to be CRM\(-\), does not inhibit the activity of human antibodies directed against AHF. In a minority of families, however, plasma has the property of inhibiting such antibodies, as if it contained immunologically cross-reacting material; such patients are said to be CRM\(+\).

The present paper suggests a third type of heterogeneity among individuals in whom functional deficiency of AHF appeared to be inherited in an X chromosome-linked manner. In patients with severe classic hemophilia, the titer of
functional AHF is invariably less than 0.01 U/ml on repeated determination. Among 20 individuals with mild or moderately severe classic hemophilia, members of 11 different families, in 18 the titer of functional AHF was stable over periods as long as 12 yr. In one patient, the titer was 0.12, 0.12, and 0.03 U/ml on three determinations within 5 yr. In the 20th patient, who may well have had the disorder described in the present report, the concentration of functional AHF varied from 0.12 to 0.34 U/ml over a period of 5 yr.

The patients described in the present report, in contrast, displayed great variability in the concentration of functional AHF from time to time. The two patients, uncle and nephew, had symptoms suggestive of moderately severe classic hemophilia, and the uncle’s maternal grandfather was said to be similarly afflicted. Unexpectedly, the concentration of functional AHF varied dramatically from time to time in the affected patients; it was detected independently in the surviving nephew in two different laboratories using somewhat different techniques. In both affected individuals, the conversion of prothrombin to thrombin during clotting appeared to be abnormally slow even at times when titers of functional AHF were within normal limits. This defect in prothrombin consumption was not corrected by infusions of small amounts of plasma.

Additional evidence for the uniqueness of the patient’s disorder was derived from studies of the effect of transfusion of cryoprecipitated AHF. After a trial infusion of cryoprecipitates into one of the patients, functional activity identified as AHF rose immediately to the predicted level and then appeared to dissipate rapidly with a half-disappearance time of 12 hr, the usual finding in classic hemophilia. After 12 hr, however, the decline in functional AHF stopped. Although this might have been interpreted as an error in the assay for functional AHF, after an intensive course of cryoprecipitate and whole blood necessitated by a surgical procedure, functional AHF remained at a normal level for at least 1 wk and even 4 mo later was significantly higher than before surgery.

Differentiation of the patient’s disorder from von Willebrand’s disease is based upon many criteria. The bleeding time in the affected individuals was normal, retention of platelets by columns of glass beads was usually normal, in one individual aggregation of platelets by ristocetin was normal, and the paradoxical rise in functional AHF which follows transfusion of normal or hemophilic plasma in patients with von Willebrand’s disease was not observed. Further, the concentration of antigenic material related to AHF was usually relatively higher than functional AHF activity in RH, a finding characteristic of classic hemophilia. Assuming that the disorder is inherited in this family as a X chromosome-linked characteristic, four obligate female carriers were tested. In three, relatively more AHF-like antigen than AHF functional activity was detected in plasma (VI-7). In the fourth (III-7), 75 yr old at the time she was tested, AHF-like antigens and functional activity were normal and in proportion.

Using the relatively crude methods presently available, no qualitative difference was detected between the AHF-like agent in RH’s plasma and that of normal individuals. RH’s plasma appeared to be CRM−, that is, it inhibited a human antibody to AHF in proportion to its titer of functional AHF and not its content of antigens recognized by heterologous antiserum.
This unusual family emphasizes the heterogeneous nature of the syndrome classified as hemophilia. Until a more rational nomenclature can be devised, it seems appropriate to designate this disorder, possibly a variant form of hemophilia, as Heckathorn’s disease. If experience is a guide, still other examples may soon be forthcoming.

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