

# VON WILLEBRAND'S DISEASE— HISTORY, DIAGNOSIS AND MANAGEMENT

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Von Willebrand's disease is a complex and in many aspects poorly understood congenital bleeding disorder. Although in the last few years exciting advances have been made in comprehending this not uncommon hemorrhagic diathesis, many facets of it still defy our detailed understanding. Because of its complexity, the disorder has historically been viewed as a thrombocytopathy, a coagulopathy and even a telangiopathy. It has been described by many different names and has been the subject of a variety of reviews, the most notable and most recent one by Bowie and associates [30]. Since that time, however, a considerable amount of new knowledge has been gained which seems to warrant that at this time an issue of this journal be devoted to this subject. This particular review will first attempt to highlight the historical events related to the disease which will help to understand some of the problems that kept us from finding a rational explanation for it. This will be followed by a discussion of the diagnostic criteria and the management of the disorder. Because the pathophysiology of the disease encompasses both abnormal platelet function and abnormal plasma clotting factor activity, especially factor VIII, and because newer investigations seem to link these two together in one functional entity, both are reviewed separately and in depth in this issue.

## HISTORY AND PATHOPHYSIOLOGY OF THE VON WILLEBRAND'S DISEASE

The name "von Willebrand's disease" relates to its description by Professor Erik von Willebrand in 1926 and 1931 [175, 176]. In these two papers, a five-year-old patient and a large number of her family members from the Åland Islands in the Gulf of Bothnia are described, many of them suffering from a severe bleeding problem, manifesting itself most commonly in epistaxis and bleedings into the skin and mucous membranes. The bleeding times were prolonged, the Rumpel-Leede test was positive, but platelet counts were normal. Also coagulation times and clot retraction were normal. Morphologically the platelets seemed to appear in many medium and large forms. The disorders showed an autosomal dominant pattern of heredity, affecting both males and females. Since at that time only hemophilia, thrombocytopenia and thrombasthenia Glanzmann [58] were known, von Wille-

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brand recognized that the family under study had a different bleeding problem and attributed it to a "disturbance of function in the thrombocytes and general alteration in the capillary wall." He proceeded to call the disease "Pseudo-hemophilia," to denote the hemophilia-like clinical bleeding problem.

It must be pointed out at this time, that Minot [113, 114] probably described the same disorder prior to the publications of von Willebrand, and for this reason Quick [135, 138] refers to the disease as the "Minot-von Willebrand syndrome."

In 1930, Morawitz and Jürgens [115] had studied a similar case in which they found with the aid of a capillary thrombometer, what they interpreted as abnormal platelet aggregation and thrombus formation. These findings prompted Jürgens to visit, together with Professor von Willebrand, the patients on the Åland Islands, where they made identical observations with the capillary thrombometer. Jürgens then suggested that the name "pseudo-hemophilia" be dropped in favor of "constitutional thrombocytopathy," to reflect the qualitative platelet abnormality in this disorder [177, 178]. This terminology, or the name "von Willebrand-Jürgens syndrome," was subsequently the most common nomenclature used in Europe to describe this disease. The pathophysiology was thus related to a congenital qualitative platelet abnormality or thrombocytopathy, the prime basis for it being the abnormal thrombus formation in the capillary thrombometer.

Many of the following events relate heavily to the use of the capillary thrombometer, respectively the lack of it for general availability. For this reason, it seems important to reflect for a moment on this apparatus. The thrombometer made use of a capillary tube through which blood was pumped back and forth by means of two paraffinized glass pumps. The time it took for a thrombus to form was recorded. Critics of this procedure readily pointed out that this apparatus measured coagulation times and denied its usefulness in measuring platelet function. Although, and in retrospect, it is difficult to assess what was measured with this apparatus, the thrombus formation in blood of von Willebrand's disease patients was markedly prolonged. In contrast, the thrombus formation time in blood of hemophiliacs was normal [86, 178]. This indeed seems to indicate that platelet function was recorded rather than fibrin formation; and with our present knowledge this statement certainly seems to be correct. Unfortunately, however, the capillary thrombometer never gained popularity as a research tool, and with its general unavailability, the concept of von Willebrand's disease being a qualitative platelet disorder remained questionable, and therefore, disputed. Nevertheless, and in spite of all developments until about 1951, the platelets of patients with von Willebrand's disease were extensively studied and the various abnormalities described up to that time are reviewed by Walsh in this issue [165]. Due to the fact that qualitative platelet function was at that time difficult to assess, and the thrombometer having fallen into disrepute, the von Willebrand's disease was during that time of 1940 to 1950 viewed primarily as a telangiopathy. It must be remembered that von Willebrand in his first papers [175, 176] spoke of a "disturbance of function in the thrombocytes and general alteration in the capillary wall," obviously based on the prolonged bleeding times and the positive Rumpel-Leede test. This concept was further maintained by Jürgens [88]. In 1941, Macfarlane [100] described distorted and bizarre nail bed capillaries in five patients with "pseudo-hemophilia." This observation was supported by several other

reports [20, 38, 54, 98, 99, 106, 133, 141], but also, observations to the contrary were made [133], especially in later years [2, 65, 92, 164]. This development led Schulman and associates to call von Willebrand's disease "vascular hemophilia" [147, 148]. Also, the term "angiohemophilia" was used to denote the same disorder [2, 13, 65, 67]. Presently it is difficult to assess whether the described morphologic abnormalities are indeed part of the von Willebrand's syndrome. Not only do all patients with von Willebrand's disease fail to display these changes, but the changes have been described in subjects not suffering from von Willebrand's disease [36, 84]. More recently, Quick [138] expressed the view that the defect in von Willebrand's disease is localized in the mechanism that maintains the tonus of the microcirculation. He based his view on the effect of aspirin on the bleeding times of patients with von Willebrand's disease, as judged by the so-called "aspirin tolerance test." In mild and subclinical cases the bleeding times will become significantly prolonged, when aspirin is ingested [136, 137].

The first report implying a missing coagulation factor, namely factor VIII, in patients with von Willebrand's disease appeared in 1953 [5, 95, 139]. This also was the year where the thromboplastin generation test was described [18], which allowed a fairly easy measure of factor VIII activity. These observations opened a new era in which the disease was primarily considered a coagulopathy, and a large number of case descriptions followed in the next few years (see [30]). A crucial event during that time was the re-examination of the original Åland Islands patients with the thromboplastin generation test in 1957 by Jürgens and associates [89] and by Nilsson and co-workers [121]. Both groups found that also these patients lacked factor VIII activity in plasma. In retrospect, already earlier reports had given indications for a disturbance in the coagulation system. Jürgens and Ferlin [87], for example, had noted an abnormal prothrombin consumption in some cases from the Åland Islands and Nickerson [118] reported shortened coagulation times in response to the transfusion of fresh blood. Interestingly, Nickerson [118] had already found that hemophilic plasma had less of an effect on the coagulation times of von Willebrand patients' plasma.

The next major event in solving the riddle of the von Willebrand's disease came in 1956 when Nilsson and her co-workers [120, 121, 123] infused human Cohn fraction I-O to patients with the disease, not only obtaining the expected rise in the factor VIII levels, but also observing a normalization in the previously prolonged bleeding times. Since Cohn fraction I-O contains fibrinogen and factor VIII, patients next were given fibrinogen free of factor VIII, and no rise in factor VIII and no correction of the bleeding times was noted [119, 122]. These studies seemed to indicate that factor VIII might be responsible for the correction of the bleeding times. However, when Cohn fraction I-O was used from which factor VIII activity had been removed by sterile filtration, the bleeding times were also corrected. Moreover, Cohn fraction I-O from hemophilia A plasma corrected the prolonged bleeding times. This same fraction from plasma of von Willebrand patients did not correct the bleeding times. Since it made no difference whether Cohn fraction I-O was prepared from platelet poor or platelet rich plasma, the correction of the abnormal bleeding times in von Willebrand patients obviously had to be due to a plasma factor not necessarily associated with the factor VIII activity. This plasma factor became subsequently known as the "von Willebrand factor" or

the "anti-von Willebrand factor." These experiments were repeated by several groups of investigators [19, 25, 44-46, 117, 166] with identical results, even adding serum to the list of blood fractions which apparently did not contain this so-called "von Willebrand factor" [46], because it failed to correct the prolonged bleeding times.

With reference to the deficiency in factor VIII activity, not only in classical hemophilia but also in von Willebrand's disease, a genetic problem seemed to exist. The factor VIII deficiency in classic hemophilia follows a recessive sex-linked pattern of heredity, while the one in von Willebrand's disease follows an autosomal dominant pattern. One is associated with normal bleeding times, the other with abnormal bleeding times.

The scientific problem was further complicated by finding that the half-life of factor VIII, transfused to von Willebrand patients was about forty hours, whereas it was only 6-8 hours in patients with hemophilia A [37]. Moreover, the levels of factor VIII in plasma of von Willebrand patients following transfusion always seemed to be higher than expected [25, 37, 44]. Real confusion arose, when hemophilic plasma, containing no factor VIII activity, transfused to patients with von Willebrand's disease, also having no factor VIII activity, not only corrected the patients' bleeding times but generated factor VIII activity [37, 44, 45]. In many instances the factor VIII levels rose higher when hemophilic plasma was transfused as when normal plasma was given. It was noted, however, that the maximum rise in factor VIII was not obtained immediately, but after 5-8 hours following transfusion. In contrast, when cross-transfusions were performed between a von Willebrand patient as donor and a hemophiliac as a recipient, the rise in factor VIII levels failed to materialize. These experiments seemed to indicate that the factor VIII deficiency in von Willebrand's disease was different from the factor VIII deficiency in hemophilia. Von Willebrand patients seemed to have a factor which could stimulate factor VIII production in the hemophiliac, but not vice versa. Since these findings were difficult to reconcile with a single genetic defect, Graham and associates [11, 61, 105] developed three hypotheses to account for these observations made so far. All are speculative. They require two genetic loci and are known as "activator hypothesis," "regulatory hypothesis" and "combining subunit hypothesis." In principle, the "activator hypothesis" postulates that a structural gene on the X-chromosome codes the synthesis of a precursor of factor VIII which must be activated by a factor whose synthesis depends on an autosomal gene. A defective structural gene would result in hemophilia A and a defective autosomal gene would cause von Willebrand's disease.

The "regulatory hypothesis" postulates that an autosomal operon produces an effector substance which regulates the X-chromosomal operon coding for factor VIII. Both operons are under repressor control. Von Willebrand's disease would, therefore, occur when an increased autosomal repressor activity results in a decreased effector substance synthesis and thus synthesis of factor VIII. Hemophilia would have to be a mutation of the X-chromosomal operon.

The "combining subunit hypothesis" postulates that the factor VIII molecule is composed of two parts, one under the control of a gene on the X-chromosome and the other under the control of paired genes on an autosome. Hemophilia A would be a mutation of the former, and von Willebrand's disease a mutation of the latter.

This hypothesis would also account for the apparently existing heterozygote and homozygote states of von Willebrand's disease.

From what has been learned in the last few years about the molecular structure of the factor VIII molecule [83], the "combining subunit hypothesis" seems to follow most closely the experimental data and developments. Snyder [155] more recently updated and slightly modified the "combining subunit hypothesis" and accounted for most clinical and experimental data so far collected on hemophilia A and on von Willebrand's disease.

In view of the before mentioned developments and the exciting observations on factor VIII, it can easily be understood that the original concept of von Willebrand's disease being a qualitative platelet abnormality, was almost forgotten. For these reasons the disease was primarily considered to be a coagulopathy. Still unexplained, however, remained the abnormal bleeding times. Also the so-called "anti-von Willebrand factor," postulated to be in plasma, was never identified. The above mentioned hypotheses tried to explain the various findings centering around factor VIII, but did not readily offer an explanation for the abnormal bleeding times.

In 1960, Hellem [69] reported decreased platelet adhesiveness in some patients with von Willebrand's disease, but interpreted these findings as a result of an existing low hematocrit. Borchgrevink [23] next observed poor platelet adhesion on the wound margins of his patients *in vivo*, and it is now well established that patients with von Willebrand's disease with abnormal bleeding times also have decreased platelet adhesion *in vitro* and *in vivo*. These reports are detailed in the contributions by Walsh [165]. As a matter of fact, abnormal platelet adhesion is one of the important diagnostic criteria for von Willebrand's disease. There seems to exist, however, a strong relationship between platelet adhesion and bleeding times, as one would expect. Thus, in the last few years, the attention once again returned to the platelet in von Willebrand's disease. Inasmuch as it is now possible to relate bleeding times to platelet adhesion, still the link to the factor VIII abnormality was missing.

In 1971 two major developments came about that had a major impact on our understanding of von Willebrand's disease. The first related to platelet function in this disorder. Howard and Firkin [75] found that ristocetin, and antibiotic, once marketed but then withdrawn, aggregated normal human platelets, but failed to aggregate platelets from patients with von Willebrand's disease. This observation has now been confirmed by many other investigators and for details the reader is again referred to the contribution of Walsh [165] in this issue. Inasmuch as the lack of platelet aggregation by ristocetin is not specific for von Willebrand's disease alone, but also seen in idiopathic thrombocytopenic purpura [76], platelet storage pool disease [167], Bernard-Soulier syndrome [77, 167], and in acute leukemias, myeloblastic and monocytic [173], it has become an important diagnostic tool for von Willebrand's disease. An additional interesting observation indicates that the ingestion of aspirin causes abnormal ristocetin platelet aggregation in normal persons [125, 167]. This could serve as a link to explain the marked prolongation of bleeding times following aspirin ingestion (the so-called aspirin tolerance test) in patients with von Willebrand's disease who have otherwise a borderline normal bleeding time. Inasmuch as one could now confirm some kind of platelet

dysfunction, related to adhesion and ristocetin induced aggregation, it still did not explain the relationship to the factor VIII abnormality.

The second new development, which occurred around 1970, relates to the immunologic properties of factor VIII. Hoyer and Breckenridge [79] were the first to initiate these studies. They used homologous antibodies for their work. The term homologous antibodies is used to describe the antibody inhibitors which develop in hemophiliacs or occasionally also in healthy individuals in response to blood or blood fraction therapy. These antibodies have been reviewed in detail in an earlier issue of this journal [150]. Investigating 34 patients from 27 families, Hoyer and Breckenridge [79] found that six of these patients had material in their plasma which antigenically reacted with the homologous antibodies. The term "cross-reacting material [CRM]," borrowed from bacterial immunology work, was used to denote these findings, and hemophiliacs with a positive antigenetic reaction were described as CRM<sup>+</sup>, while those who do not possess antigenically active material in plasma were described as CRM<sup>-</sup>. These authors also tested six patients with von Willebrand's disease and found them all CRM<sup>-</sup>. Similar results were subsequently reported by Denson and co-workers [48], Feinstein and associates [55], Meyer and Larrieu [108], Lechner [96], and Hoyer [78]. With these homologous antibodies, between 10 to 15% of hemophilia A patients seem to be CRM<sup>+</sup> or A<sup>+</sup>, as Denson and co-workers [48] labeled it. In contrast, patients with von Willebrand's disease seemed uniformly to be CRM<sup>-</sup> [78, 109]. These findings seem to indicate that there could be hemophiliacs that have a functionally abnormal factor VIII molecule rather than the absence of the factor [26, 79]. This dual abnormality is best known for congenital abnormalities of the fibrinogen molecule where a so-called afibrinogenemia can be clearly distinguished from a dysfibrinogenemia, a subject matter recently reviewed in this journal [101]. Studies of the cross-reacting material in hemophilic plasma revealed great similarities to factor VIII from normal plasma except, of course, for the lack of biologic activities [80]. With the exception of these 10 to 15% hemophiliacs, the cross-reaction of antigenic material with homologous antibodies seemed to closely follow activity patterns, i.e., low or absent biologic activity coincided with negative cross-reacting material. It must be pointed out, however, that methods for determining the cross-reacting material are also important in that, for example, the cross-reacting material cannot be determined by immunodiffusion, presumably because of the low factor VIII protein in plasma [80]. In addition, the type of antibody used is of considerable significance in that with the use of heterologous antibodies a much larger population of hemophiliacs is CRM<sup>+</sup>. However, even when heterologous antibodies are used, the species in which the antibody is produced makes a difference. Gralnick and co-workers [62], for example, used goats to produce an antibody to partially purified human factor VIII and observed that 15% of their hemophiliacs were CRM<sup>+</sup>. In contrast, Zimmerman et al. [179] and Hoyer [78] demonstrated cross-reacting material in all of their hemophiliacs studied. Both groups of investigators used rabbits to produce antibodies to highly purified human factor VIII. Hoyer [78] compared the binding properties of human antibodies with those rabbit antibodies and could demonstrate that rabbit antibodies formed a stable complex with the factor VIII protein, whereas human antibodies inactivated the biologic activity, but did not form a stable complex with the protein. Other

investigators have since confirmed that practically all hemophiliacs suffering from factor VIII "deficiency" are CRM<sup>+</sup> with heterologous antibodies to factor VIII, provided the antibodies are prepared in rabbits [55, 109, 158]. In contrast, patients with severe forms of von Willebrand's disease were found to be uniformly CRM<sup>-</sup>, regardless of which type of antibody was used, homologous or heterologous [78, 109, 158, 179]. In mild forms of von Willebrand's disease the immunologic levels and the activity levels were proportional, when heterologous antibodies were used. With respect to hemophilia, carriers of the disease would now be expected to have normal levels of factor VIII antigenic material, but only about one-half as much factor VIII activity. This was demonstrated by Zimmerman and co-workers [180]. Today this procedure is an additional diagnostic tool to detect hemophilic carrier states.

The observations that factor VIII activity is not necessarily identical with factor VIII antigenic material, has led to a different nomenclature, where the factor VIII associated antigen material is denoted as factor VIII<sub>ag</sub>, and the biologic activity of factor VIII as factor VIII<sub>coag</sub>. In von Willebrand's disease, the ratio of factor VIII<sub>coag</sub> to factor VIII<sub>ag</sub> seems always to be one, whereas in hemophilia the ratio is considerably less than one. These findings now also have led to the belief that patients with von Willebrand's disease do not synthesize an immunologically detectable factor VIII molecule, whereas, in contrast, hemophiliacs produce an immunologically detectable molecule which, however, lacks biologic activity [27]. But still, these observations did not explain the abnormal bleeding times, presumably due to the lack of platelet adhesion and the abnormal platelet aggregating response to ristocetin in patients with von Willebrand's disease.

Recent work on the purification and characterization of the factor VIII molecule, however, seems to shed some light on this interrelationship of abnormal platelet function and decreased factor VIII in von Willebrand's disease patients. The detailed data on the purification and biochemical characterization of factor VIII have been reviewed recently [11, 47], and also this issue of this journal carries a review, although not comprehensive [83]. One of the puzzling findings relative to the factor VIII molecule has been its apparently widespread molecular weight, ranging from 25,000 to 2,000,000 (see [83] for references). Molecular aggregation, of course, would be one possible explanation, i.e., small units of about 25,000 molecular weight aggregate to 2,000,000 molecular weight structures. Against this concept speaks the finding that small units (200,000 molecular weight) could only be obtained when the large molecular weight products were reduced with reducing agents [97, 104]; dispensing agents did not yield to these subunits. It has also been suggested that there may be two different species of factor VIII circulating in plasma [168], one large molecular weight and one low molecular weight form. Low molecular weight factor VIII has also been found in patients with von Willebrand's disease after treatment with factor VIII concentrates [22] and in kidney extracts [10]. Finally, low molecular weight forms of factor VIII were obtained from high molecular weight forms by gel filtration or by ultracentrifugation in the presence of high ionic strength salt solutions [128, 169], thus giving credence to the possibility that factor VIII from plasma is composed of subunits which are held together by disulfide bonds [16, 70, 97, 104, 149]. The subunits range in molecular weight from 25,000 to 240,000. It is also important that the subunit

structures were identical, whether they were derived from factor VIII from normal plasma or from hemophilic plasma [16, 104, 149]. More recently, factor VIII was dissociated at low ionic strength and in the absence of reducing agents into two components, a slow and a fast moving one. These components differed in precipitation properties on cross-immunoelectrophoresis, they both cross-reacted with heterologous antibodies and were nonidentical with each other [116]. Also the work of Wagner and associates [64, 128] showed that normal bovine and canine factor VIII is a large molecular complex composed of apparently one large carrier molecule and a small fragment with factor VIII procoagulant activity. The two fractions were dissociated in the presence of 0.25 M calcium chloride and recombined in the absence of calcium ions [42, 43]. The molecular weight of the small fragment with procoagulant activity seems to be around 100,000, while the large molecular weight carrier protein seems to have a molecular weight of about 2,000,000. The same observations were made with human factor VIII [42]. It was thus concluded that the large molecular weight inactive carrier protein component of factor VIII functions as a carrier for the small fragment which possesses the procoagulant activity [42]. Using heterologous anti-factor VIII antibodies, produced in rabbits, Rick and Hoyer [142] could now show that the large molecular weight carrier protein cross-reacted with these antibodies while the small procoagulant carrying component did not cross-react. Although no cross-reaction with the small component was observed, the antibody still neutralized its biologic factor VIII activity. Homologous antibodies, i.e., antibodies occurring naturally or acquired in human plasma, neutralized the procoagulant activity of these small factor VIII fragments. The factor VIII complex can thus be dissociated into an active carrier component which cross-reacts with heterologous antibodies to factor VIII and a small procoagulant carrying component which does not cross-react with these antibodies. Therefore, factor VIII<sub>ag</sub> and factor VIII<sub>coag</sub> are two distinct entities, although they seem to form a complex. Zimmerman and co-workers [180] added increasing amounts of solid phase rabbit antibody to normal plasma and observed an increase in the ratio of factor VIII<sub>coag</sub> to factor VIII<sub>ag</sub>. When they added increasing amounts of homologous antibody, the ratio decreased. These experiments suggest again, that not only the antigenic sites which bind the heterologous rabbit antibody and the homologous human antibody are on separate sites of the factor VIII molecule. They also indicate that the human homologous antibody binds to the activity carrying part of the factor VIII molecule. Similar results were reported by Kernoff [91].

Since all patients with hemophilia A show factor VIII related antigen with heterologous rabbit antibodies, it can now be concluded that they all have the large molecular weight carrier protein, but lack the small molecular weight procoagulant carrying component. This large molecular weight part of the molecule seems to be intact in hemophiliacs, because Cooper and Wagner [42] were not only able to recombine normal high molecular weight material with normal low molecular weight material, but also high molecular weight material from hemophilia A plasma with low molecular weight material from normal plasma. Since 10 to 15% of the hemophiliacs are CRM<sup>+</sup> or A<sup>+</sup>, i.e., they possess cross-reacting material which reacts with homologous human antibodies, they must also have the small portion of the molecule, but in an inactive form. This would mean that the CRM<sup>+</sup> hemophiliacs



have an abnormal small molecular weight protein portion circulating, while those who are CRM do not have any small molecular weight portion.

If it should be correct that antibody blocking can be demonstrated in the plasma of all CRM hemophiliacs, as suggested by Biggs [107], and that one only needs to increase the concentration of the antibody to demonstrate this, then there would be only one form of hemophilia A, namely, the one characterized by the presence of an abnormal small molecular weight fraction of the factor VIII molecule.

Since, in contrast, the majority of patients with von Willebrand's disease are CRM, i.e., their plasma does not contain antigenic material which cross-reacts with heterologous antibodies to factor VIII, they seem to lack the large molecular weight portion of the factor VIII molecule. It must be recalled that, as indicated above, there was a direct relationship between the amount of factor VIII<sub>ag</sub> and factor VIII<sub>coag</sub> in most patients with von Willebrand's disease.

It has been pointed out above that besides a decreased factor VIII level, both VIII<sub>ag</sub> and VIII<sub>coag</sub>, patients with von Willebrand's disease also have prolonged bleeding times, apparently due to poor platelet adhesion *in vivo* as well as *in vitro*. Poor platelet adhesion leads to poor platelet aggregation and, therefore, to a delayed formation of the first hemostatic plug or platelet plug. When Nilsson and associates [120, 121, 123] infused Cohn fraction I-O into patients with von Willebrand's disease, they noted not only a rise in factor VIII activity but also a normalization of the prolonged bleeding times. The bleeding times even became normal when the Cohn fraction I-O from which the factor VIII activity had been removed, was infused, and when Cohn fraction I-O from hemophilic plasma was administered. These developments seemed at that time to indicate that a plasma protein, other than the active factor VIII, was responsible for the normalization of the bleeding times in von Willebrand's disease patients, this factor being named the "von Willebrand's factor" or the "anti-von Willebrand's factor." Also, *in vitro* it could be shown that platelet adhesiveness improved in von Willebrand's disease patients' plasma when the glass beads in the column used to measure platelet adhesion, were first coated with either normal or hemophilic plasma or cryoprecipitate [107]. In contrast, plasma from patients with von Willebrand's disease did not have this effect [107]. By correlating the platelet adhesion correction with the levels of factor VIII related antigen, not only in plasmas but also in purified factor VIII concentrates, Bouma and co-workers [26, 28] concluded that the protein, correcting the prolonged bleeding times by improving platelet adhesion, was the factor VIII related antigen or, as we know it now, the large molecular weight carrier protein of the factor VIII molecule. This then would explain why hemophiliacs have normal bleeding times and normal platelet adhesion, whereas patients with von Willebrand's disease lack both.

As indicated above, Howard and Firkin [75] were the first to find that platelets from patients with von Willebrand's disease lacked aggregability when challenged with ristocetin. Although not specific for von Willebrand's disease, this test has become part of the tools with which the disorder can be diagnosed. Since normal plasma and hemophilia A plasma and serum, added to von Willebrand's disease plasma, corrected the abnormal aggregation response to ristocetin [85, 111, 170, 171], it became apparent that possibly the von Willebrand factor or the anti-von

Willebrand factor, or, as we know now, the factor VIII related antigen or the large molecular weight portion of the factor VIII molecule, might be linked to this phenomenon [144, 170, 171]. Patients with von Willebrand's disease, not having this part of the factor VIII molecule, would thus lack aggregation with ristocetin. Indeed, when heterologous antibodies, produced in rabbits, were added to normal platelet rich plasma, aggregation with ristocetin was inhibited [26, 74, 110]. In addition, the defective ristocetin induced aggregation response of washed human platelets could also be corrected by adding purified factor VIII [171]. It has been suggested that the factor VIII molecule is necessary for the maintenance of weak inter-platelet bonds, probably through electrostatic forces, when platelets aggregate [74]. Also, homologous human antibodies to factor VIII have been shown to inhibit ristocetin-induced platelet aggregation [160], although these observations are not consistent, and discrepancies have been noted [52]. It is possible that those human antibodies which are directed against both, factor VIII<sub>coag</sub> and factor VIII<sub>ag</sub>, would also inhibit ristocetin induced aggregation, while those directed against factor VIII<sub>coag</sub> only, would not have this property. Further evidence that the factor VIII antigen is involved in ristocetin-induced platelet aggregation came from studies reported by Collier and associates [40]. They produced an antibody to factor VIII in goats and could demonstrate the presence of antigen not only on normal human platelets, but also on hemophilic platelets. Platelets from patients with severe von Willebrand's disease did not have the antigen on their platelet surface.

Baugh and co-workers [12] have now shown that factor VIII antigen which inhibits the ristocetin-induced platelet aggregation, can be separated into two components, each one separately is ineffective but in combination they have this inhibiting property. This could mean that there are two plasma proteins required to give the von Willebrand factor activity or more specifically at least ristocetin-aggregation activity.

Much clarification of our understanding of hemophilia A versus von Willebrand's disease has thus come from studies related to the factor VIII molecule. Although there still seem to exist some differences of opinion in the final details of the structure, agreement seems to exist on the fact that the factor VIII molecule with its molecular weight of about 2,000,000 Daltons is composed of subunits which can be dissociated *in vitro*. Whereas several investigators seem to believe that the subunits have a molecular weight of about 240,000, which then aggregate to form an over-all molecular structure of 2,000,000 [22, 70, 97, 104, 116, 168], others have obtained evidence to suggest that two different molecular species are involved [42, 43, 64, 118]; one is termed "carrier protein" and is apparently large in molecular weight, the other is smaller and carries the biologic activity. Heterologous antibodies, as pointed out above, are apparently directed against the large molecular weight species, whereas homologous antibodies may, dependent on the antibody, be directed against the smaller unit only. The factor VIII molecule not only carries the biologic clot-promoting activity, but apparently is also involved in the platelet adhesion and aggregation. The latter platelet related activities seem to reside in the carrier unit, or the unit to which heterologous antibodies form.

These basic assumptions now allow an explanation of the many different laboratory features of hemophilia A and von Willebrand's disease, and also allow to speculate on the different genetic patterns the diseases display.

Hemophilia A, characterized by a lack of factor VIII activity, but normal platelet function, that is platelet adhesion and ristocetin-induced platelet aggregation, and thus, normal bleeding times, has antigen-related protein, when measured with heterologous antibodies, and thus seems to have an intact carrier protein unit. For this reason platelet function and bleeding times are normal. The defect in hemophilia A thus seems to reside in the small molecular weight subunits of the molecule. While 10 to 15% of the hemophiliacs have immunologically cross-reacting material in plasma, when homologous antibodies are used, they must then have an abnormal small molecular size subunit, instead of the absence of this subunit. In contrast, the majority of hemophiliacs apparently do not have the cross-reacting material with homologous antibodies, suggesting the absence of the small subunit of the factor VIII molecule. Both defects are genetically transmitted in a recessive sex-linked manner. Biggs [17], on the other hand, was able to demonstrate that all hemophiliacs have cross-reacting material, but larger than usual amounts of antibody are needed for their demonstration. If this can be shown for all hemophiliacs by others as well, then hemophilia A would be caused by an abnormal small subunit of the factor VIII.

Carriers of this disease would then have to display normal amounts of carrier protein or antigenic material, but only half of the normally expected factor VIII procoagulant activity, i.e., the ratio of factor VIII activity to factor VIII antigen would be less than one. This has been found to be true [15, 53, 180].

Von Willebrand's disease in its classic form is characterized by prolonged bleeding times, abnormal platelet adhesion and ristocetin-induced aggregation, and also by low factor VIII activity. Most patients do not have immunologically demonstrable factor VIII protein in plasma, when heterologous antibodies are used. They thus seem to have absent or low factor VIII related antigen and, therefore, low factor VIII clot promoting activity. The ratio of factor VIII activity to factor VIII antigen has always remained one. These patients, therefore, seem to lack the carrier protein portion of the factor VIII molecule. Since this part of the molecule is involved in the adhesion of platelets, not only *in vitro* but also *in vivo*, and also involved in the platelet aggregation, the bleeding times of these patients are prolonged. The synthesis of this factor VIII related antigen portion of the molecule seems to underlie autosomal dominant control which explains the different genetic patterns of von Willebrand's disease versus that of hemophilia A. The findings that hemophilic blood transfused into a von Willebrand's disease patient yields a rise in factor VIII activity [37, 44, 45], can now be explained by assuming that the carrier protein portion of the factor VIII molecule induces the synthesis of the smaller fragment, the combination of which, of course, gives the measured activity. This implies that von Willebrand's patients do not have these small fragments already available in plasma. Bloom et al. [22] and Stibbe et al. [157] have advanced similar hypotheses to account for both diseases. However, final proof must await, at this time further elucidation of the structure of the factor VIII molecule, especially its subunit structure and composition.

Whereas the above described picture for von Willebrand's disease seems to prevail in the majority of patients, different manifestations have also been observed in a smaller number of instances. Holmberg and Nilsson [72] showed that 19 of 70 patients with the typical features of von Willebrand's disease had in spite of low factor VIII activity normal factor VIII antigen levels. Interestingly, the pattern of

heredity for these patients seemed to be recessive sex-linked [73]. A similar combination of low factor VIII activity but normal factor VIII antigen was also described by other investigators [9, 76, 93]. An abnormal protein could explain these findings [63]. Apparently even other combinations such as normal factor VIII activity, normal factor VIII antigen but abnormal bleeding times and platelet adhesiveness, and low factor VIII activity, normal factor VIII antigen and normal bleeding times have been observed [53]. Even variations within a family have been described [112]. Final clarification of these rare and different forms of the classic von Willebrand's disease must again await resolution of the structure and substructure of the factor VIII molecule which itself seems to be complex.

Von Willebrand's disease has also been described in dogs [49, 50] and pigs [129]. As in man, the pattern of heredity seems to be autosomal dominant. The animals had low factor VIII levels, prolonged bleeding times and abnormal ristocetin-induced platelet aggregation [50]. Using rabbit anti-canine factor VIII antibodies, the dogs in the family studied by Dodds [50] had decreased factor VIII antigen. These dogs thus seem to resemble the classic von Willebrand's disease known in man.

#### ACQUIRED VON WILLEBRAND'S DISEASE

Acquired von Willebrand's disease has also been described in 8 patients. These were recently reviewed by Shapiro and Hulton [150] in this journal and for details the reader is referred to that article. In principle, the acquired forms resemble the congenital ones, with decreased platelet adhesiveness, low factor VIII procoagulant levels, and, where measured, low factor VIII antigen and abnormal ristocetin-induced platelet aggregation. Most patients had an underlying disease involving the immune system. Only one had a transient form of acquired von Willebrand's disease following pesticide overdose. Possibly one is dealing in all of these cases with an immune inhibitor against the factor VIII protein.

#### OTHER FORMS OF VON WILLEBRAND'S SYNDROME

Besides the classic von Willebrand's disease, characterized by platelet malfunction and factor VIII deficiency, also other forms of the syndrome have been reported. A number of patients with otherwise typical signs of von Willebrand's disease had instead of the factor VIII deficiency a factor IX deficiency [3, 8, 21, 35, 41, 51, 57, 60, 65, 67, 102, 103, 154, 156, 161]. Also combined factor VIII and IX deficiencies with otherwise typical von Willebrand's syndrome features have been noted [71, 81, 102, 153, 163]. In addition, factor VII deficiencies [6, 89, 143, 172] have been observed; one of the reports [89] related to the original von Willebrand families on the Åland Islands. Factor V deficiencies [144], factor X deficiencies [29], and factor XI deficiencies [56, 90, 140, 174] have also been noted, and finally combinations of factor IX and XI [34] as well as combinations of factor VIII and XI [34] have been described. It is at present difficult to assess

whether these bleeding problems are truly part of von Willebrand's syndrome or whether they represent different defects.

### CLINICAL MANIFESTATIONS AND DIAGNOSIS

It was pointed out above that von Willebrand's disease is an inherited bleeding problem which is transmitted in an autosomal dominant manner. Both sexes are equally affected. Clinically and in comparison to classic hemophilia, the over-all hemorrhagic tendency is less severe. Even within a given family the severity of the disease may vary widely [32]. The bleedings are mainly from the skin and mucous membranes, and epistaxis, especially in early life, is one of the most common clinical bleeding manifestations. Bleedings from gums and after shedding of deciduous teeth is frequently encountered. Post-traumatic and postsurgical bleedings are prominent. Prolonged menstrual bleedings and postpartum hemorrhages are also common. In severe forms of von Willebrand's disease, gastrointestinal bleedings and hematuria have been encountered [32, 126]. In contrast to classic hemophilia, hemarthroses are relatively rare. These are encountered only in patients with severe forms of the disorder.

As described earlier, the patients with classic von Willebrand's disease have prolonged bleeding times, abnormal platelet adhesion, defective ristocetin-induced platelet aggregation, and abnormal factor VIII levels, both activity and antigenically related material.

The *bleeding times* are generally abnormal with both the Duke and the Ivy procedures as well as with modifications of both tests [24]. In performing these tests, standardized methods must be adopted and differences in the sensitivities of different tests must be recognized. Generally speaking, the Ivy method seems to be more sensitive than the Duke procedure in patients with von Willebrand's disease [1, 24, 126]. In mild forms, the Ivy bleeding times may be abnormal, while the Duke bleeding times are normal. Also in response to therapy the Duke bleeding time may become normal while the Ivy bleeding time stays abnormal [24, 25, 45, 166]. There seems to be some correlation between the level of factor in plasma and prolonged bleeding times [1, 130], especially when the factor VIII levels are very low. Bleeding times may also vary in one and the same patient when performed at different time intervals. In patients with mild von Willebrand's disease who have normal or borderline normal bleeding times, *aspirin tolerance tests* [136] may be performed. By having the patient ingest as little as 0.65 g of aspirin, bleeding times will be prolonged two hours after intake [136].

Abnormal *platelet adhesiveness* is generally also a typical finding in patients with von Willebrand's disease. But again, variations in technic used for measuring platelet adhesion are important to recognize. For example, the Wright rotator method, the Hellem glass bead column and its modifications are in most instances of von Willebrand's disease normal [124]. In contrast, the Salzman glass bead column technic, its modification by O'Brien and Heywood [124] and its modification by Bowie and associates [31] is generally much more frequently abnormal in patients with von Willebrand's disease. It must be recognized, however, that on rare occasions the Salzman test may also be abnormal in healthy

individuals [1]. In addition, difficulties in reproducibility of this technic have been encountered [1]. Some correlation between platelet adhesion and factor VIII levels has been noted, but this correlation may be weak [1, 7]. It can be improved when concentrates from von Willebrand's plasma are used [107].

Measuring *ristocetin-induced platelet aggregation* is another helpful diagnostic tool in assessing von Willebrand's disease. As pointed out before, since the earlier reports by Howard and Firkin [75] who demonstrated that platelets from normal individuals would aggregate with ristocetin, an antibiotic, and that platelets from patients with von Willebrand's disease failed to respond to ristocetin, many investigators have confirmed this observation. Again this phenomenon is not exclusively found in von Willebrand's disease, but together with other tests, it is an important investigative tool. Also this aggregation response closely correlates to the level of factor VIII in plasma, more specifically to the level of immunologically determinable factor VIII protein. Meyer et al. [111] suggested two procedures, one using platelet-rich plasma and the other using washed normal platelets to which plasma or fractions containing the "von Willebrand factor" are added. Aggregation of the platelets is in both tests measured with a platelet aggregometer. Correlating ristocetin-induced aggregation with platelet adhesion, the authors found good correlation. They felt that these aggregation studies are easier to perform than platelet adhesion tests. Their data also correlate fairly well with the levels of factor VIII related antigen in plasma. Similar data were recently obtained by Weiss [167] and Olson and co-workers [125]. Both groups stressed the usefulness of ristocetin-induced platelet aggregation in screening for von Willebrand's disease, but cautioned that normal aggregation may be found in exceptional cases of von Willebrand's disease, and abnormal aggregation in patients not necessarily suffering from the disease. This includes patients on aspirin. The simple screening of aggregation of patients' platelet-rich plasma by adding ristocetin may then be complemented by specific assays such as described by Meyer et al. [111].

It should be pointed out that platelets of von Willebrand's disease patients do aggregate normally with ADP, collagen, epinephrine and all other aggregation-inducing agents [126]. Ristocetin is the only aggregating agent known today that fails to aggregate von Willebrand's patients platelets.

Recently, Brinkhous and associates [33] described an aggregation test that does not require an aggregometer; this test simply needs a magnifying glass to determine platelet aggregation macroscopically. Washed paraformaldehyde-fixed platelets which are thus devoid of their energy generating mechanism, are aggregated by ristocetin in the presence of normal plasma or hemophilic plasma, but not by von Willebrand patients' plasma. The fixed platelets can be stored for weeks and therefore do not present a problem of preparation each time the test is to be performed. The time it takes for the platelets to aggregate (snowstorm effect) is measured. A close correlation was observed between platelet aggregation and factor VIII activity in patients with von Willebrand's disease.

Measurements of *factor VIII activity* and antigen are also helpful in diagnosing von Willebrand's disease. As was pointed out earlier, low factor VIII levels are found in most patients with von Willebrand's disease. The activity can be measured by either one stage procedures using known hemophilia A plasma as substrate and the partial thromboplastin time as the test system, or by the thromboplastin

generation test [132]. In comparison to classic hemophilia, the factor VIII levels are generally less severely depressed in von Willebrand's disease. In most instances, the levels of factor VIII activity range between 1% and 50% [11, 44, 126, 130], but also levels of 50% to normal are encountered [1, 130]. Generally speaking, some degree of correlation seems to exist between factor VIII activity levels and platelet adhesion, ristocetin induced platelet aggregation, and bleeding times, as was pointed out earlier.

In addition to factor VIII activity, *factor VIII antigen* can be determined using heterologous antibodies—antibodies to human factor VIII produced in rabbits. As was described earlier, patients with von Willebrand's disease were found to have decreased levels of antigen in plasma [66, 78, 109, 158, 179], the antigen levels closely correlating with the activity measurements. Patients with von Willebrand's disease thus seem to lack the large molecular weight carrier protein of the factor VIII molecule, as was mentioned above. This is in contrast to classic hemophilia, where generally normal factor VIII antigen levels are encountered with a decrease of factor VIII activity levels. Again caution must be exercised as to the methodology for the factor VIII antigen determination as well as to the type of antibodies used, heterologous or homologous.

In addition to the above named special tests for diagnosing von Willebrand's disease, *other abnormal test results* can be found in these patients. These abnormalities relate closely to either the lack of factor VIII activity or the disturbance in platelet function. They are, therefore, secondary to the basic findings and for this reason not diagnostic. Low factor VIII levels would obviously not only result in prolonged whole blood clotting times [14] and partial thromboplastin times [94], but also in impaired prothrombin consumption [151]. By the same token and for the same reason, the reaction times of thromboelastogram tracings will be found increased [146]. As an indication of defective platelet function, the speed of clot formation and the maximal thromboelasticity of the thromboelastogram tracings are abnormal [146].

As was mentioned before, not all of these findings need to be present in a classic von Willebrand's disease patient because they may either be variations of the classic form or they present changes from the typical findings in the daily life of a patient. These changes may be variable, but many relate to changes in the factor VIII levels themselves. Factor VIII levels rise, for example, in pregnancy and this has been observed in normal women as well as women with von Willebrand's disease [152, 159]. In our own experience three patients with mild von Willebrand's disease became asymptomatic while on oral contraceptive medication. A rise in factor VIII activity has been described by several investigators in women on anovulatory hormones [11, 131].

Recently, Bachner and co-workers [7] have studied the long-term effect of both estrogen and progesterone therapy in 14 women with von Willebrand's disease. The time of treatment ranged from 6 months to more than 4 years. Independent of dose and type of medication, they observed a marked improvement in the clinical manifestations, such as epistaxis, bruising and melena. Of the laboratory tests conducted, only the bleeding times seemed to become shortened; reproducible increases in factor VIII activity were not noted, and no changes were seen in platelet function parameters. Ulutin [162] made similar observations. These

findings are in contrast to those reported by Glueck and Flessa [59] and Ozsoylu and Corbaciuglu [129], who found significant changes in factor VIII levels and bleeding times in von Willebrand's disease patients following treatment with oral contraceptives. There exists the possibility that differences in hormone dose may be responsible for these discrepancies. But also other stimuli, such as exercise, inflammatory states, hyperthyroidism and epinephrine release have been found to raise the factor VIII activity levels in plasma, as reviewed by Ingram [82]. Any of these conditions can conceivably affect the symptoms and laboratory findings in patients with von Willebrand's disease, especially when they are suffering from mild or moderate forms of the disorder.

### THERAPY OF VON WILLEBRAND'S DISEASE

Since classic cases of von Willebrand's disease seem to represent a defect in the factor VIII molecule, more specifically the large molecular weight protein part of the molecule, blood or blood fractions containing this part of the factor VIII molecule are expected to correct the hemostatic defect. For this reason, fresh frozen plasma, Cohn fraction I-O, cryoprecipitate and commercial factor VIII concentrates will not only correct the clinical bleedings, but also normalize most of the abnormal laboratory findings. Only the Ivy bleeding times may remain prolonged following treatment, while the Duke bleeding times, platelet adhesiveness, ristocetin-induced platelet aggregation and factor VIII activity and antigen may become normal [67].

In contrast to classic hemophilia, where in response to treatment the highest level of factor VIII activity is reached immediately following infusion, the peak activity in von Willebrand patients is reached only after about 4-8 hours in severe cases and 12-48 hours in milder forms [126]. In addition, the level obtained is usually far greater than anticipated from the dose of factor VIII given. For this reason, much smaller doses of factor VIII are required to treat von Willebrand's disease, as compared to classic hemophilia. Hardisty and Ingram [68] recommended as little as 5 ml/kg of plasma body weight as the priming dose, followed by 5 ml/kg every 24-48 hours. This hyper-responsiveness to factor VIII therapy can also be achieved with aged plasma, in which factor VIII activity is greatly diminished [126], with hemophilic plasma, serum or even adsorbed serum, as was pointed out above. Owen and associates [126] even found a better response with hemophilic plasma than with normal plasma. As was mentioned before, all of the fractions of blood listed contain the carrier protein of the factor VIII molecule which apparently is responsible for stimulating the synthesis of factor VIII activity when infused into patients with von Willebrand's disease. Furthermore, and in contrast to classic hemophilia, the factor VIII level generated in von Willebrand patients does not disappear within a few hours, but rather remains present for about 24-48 hours.

The diagnosis of von Willebrand's disease by means of laboratory tests may at times be difficult and confusing because of these many variations. For this reason, the patient's factor VIII response to therapy may at times become the most important criterion for the proper diagnosis [126].



As would be expected, no normalization of the hemostatic defect can be achieved in von Willebrand's disease with platelet concentrates.

Those forms of von Willebrand's syndrome which have presented with other coagulation factor deficiencies have responded well to fresh frozen plasma and whole blood, but the effect of fractions, such as factor IX concentrates, on patients with factor IX defects have not been reported. Nothing is known yet about whether these fractions affect abnormal bleeding times as well.

In conclusion, it can be stated that the last few years have yielded us information that has greatly enhanced our knowledge of von Willebrand's disease, not only from the diagnostic point of view, but also from the management point of view. The unraveling of the pathophysiology of the rare forms of von Willebrand's syndrome remains an uncompleted task.

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