Dominant Inheritance of Hemophilia A in Three Generations of Women

By John B. Graham, Emily S. Barrow, Harold R. Roberts, William P. Webster, Philip M. Blatt, Philip Buchanan, A. I. Cederbaum, J. P. Allain, D. A. Barrett, and H. R. Gralnick

A bleeding diathesis is described which is phenotypically indistinguishable from hemophilia A and which has been transmitted as a dominant trait in three generations of women in a North Carolina kindred. The abnormal phenotype is characterized by clinical mildness and slightly abnormal clotting time, prothrombin consumption, and partial thromboplastin time. Bleeding time, platelet count, clot retraction, tourniquet test, and prothrombin time are normal. Concentrations of factors I, II, V, VII, IX, X, and XII are normal, while factor VIII activity is reduced to 2%-5% of control values. De novo synthesis of factor VIII does not occur after transfusion; factor VIII-related antigen is normal; patients' plasmas aggregate platelets normally in the presence of ristocetin, and a typical protein pattern is seen when a chymotryptic digest of cryoprecipitate of the proband is examined by SDS-polyacrylamide gel electrophoresis. Six possible genetic explanations are entertained. Balanced X-autosomal translocation of hemophilia A heterozygotes has been excluded by cytogenetic analysis of metaphase chromosomes. Classic von Willebrand's disease (vWD) is probably excluded on the basis of the laboratory data, and extreme lyonization of hemophilia A heterozygotes on probabilistic grounds. The genetic possibilities which cannot be excluded include a previously unrecognized variant mutation at the vWD locus, a dominant mutation at the hemophilia A locus on the X chromosome, and dominant mutation at a hypothetical fourth locus involved in factor VIII synthesis and control.

Evidence acquired from a variety of sources suggests that the genetic control of blood coagulation factor VIII is very complex. Population geneticists have shown that the distribution of plasma factor VIII levels within the normal population is consistent with the activity of a polygenic system and that the levels within and between members of hemophilia A families are consistent with the existence of multiple alleles at the hemophilia A locus on the X chromosome. Family studies have shown that coagulant factor VIII activity is reduced in some kindreds as a result of mutation at a locus on an autosomal chromosome (von Willebrand's disease), conclusive genetic evidence that at least two loci are involved. The existence of persons having a combined defect of factors V and VIII, a phenotype radically different from all others, limited to a single sibship and apparently inherited as an autosomal recessive trait, implies that at least a third locus is involved in the control of factor VIII. Barrow and Graham have emphasized that the genetic situation is probably
even more complex, because kindred with factor VIII defects exist whose patterns of inheritance differ from the patterns described above. These genetic complexities, plus the evidence concerning molecular variants of factors I, VIII, IX, and X have forced the blood coagulation establishment to develop additional genetic nomenclature to accommodate the new knowledge. Thus the nomenclaturists have recommended that coagulation deficiency phenotypes in whom abnormal products are demonstrated (i.e., which are CRM\(^+\)) be regarded as phenotypes produced by rs alleles, while the purely deficient phenotypes (i.e., CRM\(^-\)) be regarded as the fruit of aa\(^-\) alleles, etc.

The purpose of this communication is to describe a unique kindred of women transmitting factor VIII deficiency. Their abnormal phenotype is indistinguishable from hemophilia A and has been transmitted for three generations as a dominant characteristic. We shall indicate the mechanism which might be responsible for this hereditary pattern, describe our efforts to resolve the dilemmas posed by this family, and emphasize the importance of reporting such ambiguous phenotypes and kindred.

**MATERIALS AND METHODS**

Blood was obtained by venipuncture on numerous occasions from the proband and on at least one occasion from her mother, daughter, granddaughter, and other available members of her family. The earlier samples were obtained by a two-syringe technique wherein the blood in the first syringe was discarded and the blood in the second was mixed with 3.2% sodium citrate in a ratio of 8:1. In recent years, 4.5-ml samples of blood have been collected by the vacutainer method into 0.5 ml of 3.8% sodium citrate. In both instances, the citrated blood was centrifuged immediately at 1000-2000 g, the plasma pipetted into 9-mm glass tubes, and the plasma either tested within 1 hr or frozen and stored at \(-20^\circ\)C or lower for subsequent analysis. Plasma samples shipped to Connecticut and Sweden for antigen determination were quickly frozen at \(-70^\circ\)C after centrifugation of the citrated blood and were transported in containers filled with solid CO\(_2\).

The proband was plasmapheresed in July 1974 using the Fenwal system, and approximately 250 ml of her plasma containing 50 ml of 4% sodium citrate was quickly frozen at \(-70^\circ\)C, then shipped in solid CO\(_2\) to the NIH for study of her cryoprecipitate.

Peripheral blood for preparation of lymphocyte cultures was obtained using heparinized syringes, and the microcultures which were established immediately were incubated at 37°C for 3 days using a modification of the usual Moorhead technique. Cells were harvested from the cultures after 3 days of incubation, and air-dried slides were prepared for examination of the chromosomes by staining with Giemsa using a modified Seabright technique.

Tests of hemostasis were done by the methods in use at the Clinical Coagulation Laboratory of the North Carolina Memorial Hospital, most of them have been described elsewhere. Assays for plasma levels of factors V, VIII, IX, and XII were carried out using partial thromboplastin-type assays in which the correctable ability of the test plasma was compared with that of a standard, pooled normal control plasma using a specifically defective plasma as substrate. Factor VIII level was also assessed by a modified TGT procedure. Concentrations of factors VII and X were assessed by comparing the corrective effect of the test plasma with a pooled normal control plasma against a specifically deficient substrate plasma using a standard prothrombin time test.

Cryoprecipitates were prepared at the NIH from plasmas of single-unit plasmaphereses performed on 13 normal individuals, three patients with X-linked hemophilia, and the proband of this family. The blood was collected in plastic bags containing 1/9 volume 4% sodium citrate (Fenwal Laboratories, Morton Grove, Ill.), and after centrifugation at 5000 g at \(4^\circ\)C for 15 min, the plasma was removed and respun. The cell-free plasma (240 ± 20 ml per 1 U of blood) was then frozen at \(-30^\circ\)C. After thawing overnight at \(4^\circ\)C, the cryoprecipitate was centrifuged, recovered, and dissolved in supernatant plasma in a final volume of 11-12 ml. The solution of cryoprecipitate was tested immediately. It was digested at \(24^\circ\)C with alpha-chymotrypsin (Worthington
Biochemical Corp., Freehold, N.J.) which had been dissolved just before use in 0.001 M hydrochloric acid at a concentration of 10 mg/ml. Initially, 0.250 ml of chymotrypsin was added to the cryoprecipitate to a final concentration of 205 μg/ml of cryoprecipitate. Each subsequent addition of 0.03 ml, made at 15-30-min intervals, amounted to a final enzyme concentration each time of 25 μg/ml of cryoprecipitate. After treatment, cryoprecipitate was filtered through a 2.5 x 40-cm Pharmacia column packed with Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). The columns were eluted, at a pressure of approximately 20 cm of H₂O and temperature of 4°C, with a buffer containing 0.05 M Tris (Mann ultra-pure) and 0.1 M NaCl adjusted with HCl to pH 7.35. The flow rate of the column averaged 14 ml/hr, and 1.6-1.8-ml fractions were collected. Protein was monitored by absorbance at 280 nm, and protein concentration was measured by the method of Lowry et al. The void volume of the 2.5 x 40-cm column was 48 ml. Similar eluates were collected from columns receiving digested cryoprecipitates from normal, hemophilic, and the proband's plasmas. Polyacrylamide-gel electrophoresis was performed on the collected fractions as previously described.

The levels of factor VIII-related antigen were assessed in three laboratories by different techniques. The ability to neutralize a human anti-factor VIII inhibitor was determined in Chapel Hill by the method described by Barrow and Graham. The ability of two of the abnormal plasmas to precipitate with a rabbit anti-factor VIII antibody was compared with normal controls by Dr. Lars Holmberg using the Laurell method. The plasmas of the three affected females were compared with plasmas of normal controls by Dr. Leon Hoyer using a radioimmunoassay.

The ability of the whole plasma of the proband and her daughter to cause the aggregation of fixed, washed human platelets in the presence of ristocetin was examined by a method recently described. The location of the ristocetin-aggregating factor in the plasma of the proband was further defined by gel filtration. Ten milliliters of plasma from a normal control, a patient with hemophilia A, and the proband were filtered separately through a Bio-Gel A-15 M column (2.5 x 40 cm) using a Tris-HCl buffer, 0.05 M, pH 6.8, containing 0.15 M NaCl. Three protein peaks were eluted in each instance. Samples from each peak of each plasma were pooled and tested separately in the ristocetin platelet aggregation test using agglutination of fixed, washed platelets as the end point.

RESULTS

Medical Histories of Pertinent Members of the "Be" Kindred

The proband, VIII-2, was born in 1927 and was first seen in Chapel Hill by Dr. Jessica Lewis in January 1954 at age 27 because of prolonged bleeding following tooth extraction. She was next seen in 1960, at age 33, by Dr. John Graham while being treated at the North Carolina Memorial Hospital for hematuria. Since 1960 she has been treated in our hospital on four occasions by Dr. Harold Roberts, who has also followed her condition between admissions in the outpatient clinic. Her dental problems have been dealt with when necessary by Dr. W. P. Webster.

The patient has suffered since childhood from excessive bleeding. This problem was first noted when she began cutting deciduous teeth, and her childhood was characterized by numerous bruises and free bleeding from minor cuts. She bled excessively when teeth were extracted and bled for 4 wk after tonsillectomy at age 15. While she did not bleed excessively at childbirth, she bled for 3 mo following dilation and curettage, an episode which required 17 U of whole blood and 1 U of plasma. Following the extraction of a third molar at age 27, she required several blood transfusions. On May 23, 1967, she was treated at North Carolina Memorial Hospital for relief of a nasal obstruction which had been caused by a blow to the nose 5 mo previously. After preparation for surgery with 2 U of cryoprecipitate, her left turbinate was electrocoagulated with-
out difficulty. She was readmitted to the hospital on November 12, 1968, with hemorrhia, which ceased after receipt of 17 bags of cryoprecipitate during a 3-day period, most of it on the day of admission. Her latest admission was on January 4, 1971, again for hemorrhia which ceased after administration of 80 bags of cryoprecipitate.

The proband has been doing well since her last admission, regulating her menses with Ovral and only rarely experiencing breakthrough bleeding. She has never had hemarthrosis nor gastrointestinal bleeding, and there is no limitation of motion of her joints.

The mother of the proband, VII-1, was born in 1898 and is living and well. She has no history of excessive bleeding, even though she has been involved in several accidents and operations, and has never required transfusion. Physically traumatic events in her life have included childbirth three times, fracture of both bones of the right lower leg, cholecystectomy in 1940, removal of leiomyomata of the uterus in 1942, removal of a small tumor of one hip in 1966, and multiple dental extractions.

The father of the proband, VII-2, was born in 1892 and died in 1964 of carcinoma of the prostate. He was admitted several times to hospitals of the Veterans Administration at Salisbury and Durham, N. C., where a history of impaired hemostasis was never obtained and excessive bleeding was never observed. A prothrombin time performed in 1958 was recorded as 100% of control, and he had bladder cystography in 1958 without hemorrhage. The cystography was followed by transurethral prostatectomy, bleeding from which cleared in a few days, the catheter having been removed without excessive bleeding on postoperative day 6. A right inguinal hernia was surgically repaired in 1962, and bilateral orchidectomy was performed in 1963 for therapy of the prostatic carcinoma. Neither were accompanied by excessive bleeding.

The daughter of the proband, IX-1, was born in 1946 and has evidenced a slight tendency toward excessive bleeding, but much less than her mother, although her factor VIII level is almost as low. She underwent tonsillectomy at age 6 and appendectomy at age 9 without difficulty and gave birth to a daughter (X-1) at age 26 without requiring replacement therapy.

The granddaughter of the proband, X-1, was born in 1972. She has had a normal infancy and has shown no evidence of a bleeding tendency.

Laboratory Data

A complete battery of tests was carried out on the proband, VIII-2, several of them many times, and typical observations are recorded in Table 1. It will be noted that her whole-blood clotting time was slightly prolonged, and prothrombin consumption was slightly impaired. The partial thromboplastin time was clearly prolonged, presumably due solely to reduction of factor VIII, since assays of the other coagulant factors showed values in the normal range.

The bleeding time was normal on all of many occasions, and the tourniquet test was negative; platelets have been present in normal numbers and have been fully effective in clot retraction. Factor VIII levels have ranged from less than 2% to as high as 4% of control during the 20 yr of observation, except after transfusion, and it does not seem to matter whether the substrate plasma for
Table 1. Laboratory Data on Pertinent Members of the "Be" Kindred

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
<th>Proband (VIII-2)</th>
<th>Proband's Daughter (IX-1)</th>
<th>Proband's Granddaughter (X-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood clotting time (min),</td>
<td>8-10/25-30</td>
<td>13.5/45; 9; 12;</td>
<td>10.5/69</td>
<td></td>
</tr>
<tr>
<td>glass/silicone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin consumption (%)</td>
<td>&gt;80</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>consumed in 1 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial throm. time (secs),</td>
<td>50-80</td>
<td>157/77/86</td>
<td>121/57/62</td>
<td></td>
</tr>
<tr>
<td>subject/control/mixture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding time (min) (Duke)</td>
<td>2-4</td>
<td>2; 2.5</td>
<td>3.5; 4.5</td>
<td></td>
</tr>
<tr>
<td>Tourniquet test</td>
<td>Negative</td>
<td>Neg. x 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count x 10^9</td>
<td>150-450</td>
<td>312; 178</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clot retraction</td>
<td>Good</td>
<td>Good</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin time (sec),</td>
<td>11.5-14</td>
<td>11.5/12</td>
<td>12.2/12</td>
<td>12.7/12</td>
</tr>
<tr>
<td>subject/control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clotting factor assays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor I (fibrinogen)(mg/100 ml)</td>
<td>200-400</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor II (prothrombin)</td>
<td>350-500</td>
<td>450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Iowa units)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor V (proaccelerin) (%)</td>
<td>50-150</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VII (proconvertin) (%)</td>
<td>50-150</td>
<td>66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIII (AHF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>one-stage (%)</td>
<td>50-150</td>
<td>&lt;2</td>
<td>4; 5</td>
<td>3; 2</td>
</tr>
<tr>
<td>two-stage (%)</td>
<td>50-150</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor IX (Christmas)%</td>
<td>50-150</td>
<td>110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor X (Stuart)%</td>
<td>50-150</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor XII (Hageman)%</td>
<td>50-150</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIII-related antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neut. of human inhibitor (%)</td>
<td>50-150</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ppt. of rabbit anti-VIII (%)</td>
<td>50-150</td>
<td>76</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>Radioimmune, rabbit Ab. (%)</td>
<td>50-150</td>
<td>72</td>
<td>46</td>
<td>67</td>
</tr>
<tr>
<td>Ristocetin aggregation of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal platelets (%)</td>
<td>50-150</td>
<td>66</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

Unless otherwise indicated, laboratory data were collected in the Clinical Coagulation Laboratory of the N. C. Memorial Hospital, Chapel Hill.

*Per cent of pooled normal control.
†Laboratory of J. B. Graham and E. S. Barrow. Highly potent inhibitor from male with hemophilia A used.
§Laboratory of L. Holmberg, Malmo, Sweden.
‖Laboratory of L. Hoyer, Farmington, Conn.
| Laboratory of J.-P. Allain, Chapel Hill and Paris.

the factor VIII assay has been activated with kaolin prior to use. Almost identical factor VIII values were obtained on the same sample with assays of the one- and two-stage types. The subject's plasma contains factor VIII-related antigen in full amount, as assessed in three different systems, and her plasma aggregates platelets normally in the presence of ristocetin.

Filtration of the proband's plasma through Bio-Gel A-15 M showed that her ristocetin-aggregating capacity is equal to that of a normal control and a man with hemophilia A (Table 2). In all three subjects, more than 85% of the activity was present in the void volume.

Since the proband (and her daughter and granddaughter) can be presumed
on genetic grounds to be heterozygous for a mutant gene (see below), an attempt was made to detect an abnormality of the factor VIII-like protein in her plasma. Experiments showed that the protein elution pattern of her cryoprecipitate on Sepharose 4B could not be distinguished from that of cryoprecipitates prepared from normals or patients with X-linked hemophilia A. Specifically, a small protein peak with factor VIII activity eluted at the void volume of the normal cryoprecipitate (O.D. 280, 0.169 ± 0.058, mean ± 1 SD, n = 13). The peak protein in the void volume of the proband's cryoprecipitate was 0.113. This small protein peak was followed by return of the protein concentration of ensuing column fractions nearly to base line, followed by an abrupt rise in protein content as the majority of the cryoprecipitate proteins eluted from the column. A very small amount of procoagulant activity was found in the void volume fraction of the proband, with a peak factor VIII activity of 3% (normal, 20%-40%). (Male patients with hemophilia of the X-linked variety have had factor VIII levels which varied from 1% to 4%). When the column fractions from the proband's cryoprecipitate were pooled, concentrated, and subjected to polyacrylamide-gel electrophoresis, a protein was found in her void volume fraction which did not enter a 5% polyacrylamide gel in the presence of sodium dodecyl sulfate, an identical gel pattern being observed with cryoprecipitates

Table 2. Recovery of Ristocetin-aggregating Factor From Bio-Gel A-15 M Eluates of Plasma

<table>
<thead>
<tr>
<th>Type of Plasma</th>
<th>Peak 1 (V0)</th>
<th>Peak 2</th>
<th>Peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.22</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>0.23</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Proband</td>
<td>0.17</td>
<td>0.03</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*One unit is amount of ristocetin-aggregating factor in 1 ml of plasma.

Fig. 1. Factor VIII levels of the proband following administration twice of two bags of the cryoprecipitate from normal plasma. Infusions were done 10 hr apart.
of normals and X-linked hemophiliacs. After reduction of the fractions from each genotype, and electrophoresis, a single band was seen in each, and each band had an estimated molecular weight of 235,000.

The response of the proband to transfusion of normal cryoprecipitate was examined carefully to assess the possibility that the hemophilic phenotype might be a variant of von Willebrand's disease. In preparation for electrocoagulation of her left turbinate in 1967, she was given two bags of cryoprecipitate followed by two additional bags 10 hr later. The factor VIII levels following each transfusion are shown in Fig. 1. It will be noted that each time there was an immediate, maximal rise in plasma factor VIII and that the factor VIII level diminished rapidly after transfusion, with a half-time of about 8 hr.

Only selected laboratory tests were carried out on the proband's daughter (IX-1) and her granddaughter (X-1). These are also shown in Table 1, and it will be noted that there are no exceptions to the conclusion that the daughter and granddaughter have the same phenotype as the proband, i.e., CRM*, factor VIII deficiency.

**Genetic Studies**

**History of the “Be” kindred.** The “Be” kindred (Fig. 2) is of predominantly German origin, and the family has resided in the counties of Cabarrus and Rowan, North Carolina, since at least 1727 when Johannes “Pe” emigrated from the Rhineland Palatinate. The origins and dates of emigration of the “Be” segment of the kindred are less certain, but the surname has been listed in the

<table>
<thead>
<tr>
<th>GENERATION</th>
<th>NUMBER OF MEMBERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>(2)</td>
</tr>
<tr>
<td>II</td>
<td>(4)</td>
</tr>
<tr>
<td>III</td>
<td>(11)</td>
</tr>
<tr>
<td>IV</td>
<td>(9)</td>
</tr>
<tr>
<td>V</td>
<td>(10)</td>
</tr>
<tr>
<td>VI</td>
<td>(26)</td>
</tr>
<tr>
<td>VII</td>
<td>(58)</td>
</tr>
<tr>
<td>VIII</td>
<td>(87)</td>
</tr>
<tr>
<td>IX</td>
<td>(97)</td>
</tr>
<tr>
<td>X</td>
<td>(3)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>(307)</strong></td>
</tr>
</tbody>
</table>

**Fig. 2.** The “Be” kindred transmitting dominant hemophilia A. Females are represented as circles, males as squares; abnormal females are shown as solid circles. Only the portions of the pedigree pertinent to the question of consanguinity of the parents of the proband and her descendants are shown. The proband is indicated by the arrow.
censuses of these counties since at least 1820. Although all the surnames have now been anglicized, the surnames, "Pe," "Be," "Dr," and "Fo" are almost certainly of Germanic origin.

Some information was obtained on 307 members of the kindred distributed over ten generations—162 males and 140 females, the sex of five being unknown. This sex ratio is not different from unity since $\chi^2 = 0.94$, $p > 0.30$. There was no history of a bleeding tendency among either the men or the women of the family prior to the eighth generation, and only the female proband (VIII-2) has clearly suffered from a hemorrhagic diathesis.

A systematic search was made to discover whether the father and mother of the proband were genetically related, since both bore the surname "Be," and homozygosity for an autosomal recessive trait is a possible mode of inheritance. All of the ancestors of both parents of the proband were identified for the preceding three generations, and a common ancestor was not discovered. Nevertheless, her parents probably shared a common ancestor in an earlier generation because of the small number of the original German settlers and the coincidence of surnames.

Coagulant factor VIII assays were done on all of the close relatives of the proband who could be found and would cooperate, an especial effort being made to test all men. Factor VIII levels on 12 members of the kindred, including the three affected women, are shown in Fig. 3. It will be noted that all except the proband and her descendants, including four males, have normal factor VIII levels.

Cytogenetic studies. Detailed chromosome analyses were performed for a specific reason bearing upon the inheritance of hemophilia in women. Dominant hemophilia A might conceivably have occurred in three generations of fertile females heterozygous for X-linked hemophilia A if they possessed and had transmitted a balanced translocation between an X chromosome bearing

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**Fig. 3.** The plasma factor VIII levels of the close relatives of the proband are indicated by the numbers below the pedigree symbols. Females are represented by circles, males by squares, and affected females by solid circles. The left marginal Roman numerals representing generations and the Arabic numerals within the solid circles and representing individuals are identical with those in Fig. 2. The proband is again indicated by the arrow.
X-CHROMOSOMES OF 3 HEMOPHILIC WOMEN

Fig. 4. The X chromosomes of the proband (VIII-2), her daughter (IX-1), and her granddaughter (X-1), all of whom have factor VIII levels less than 5% of normal. The chromosomes have been stained with Giemsa, and a single G-band is present in the short arms, and two G-bands are present in the long arms of all of them. All three women were 46, XX, and no abnormal chromosomes or bands were present.

the hemophilia allele and an autosomal chromosome, a genetic mechanism which would appear to be dominant for reasons which will be discussed later.

Chromosome preparations were made from cultured lymphocytes of the proband's mother (VII-1), the proband (VIII-2), the proband's daughter (IX-1), and her granddaughter (X-1). Air-dried slides were stained with Giemsa, and the chromosomes were counted and karyotyped. All four females showed a 46, XX chromosome complement. With Giemsa staining, the short arms of the X chromosome are expected to contain one G-band and the long arms to contain two. Normal banding patterns for the X chromosomes of the three affected females can be seen in Fig. 4; there is no evidence of a translocation.

DISCUSSION

We have described our studies of the dominant transmission of hemophilia A in three generations of the women in one family. Our data raise two major questions: (1) Do the studies really show that the women are transmitting hemophilia A? (2) If the women are transmitting hemophilia A, what genetic mechanism is responsible?

The phenotypes of the proband and her descendants closely resemble hemophilia A in several respects. From the laboratory standpoint (Table 1), the proband shows slight prolongation of whole-blood clotting time and slight impairment of prothrombin consumption. Bleeding time, tourniquet test, plate-
let numbers, and clot retraction are entirely normal. Prothrombin time is normal, partial thromboplastin time is moderately prolonged, and there is no suggestion of an inhibitor. Specific coagulant assays for factors I, II, V, VII, VIII, IX, X, and XII show that only factor VIII is significantly depressed, and there is not a delayed rise of factor VIII following transfusion with normal cryoprecipitate (Fig. 1). When the proband’s plasma is examined by SDS-polyacrylamide-gel electrophoresis of a purified void volume fraction of cryoprecipitate, it shows bands not different from normal controls and hemophilic men. Factor VIII-related antigen is present in full amount. Ristocetin aggregation of platelets is effected successfully by the plasma of the proband and her daughter, and the Ristocetin-aggregating activity is found almost exclusively in the void volume just as it is in controls and hemophiliacs.

From the clinical standpoint, the correspondence of the hemorrhagic diathesis with that of hemophilia A is less perfect. The proband, whose basal factor VIII level appears to be about 2% of normal, does not have symptoms which are as severe as those usually seen in men hemizygous for hemophilia and having similar factor VIII levels. Probably of even greater significance is the fact that her daughter and granddaughter are essentially asymptomatic, although their factor VIII levels are consistently less than 5% of normal. Our conclusion—admittedly based largely on intuition—is that the discrepancy between clinical symptoms and clotting factor level in these women indicates that their phenotype is not exactly the same as hemophilia A, although this conclusion does not necessarily imply that mutation has occurred at a locus other than that responsible for hemophilia A. We shall continue to refer to the phenotype as hemophilia A until we discover a parameter with which to make a distinction which is more objective than intuition.

From the genetic standpoint, it is clear that the proband is the first affected person in her large kindred and that the trait is being transmitted in the fashion usually referred to as dominant. Furthermore, it is assumed that a mutation occurred in a germ cell of one of her parents. (Qualifications concerning use of the term “dominant” are discussed in Appendix II of Reference 2.) Although we do not know whether the trait is autosomal or X linked, the affected women must be considered heterozygous for an abnormal factor VIII allele because of the outbreeding in each generation. Even though the parents of the proband may have been related in the distant past, the fathers of both the daughter and granddaughter were normal and were unrelated to the “Be” kindred.

As to the genetic mechanism responsible, the six most likely possibilities are (1) Our subjects have von Willebrand’s disease (vWd), (2) Our subjects have a variant of vWd due to an unusual mutation at the vWd locus, (3) A balanced X-autosomal translocation has occurred in a heterozygote for hemophilia A and has been transmitted for three generations, (4) Extreme lyonization has produced a hemophilic phenotype in three successive generations of heterozygotes for hemophilia A, (5) A dominant mutation has occurred at the hemophilia A locus on the X-chromosome, and (6) We are observing a dominant mutation at a previously unrecognized locus, the fourth factor VIII locus.

It is quite unlikely that our subjects have the vWd phenotype. They have normal bleeding times and are CRM+ for the factor VIII-related antigen. Their plasmas aggregate platelets normally in the presence of Ristocetin, and this
activity is located in the usual plasma fraction. When the proband was transfused with cryoprecipitate, she responded as a hemizygote for hemophilia A would have, i.e., there was no suggestion of de novo synthesis of factor VIII.

Linkage studies of vWd would be very helpful. Except for linkage studies, we cannot imagine how to test the possibility that these women are suffering from a previously undescribed mutation at the vWd locus, one which has resulted in a CRM* hemophilia A phenotype. If we were to succeed in demonstrating that the locus responsible for this phenotype had the same linkage relation to a vWd-linked marker as does classic vWd, we should have positive proof. Unfortunately, we are not yet certain that the trait in our kindred is in fact autosomal, and no one has yet linked any other genetic marker to the vWd locus.

The most plausible chromosomal mechanism which might account for the transmission of dominant hemophilia A is a balanced X-autosomal translocation occurring in heterozygotes for X-linked hemophilia A. The pattern of transmission in our kindred would require that such a translocation be balanced, since both of the adult women are fertile and of normal intelligence, and the baby also appears to be normal. (Chromosomal imbalance usually produces infertility and/or mental retardation.) A balanced X-autosomal translocation has been reported in two generations by Buckton et al.,13 who described one involving the short arm of the X and the long arm of chromosome 14. They showed that the females with the balanced chromosomal complement were fertile and that the cytologically normal X chromosome replicated late. Cohen et al.,14 who reviewed all the reported human cases of balanced carriers of X-autosomal translocations, pointed out that the normal X chromosome is always the late replicator. This finding indicates that the morphologically normal X resides in the Barr body, which implies that the translocated X is active in cellular metabolism. Sequestration of the cytologically normal chromosome in the Barr body under these circumstances is expected, because if the translocated chromosome preferentially entered the Barr body, chromosomal balance would result. Chromosomal balance implies not only that there has been a reciprocal translocation, but that the individual possesses both of the translocated chromosomes and that both of the structurally abnormal chromosomes function in cell metabolism.

Assuming that our women are heterozygous and have a balanced translocation, the dominant pedigree pattern further requires that the hemophilia allele reside on the X chromosome bearing the translocated autosomal DNA, while the normal allele at this locus resides on the cytologically normal chromosome. The transmission of the hemophilia phenotype under this cytogenetic hypothesis will be dominant, even though the allele is one which is recessive in heterozygotes having morphologically normal chromosomes. The dominant mode of inheritance results because, for reasons stated above, all of the normal alleles are sequestered in the Barr bodies. (A moment's reflection will reveal that an iso-X chromosome in a hemophilia heterozygote would follow a recessive pattern since it is known1 that the iso-X preferentially goes to the Barr body.) The beauty of the balanced X-autosomal translocation hypothesis is not that it provides an explanation for this pattern of inheritance, but that it is testable. Our finding of 46 normal chromosomes in the three affected females excludes this
chromosomal mechanism as that responsible for dominant hemophilia A in this family.

The occurrence of extreme lyonization, i.e., random inactivation of all or almost all of the normal alleles in a heterozygote by sequestration in the Barr bodies has now been unambiguously demonstrated in at least three X-linked conditions: hemophilia B,\textsuperscript{15} deutan color blindness,\textsuperscript{16} and Duchenne muscular dystrophy,\textsuperscript{17} and has probably been demonstrated in hemophilia A.\textsuperscript{1,18} While extreme lyonization might have produced the pattern of inheritance in this kindred, the probability that this rare event has occurred three times by chance in a single kindred is very slight. Graham, Barrow, and Elston\textsuperscript{19} have pointed out why extreme lyonization is expected to occur more frequently in hemophilia B than in hemophilia A and have estimated that the probability of extreme lyonization in hemophilia is of the order of $10^{-3}$--$10^{-5}$. The probability that a single kindred would be encountered in which this event had occurred by chance in three successive generations is, therefore, the third power of the individual probability, or $10^{-9}$--$10^{-15}$, a possibility which can reasonably be dismissed.

The possibility that a dominant mutation has occurred at the hemophilia A locus on the X chromosome cannot be excluded. However, such a mutation has not been reported previously. Since it is believed that the X locus bears a structural gene coding for a peptide which enters into the factor VIII complex\textsuperscript{20} and since heterozygous women necessarily produce two products, the phenomenon of genetic dominance might be observed if the peptides produced from abnormal alleles by some of the cells of a heterozygote interfered with the activity of normal peptides produced from the normal alleles of other cells. With this possibility in mind we have examined the plasma of the proband repeatedly under a variety of circumstances for evidence of an inhibitory product. When normal plasma is mixed in various proportions with the proband's plasma, 1 : 9 : 9 : 1, the change in the factor VIII assay values is not different from that obtained when normal plasma is similarly mixed with plasma from an unrelated hemophilic man. Also, there is no evidence that the proband's plasma at any concentration inhibits the prothrombin time of normal plasma when tested with human, rabbit, or dog thromboplastin.

A more strenuous attempt was made to detect an abnormal molecular product resembling factor VIII. The factor VIII-related protein in the proband's cryoprecipitate, however, appeared to be identical with that seen in patients with X-linked hemophilia and in normal individuals.\textsuperscript{21-23} Although a less than normal amount of procoagulant activity eluted at the void volume of the patient's cryoprecipitate, this was associated with a normal amount of protein which, when concentrated and subjected to polyacrylamide-gel electrophoresis, revealed the typical subunit of the human factor VIII protein.

In principle, the possibility of a dominant mutation at the X locus for hemophilia could be tested by studying the linkage of the trait to G-6-P-D or color-blindness, the loci on the X chromosome adjacent to the hemophilia A locus.\textsuperscript{24} Unfortunately, such studies require the existence of compound heterozygotes, matings of the proper types, and large numbers of progeny, all of which are lacking in our kindred. An alternative test of dominant X linkage would be to compare the phenotypes of affected males and females, since in X-linked,
dominantly inherited hypophosphatemia and vitamin D-resistant rickets, the
hemizygous males are usually more severely affected than heterozygous fe-
males.25 Unfortunately, there are no affected males in the kindred. Of course,
the occurrence of male-to-male transmission of the abnormal phenotype would
by itself have excluded X linkage.

Finally, there is the possibility that we are observing a dominant mutation at
a previously unrecognized factor VIII locus. The segregational data give no
hint as to whether such a mutant gene is located on the X or an autosomal
chromosome. The latter is more likely a priori simply because more of the
DNA is present in autosomal chromosomes than X chromosomes. There is
nothing in the phenotype, however, to suggest that our subjects are different
from men with hemophilia A except the mildness of their symptoms. We re-
gard such phenotypic evidence as insufficient to conclude that we are dealing
with a genetic mutation at a previously unrecognized locus.

The published kindred most nearly resembling the one we have described is
the family of Dutch hemophiliacs first reported by Hensen et al. in 1965.26
Father-to-son transmission of hemophilia A was present, a finding which ex-
cluded X linkage, and the phenotype appeared at first not to be vWD because
of the normal bleeding times and the apparent absence of de novo synthesis
of factor VIII following transfusion. More recently, Veltkamp and his associ-
ates27,28 have reported that de novo synthesis does indeed occur in these subjects
following transfusion and that, unlike our kindred, the affected persons have
reduced levels of factor VIII-related antigen. They have concluded that their
kindred is probably transmitting a variant of vWD.

We shall continue to refer to the phenotypes of our subjects as hemophilia A,
because objective evidence that they do not have this phenotype is lacking. We
find it very easy to represent the genetic state of our subjects accurately using
the new international rules for symbolic representation of phenotypes and
genotypes in blood coagulation,2 in spite of the uncertainty about the chromo-
somal location of the locus. The phenotypes of our subjects can be recorded as
F VIII rs, since we know that they have CRM+, factor VIII deficiency. Since,
from the pedigree pattern, we infer that they are heterozygous but we are not
certain of the locus involved, we record their genotypes as VIII-0-aa+/rs.

We have been studying this kindred for almost 20 yr. Our relationships are
good, and we plan to examine the affected members with each additional
diagnostic test as new tests become available, hoping to discover a phenotypic
characteristic which distinguishes them from hemophilia A. We urge other
workers who may have studied similarly ambiguous kindred or patients to
publish their data, because the collating and comparison of such material might
produce the clarification which is needed.

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