Hemophilia: New Information about the "Royal Disease"

Because hemophilia is both incurable and unpredictable, it imposes a staggering burden—physical, emotional, and financial—on its victims and their families. Lack of a reliable test for identifying carriers of this sex-linked genetic disorder has hampered adequate counseling of a woman, usually from a family with a history of hemophilia, who wants to know her chances of having a hemophilic son. Recently, however, research has shed new light on the nature of the defect causing the disease. One of the benefits of this research is that it appears to be leading to the development of a method that can reliably detect hemophilia carriers.

Classic hemophilia (hemophilia A) is one of a number of diseases characterized by excessive, even life-threatening bleeding. The gene for the disease is recessive and carried on the X chromosome. Females, who have two X chromosomes, rarely have the disease although they may carry the abnormal gene and transmit it to their children. Males, however, have only one X chromosome and invariably develop hemophilia if that chromosome carries the abnormal gene. Children of carriers have a 50 percent chance of inheriting the gene; each son who receives it will be a hemophiliac and each affected daughter will be a carrier. Up to 25 percent of all cases of hemophilia appear in families with no history of the condition; many of these may result from new mutations.

Because of its incidence in many of the royal families of Europe, hemophilia came to be known as the "royal disease." The most famous case, of course, was that of Alexis, the Tsarevich of Russia. The desperation of his mother, Alexandra, about her son's condition brought her under the influence of the notorious Rasputin and contributed to the onset of the Russian Revolution. Alexandra was the granddaughter of Queen Victoria of England, who was herself a carrier. Hemophilia, however, is not restricted to royalty. About 20,000 individuals in the United States have moderate to severe forms of the disease.

Since the 1930's clinicians have known that the blood-clotting defect that causes classic hemophilia can be corrected by infusing preparations of a material found in normal plasma. This material, now called antihemophilic factor (AHF) or factor VIII, is one of the numerous factors required for normal blood clotting. Since biological clotting tests detected little or no AHF activity in the plasma of hemophiliacs, most investigators concluded that these individuals either could not make factor VIII or that they make it in reduced quantities.

In 1957, Jacob Shanberge of the Mount Sinai Medical Center (Milwaukee) and Ira Gore of the University of Alabama presented evidence that hemophiliacs do make factor VIII but that it is somehow defective and inactive. This view gained little acceptance until the early 1970's when the availability of immunoassays (in which antibodies that react with factor VIII are used) permitted the detection of immunologically active but biologically inactive factor VIII in hemophilic plasma.

Factor VIII Antigen

Antibodies to the clotting factor appear in 5 to 20 percent of hemophiliacs treated with it and sometimes arise spontaneously in normal individuals, who consequently suffer bleeding problems. In early studies in which antiserum from these patients was used, only 10 percent of hemophilia patients appeared to have material that reacted with human antibodies to factor VIII. However, Oscar Ratnoff of Case Western Reserve University School of Medicine and Theodore Zimmerman, now at Scripps Clinic and Research Foundation, found that all hemophiliacs have antigen that reacts with rabbit antiserum to human factor VIII. Zimmerman said that more recent experiments indicate that the same also holds true for human antiserum. The concentrations of immunologically reactive material in the patients is equivalent to or greater than that in normal plasma.

These observations suggested a way of improving the identification of hemophilia carriers. Since carriers have one normal and one defective gene, they should have at least as much factor VIII antigen as normal persons but only about half the clot-promoting activity. In older tests, only biological AHF activity was measured; the average for carriers was about 50 percent of the normal value, but the data varied too widely to identify carriers with a high degree of confidence.

Zimmerman and Ratnoff hypothesized that combining the immunological assay with the biological one would allow much more reliable detection of the carrier state. They found that hemophilia carriers have a relative deficiency of functional AHF activity compared to antigen concentration. Normal individuals do not display such a relative deficiency. On the basis of this difference, the investigators correctly identified 92 percent of the carriers they tested.

Several other investigators have devised similar tests. They include Bonno Bouma of University Hospital, Utrecht, and Leonard Hoyer of the University of Connecticut School of Medicine (Farmington), in addition to Ratnoff and Zimmerman. Ratnoff reports that he can identify carriers with a degree of confidence of 95 percent or higher but in the other laboratories the confidence levels may be as low as 80 percent. The reliability of the test result is of major importance to the woman who wants to use the results when making her decision about whether or not to have a child.

In order to directly compare the results of the procedures used in the four laboratories, the National Heart and Lung Institute (NHLI) and the National Hemophilia Foundation sponsored a workshop at Case Western Reserve on 11 to 16 November. During the workshop, single blood samples were taken from each of 53 individuals (35 hemophilia carriers and 18 normal persons) and divided into four portions for analysis by each of the participating investigators. The investigators did not know which of the subjects were carriers.

Officials of NHLI, who did know the identities of the carriers, have recently decoded and analyzed the data produced by the workshop participants. According to Harvey Klein of NHLI, the workshop results confirmed that hemophilia carriers can be more reliably identified by methods that combine analyses for AHF biological and antigenic activity than by previous methods. The degrees of confidence with which the four investigators identified the carriers continued to range from 75 to 95 percent. Klein said, however, that each of the four groups used different assumptions and mathematical models to establish the criteria for separating carriers from normals. So it is still unclear whether the discrepancies in their results are real—due to slight differences in analytical

techniques—or whether they are the result of the disparate ways in which the investigators handled the data. In order to resolve this dilemma, each researcher is now analyzing the data of the others by his own method.

Factor VIII is involved not only in the etiology of hemophilia but also in that of von Willebrand's disease (VWD), another genetic condition characterized by excessive bleeding. Patients with severe VWD have reduced biological AHF activity and reduced factor VIII antigen. These observations must be reconciled with the fact that the gene for VWD is definitely distinct from that for hemophilia. It is dominant and autosomal (not sex-linked). The condition may afflict both males and females with varying degrees of severity, but VWD is generally less debilitating than hemophilia.

There are other important differences between VWD and hemophilia. Platelets (small disk-shaped cells necessary for blood clotting) from patients with VWD appear to be less "sticky" than normal ones; they do not adhere to glass beads nor do they clump in the presence of the antibiotic ristocetin. Normal platelets and also those from hemophilia victims do both these things. Addition of preparations of normal factor VIII, or even of the biologically inactive antigen material from hemophiliacs, to the plasma of VWD patients corrects these platelet defects. Thus factor VIII appears to have at least two functions in blood clotting—one involving platelets and one, the defect of classic hemophilia, that does not.

Elucidation of the structure of factor VIII should clarify the relationship between hemophilia and VWD, but, at present, there is a major controversy about the structure of the molecule. Everyone agrees that it is a glycoprotein. Estimates of its molecular weight have varied dramatically—from 25,000 to 2,000,000—but the current consensus is that factor VIII in plasma has a molecular weight between 1,000,000 and 2,000,000.

Such a large protein is likely to be composed of smaller subunits, and it is here that the controversy arises. One group, including Robert F. Wagner and Herbert Cooper of the University of North Carolina (Chapel Hill), Harvey Weiss of Columbia University College of Physicians and Surgeons, and Hoyer, thinks that factor VIII consists of two different components held together by non-covalent bonds.

Wagner and his colleagues found that factor VIII dissociates into a component of high molecular weight (at least 1,000,000) and one of low molecular weight (approximately 100,000) in solutions of high ionic strength. According to these investigators, the clot-promoting activity is associated with the smaller material. They suggested that the larger component serves as a carrier for the smaller one. Hoyer showed that the antigenic activity of the large component closely resembles that of factor VIII antigen found in normal and hemophilic plasmas. Moreover, the large component fraction corrects the defect in platelet aggregation that is characteristic of VWD.

According to Wagner, the two components rapidly reassociate when the ionic strength of the solution is lowered. The small active fragment from normal plasma also reassociates with the large component from hemophilic plasma to give a large molecule with biological AHF activity. But when a preparation from VWD plasma that corresponds to that containing the large component was mixed with the small fragment, a large biologically active molecule did not form.

Structure of Factor VIII

These results are interpreted as showing that factor VIII consists of a small component with antihemophilic activity plus a large one that is the antigenic material present in hemophiliacs, but much reduced in quantity in VWD patients. The large component affects platelet aggregation. At least two genes, one on the X chromosome and one autosomal, would thus be required to specify the synthesis of factor VIII.

Other workers, however, question the validity of the observation that factor VIII dissociates into two dissimilar components. This group includes Patrick McKee and Mary Ellen Switzer of Duke University Medical Center and Earl Davie of the University of Washington School of Medicine.

McKee and Switzer say that they have found no evidence for the dissociation of factor VIII under conditions that usually cause dissociation of protein subunits held together by non-covalent links. Only when they first treated the material with mild reducing agents that break disulfide bonds did they observe dissociation. But the subunits were identical and had a molecular weight of about 200,000. McKee and Switzer concluded that factor VIII is composed of identical subunits joined by disulfide bonds and that the whole molecule produces both antihemophilic and von Willebrand activities.

According to Davie, factor VIII has a molecular weight of about 1,200,000 and consists of identical subunits held together by disulfide bonds. In one type of experiment, he found the molecular weight of the subunit to be 100,000; when determined by another method, it was 240,000. Davie thinks that the second figure may be too high because glycoproteins may not behave like other proteins in such experiments.

Any model for factor VIII structure must be consistent with the genetics of hemophilia and VWD. McKee and Switzer have suggested that the sex-linked hemophilic defect may be located in the protein portion of the glycoprotein whereas the autosomal von Willebrand defect could be related to the structure or attachment of the carbohydrate moiety of the molecule. Thus at least two genes could be involved in the synthesis of factor VIII.

A final observation that must be explained involves the unusual response of VWD patients to transfusion with factor VIII preparations. When hemophiliacs are transfused, the antihemophilic activity of their plasma reaches the peak predicted on the basis of the amount of factor VIII administered and then declines rapidly. When VWD patients are transfused, either with normal factor VIII or with antigen prepared from hemophilic plasma, the antihemophilic activity of their plasma increases beyond the predicted amount. The factor VIII antigen or platelet-aggregating activity, however, only reaches the expected level.

Wagner hypothesizes that the large carrier component of the factor VIII molecule stimulates the synthesis or release of the small antihemophilic component. McKee and Switzer propose that the material produced in response to transfusion can correct the clotting defect of hemophilic plasma but still lacks the correct carbohydrate moiety necessary for interaction with platelets and with antibodies.

The controversy between the two groups of investigators graphically illustrates the hazards of determining the structure of a large glycoprotein found in very small concentrations in a medium as complex as plasma. Reconciliation of their differences is not possible at this time, but the current dispute does not appear to be hindering the application of new knowledge about hemophilia to more reliable tests for carrier detection.—JEAN L. MARX