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HEMOPHILIA AND VON WILLEBRAND'S DISEASE

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The relative importance of factor VIII in hemostasis is obvious from the problems observed in the two common deficiency states of this coagulation factor, Hemophilia A and von Willebrand's disease. Effective replacement therapy is available and has significantly improved the clinical status of these patients. However, along with the control of hemostasis serious complications associated with therapy have been observed. Within the last decade, our understanding of the structure and function of the factor VIII complex has advanced significantly. From these studies a clearer picture of the abnormalities of the factor VIII complex associated with these diseases has emerged. The application of newer technologies to the study of hemophilia offers the possibility of different modes of diagnosis and treatment. Today I would like to discuss our current understanding of the structure of the factor VIII complex, hemophilia A and von Willebrand's disease. Hemophilia B, another X-linked bleeding disorder that results from factor IX deficiency, will not be discussed.

The Factor VIII Complex

Considerable confusion has existed about the molecular structure of the factor VIII complex (1,2). This was mainly the result of trying to understand how both hemophilia and von Willebrand's disease could both result in the deficiency of factor VIII although they were clearly two distinct genetic diseases with different clinical presentations and modes of inheritance. Hemophilia A is inherited as an X-linked recessive disorder, whereas von Willebrand's disease is inherited in an autosomal dominant fashion. As shown in Fig. 1, a difference was also apparent when an antibody raised in rabbits against factor VIII was used to study these two groups of patients.

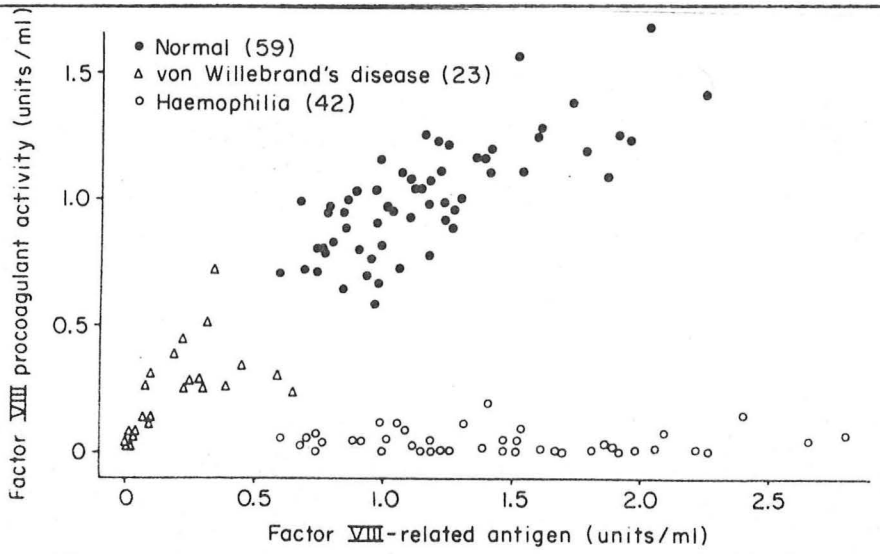

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Fig. 1. The relationship of factor VIII procoagulant activity and factor VIII-related antigen in the plasmas of normal individuals, patients with von Willebrand's disease and patients with hemophilia.

In patients with von Willebrand's disease, the level of the immunologically detected material, named factor VIII-Related Antigen, was lowered to a similar extent as the factor VIII procoagulant activity. In patients with hemophilia, normal or elevated levels of cross-reacting material were detected although the level of factor VIII procoagulant activity was very low. This led to the hypothesis that these diseases affected the factor VIII molecule in different ways - in von Willebrand's disease, a low amount of a normal molecule was present,

whereas in hemophilia, a functionally abnormal molecule was synthesized. It is now known that this is not the case. The factor VIII complex is composed of two different molecules that account for its different roles in hemostasis. The components of the factor VIII complex as well as the assays which measure each of these components are listed in Table I.

TABLE I. COMPONENTS OF THE FACTOR VIII COMPLEX

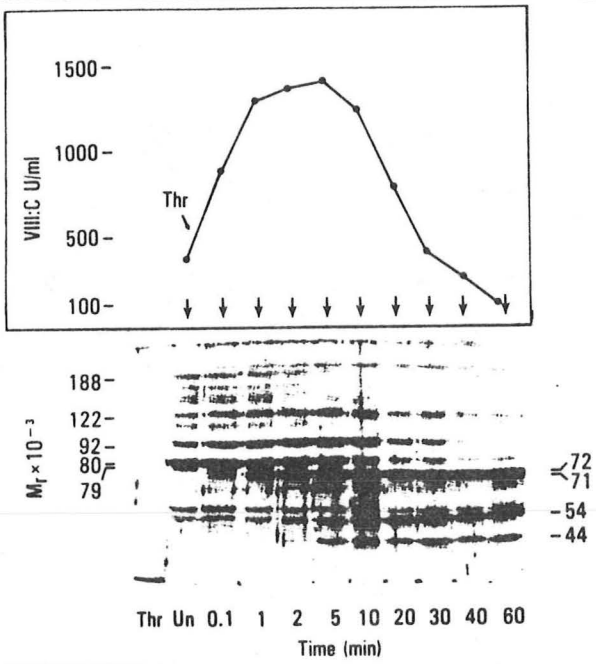
Factor VIII Procoagulant Protein
- Factor VIII procoagulant activity (VIII:C)
- Factor VIII procoagulant protein (VIII:CAg)

von Willebrand Factor (vWF) Protein
- Factor VIII-related antigen (VIIIR:Ag)
- Ristocetin cofactor (VIIIR:RC)

Considerable evidence now supports the model that the factor VIII complex is composed of these two molecules. The two proteins can be separated by chromatography in high ionic strength buffers or in the absence of calcium (1-4). The functional activity of each protein can be measured in the absence of the other molecule and specific antibodies recognize each protein independently (5-9). Recently, complementary DNA (cDNA) copies of the messenger RNAs for each of these proteins have been cloned and characterized (10-13). The factor VIII procoagulant protein is responsible for the procoagulant activity (VIII:C) measured in routine coagulant assays. This protein is recognized by human antibodies from hemophiliacs who develop antibodies to factor VIII or from patients with spontaneous inhibitors of factor VIII (5). The immunologically detected antigen is designated VIII:CAg. It is this part of the factor VIII complex that is diminished or absent in patients with hemophilia (2). The von Willebrand factor protein is recognized by antibodies against factor VIII raised in rabbits and is designated VIIIR:Ag. It was this material that was recognized in the early studies of the plasma from patients with hemophilia. This protein is functionally measured as the ristocetin cofactor (VIIIR:RC) and can support the ristocetin-induced aggregation of washed normal platelets.

The factor VIII procoagulant protein accounts for about 1% of the mass of the factor VIII complex. It is encoded by a gene on the X chromosome and appears to be synthesized in the liver, primarily in the sinusoidal endothelial cells (14). Because of the relatively low levels of this protein in plasma it has been relatively difficult to purify. Estimates of its molecular weight vary from 250,000 to less than 50,000 (5-9). Often the purified preparations from either human or bovine plasma contain a collection of proteins which occur even when the protein is purified in the presence of protease inhibitors. When the purified procoagulant protein is treated with thrombin, procoagulant activity is initially enhanced and then inactivated. As shown in Fig. 2, the changes in the VIII:C activity associated with thrombin treatment correspond to the generation and degradation of a 92,000 dalton polypeptide (15,16).

The complexity of the factor VIII procoagulant protein structure and activation has been somewhat resolved by cloning the gene for this protein (10-13). Two groups, both associated with different genetic engineering companies, recently published this work. Full-length cDNAs corresponding to the 9 kilobase (1 kilobase = 1000 base pairs) factor VIII mRNA were selected to determine the nucleotide sequence and deduce the primary amino acid sequence of the protein. These studies show that the protein is 2351 amino acids in length. A 19 residue signal sequence is cleaved from the amino-terminus of the protein to yield a mature protein of 2332 amino acids with a calculated molecular weight of 264,763. Within



from ref. 16

Fig. 2. Changes in VIII:C activity and polypeptide pattern of reduced VIII:C following treatment with thrombin.

the sequence of the factor VIII molecule, three types of domains are apparent. As shown in Fig. 3, the A domain is 330-380 amino acids long and is repeated three times within the sequence, twice at the amino-terminal end of the molecule and again near the carboxy-terminus of the molecule. At the far carboxy-terminus, the C domain of about 160 amino acids is duplicated. A unique sequence of 925 amino acids, designated the B domain, divides the repeated sequences. Of interest, the A domains of the factor VIII molecule are about 30% homologous to the triplicated domain structure of another plasma protein, ceruloplasmin.

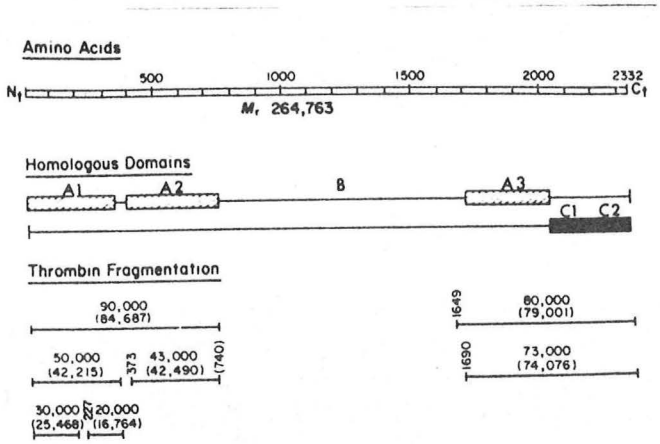


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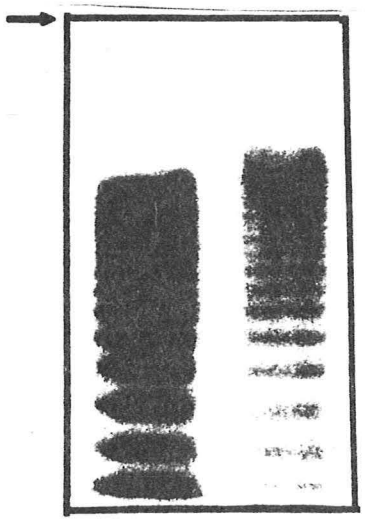
Fig. 3. Line diagram of the factor VIII procoagulant protein.

In order to understand the mechanism by which proteolysis results in activation of the procoagulant activity, Vehar et al. (12) isolated polypeptides resulting from partial thrombin digestion and subjected them to amino-terminal amino acid sequencing. By aligning these sequences with those predicted from the entire amino acid sequence deduced from the cDNA they were able to localize the proteolytic fragments. The 90,000 dalton peptide associated with the activation of the coagulant activity is derived from the amino-terminal end of the molecule and contains the first two A domains. An 80,000 dalton peptide is released from the

carboxy-terminal end of the molecule and contains the last of the A repeats as well as the repeated C domains. Smaller peptides are the resultant of further proteolysis within these larger fragments. It appears that the activation of the factor VIII molecule is accomplished by cleaving the B domain from the intact molecule, the remaining fragments being catalytically active. Recently, Fulcher has shown that both the 92,000 and 80,000 dalton peptides are required for procoagulant activity. A monoclonal antibody that recognizes an antigenic site on the 92,000 dalton fragment, inactivated the coagulant activity. Another monoclonal antibody that recognized the 80,000 dalton fragment could also inactivate the coagulant activity (8).

The von Willebrand factor (vWF) protein represents most of the mass (>99%) of the factor VIII complex. The most striking feature of the protein as it circulates in plasma is its large size. Early studies showed that the factor VIII complex in plasma has a molecular weight of greater than 1 x 10^6 (17). The complex retains this large size in the presence of denaturing agents and detergents, however can be dissociated into its subunit structure by reducing agents. This suggested that the subunits were held together by disulfide bonds between the subunits. Analysis of the factor VIII structure by gel electrophoresis and antibody blotting indicates that the heterogeneous population of vWF protein multimers circulates in plasma (18). This pattern is shown in Fig. 4. These multimers vary in size from 850,000 to greater than 12 x 10^6 daltons.



from ref. 18

Fig. 4. Multimer Structure of von Willebrand factor in normal plasma (left) and normal platelets (right).

The vWF protein is encoded by the vWF gene on chromosome 12 (19). The vWF protein is synthsized in both megakaryocytes and endothelial cells. In addition to the vWF protein circulating in plasma associated with the factor VIII complex, vWF multimers are stored in platelets and in a special organelle in endothelial cells, the Weibel-Palade bodies, and are incorporated into the extracellular matrix surrounding endothelial cells. The interaction of vWF protein with platelets is responsible for ristocetin-induced platelet aggregation. This affect is mediated through interaction of vWF protein with GP Ib, a cell surface glycoprotein of the platelet. Antibodies against this glycoprotein can inhibit this reaction, and platelets from patients with the Bernard-Soulier (giant platelet) syndrome that lack GP Ib have abnormal ristocetin-induced binding of vWF to platelets (18).

The biosynthesis of von Willebrand factor within endothelial cells involves multiple steps within many intracellular compartments. Initial studies by Lynch and later studies by Wagner have shown that vWF protein is initially synthesized as

a precursor of 260,000 daltons which dimerizes within the endoplasmic reticulum (20,21). The dimers are then transported to the Golgi apparatus where the carbohydrates are processed from a high mannose to complex type, the apparent molecular weight now increasing to 275,000. The dimers now form large multimers by disulfide bond formation and the subunit polypeptide is cleaved to yield a 220,000 dalton vWF subunit. Most of the secreted form of the vWF protein is composed of the 220,000 molecular weight subunit (22-24). The vWF protein that is stored within the cell has the same distribution of multimers as that found in the plasma. This pathway is outlined in Fig. 5.

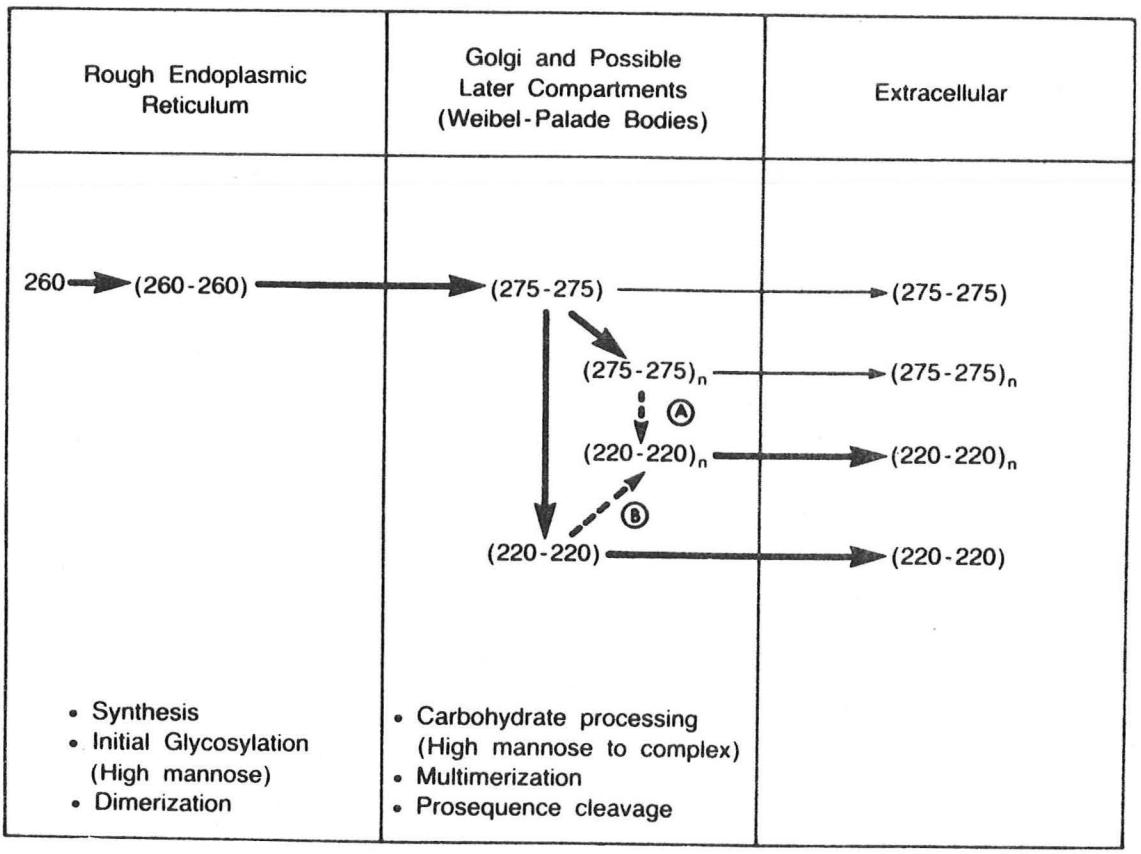


Fig. 5. Pathway for the synthesis and secretion of von Willebrand factor in endothelial cells.

Recently cDNAs for vWF protein have been isolated by both Lynch and Ginsburg that span over 8 kb of the 9.5 kb mRNA (19,25). Limited DNA sequence from these cDNAs has shown that the carboxy-terminal sequence that has been determined from analysis of the purified protein agrees with the predicted protein sequence of vWF prior to the termination codon. This indicates that the proteolytic processing of the vWF molecule must occur at the amino-terminal end of the polypeptide. Determination of the entire sequence of the cDNA and thus the entire primary amino acid sequence of the vWF protein should provide a great deal of insight into the structure and function of this protein.

Hemophilia A

The recognition of congenital bleeding disorders dates back to the writings of ancient Egypt. The first description in the medical literature of hemophilia is credited to John Conrad Otto in the early 1800's. Hemophilia A is inherited in a

classic X-linked recessive pattern. It is estimated that hemophilia A occurs in about 2 per 10,000 males (26). Approximately one-third of the cases arise by the occurrence of a new mutation. Females are usually carriers and have a 50% chance of passing the defective gene to their offspring. Males that inherit the hemophilic gene from their mothers are affected whereas females are carriers. The daughters of men with hemophilia are obligate carriers, while their male offspring are unaffected as they have inherited the Y chromosome from their father. Fig. 6 lists the pedigree of one of the most famous families with hemophilia, the decendants of Queen Victoria of England (27). (It is unknown whether the hemophilia in this family was due to factor VIII or factor IX deficiency). Queen Victoria was a carrier of the hemophilia gene, probably the result of a new mutation. She passed the defective gene to one son, Leopold, and two daughters, Alice and Beatrice. Through the marriages of her daughters the mutant gene was introduced into the bloodlines of the Russian czars and the ruling family of Spain.

The frequency and severity of bleeding episodes in hemophilia can be predicted from the factor VIII procoagulant level (26,28). The reference standard of 100% corresponds to 1.0 unit per ml of factor VIII activity. Individuals with factor VIII levels less than 1% are severely affected and suffer major bleeds requiring replacement therapy several times per month, whereas patients with factor VIII levels greater than 20% might only have difficulty after major surgery and are often not diagnosed. As shown in Table II, patients with factor VIII levels between these two levels have varying degrees of bleeding problems.

The primary bleeding problems of hemophiliacs relates to joint and muscle hemorrhages which can lead to disabling long-term sequelae as well as prolonged and dangerous postoperative bleeding. Excessive bleeding from cuts and abrasions is usually not a problem due to normal platelet function. In addition to bleeding problems, the hemophilic patient and his family face a large array of social and economic problems (26). These will not be addressed in this discussion but constitute a major area in which these families need support.

TABLE II. RELATIONSHIP OF PLASMA FACTOR VIII LEVELS TO THE SEVERITY OF BLEEDING MANIFESTATIONS

	Plasma level of Factor VIII	Bleeding Manifestation
	>40	None
Mild	10-40	Bleeding after major injury
Moderate	5-20	Bleeding after minor injury and surgery
Moderately Severe	1-5	Severe bleeding after minor injury Occasional hemarthroses
Severe	<1	Spontaneous hemarthroses and muscle hemorrhages

Bleeding Episodes

Bleeding into the joints (hemarthrosis) is one of the most common problems found in hemophilia (26,28-32). This bleeding is often spontaneous or associated with only minimal trauma. The knees, elbows, ankles and wrists are the most commonly affected joints. The onset of bleeding is associated with an "aura" that last 1-2 hours. A sense of warmth, tingling or anxiety may be noticed by the patient. Discomfort and limitation of the movement of the joint then occur, followed by pain, joint swelling, warmth and severe limitation of motion. With repeated episodes of bleeding into the joint, especially when therapy has been inadequate or delayed, a chronic synovitis can develop. This can lead to an

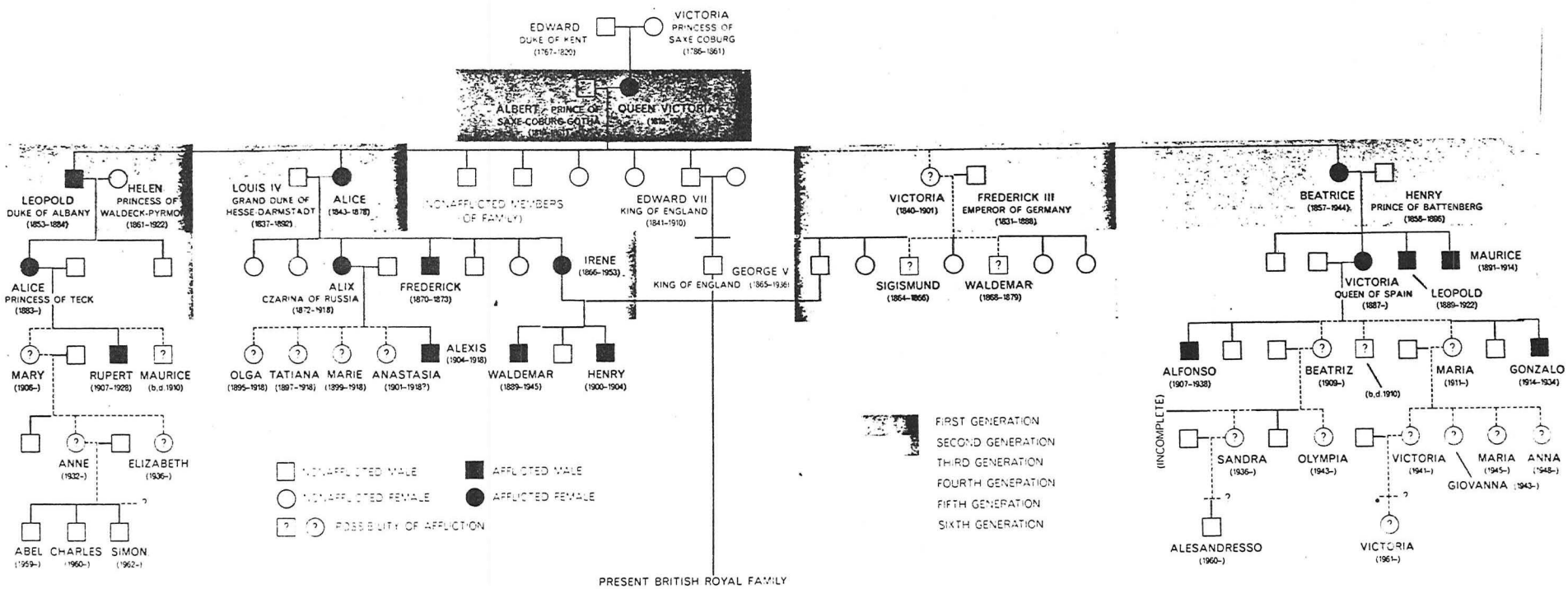


Fig. 6. Six-Generation Record of Known and Possible Hemophilia among the Decendants of Queen Victoria.

increased frequency of hemorrhages into the joint and the further development of hemophilic arthropathy.

Intramuscular hematomas also commonly occur (26,28). Small hematomas may resolve spontaneously, however larger hematomas can cause several problems. A large loss of blood, such as that associated with a retroperitoneal bleed, can cause a significant drop in the hematocrit. Nerve compression can result from bleeds into the forearm, calf and iliopsoas muscle. Bleeding into the muscles and soft tissue of the throat and neck can obstruct the airway. Pseudotumors can result from hematomas that have been inadequately treated. These can become organized, grow by rebleeding and result in pressure necrosis of surrounding bone. One of the most frequent presentations of an intramuscular hematoma is bleeding into the iliopsoas muscle. Examination reveals tenderness in the groin and iliac fossa and if the bleed is large, a mass can be appreciated. Pain is quite intense and can be confused with appendicitis. To minimize the distention of the muscle, the leg is held in flexion at the hip. Compression of the femoral nerve results in paresthesias over the anterior thigh.

Hematuria unrelated to trauma is very common and most episodes have no identifiable cause. Epistaxis is an occasional problem in some patients. Bleeding often occurs after the loss of teeth, the eruption of new teeth, or following dental work. Gastrointestinal hemorrhage is rare, and an etiology for the bleeding should be sought.

One of the most dangerous complications of hemophilia is intracranial bleeding. Cerebral hemorrhage was the most common cause of death in a recent report from the hemophilia centers in the United Kingdom, accounting for 29% of the deaths from 1976-1980 (33). In another series, 71 episodes in CNS bleeding were found out of a population of 2500 patients over a ten year period (34). Only half of these episodes were associated with trauma. As shown in Table III, CNS bleeding occurred in all ages and was more common in patients with factor VIII levels of <1%. Approximately one-half of the patients had a symptom-free period of greater than 24 hours between the trauma and the onset of symptoms. The mortality rate was 34%. Among the survivors, 15 of 37 patients were left with significant neurologic sequelae.

TABLE III. AGE AND SEVERITY OF HEMOPHILIA AT ONSET OF CNS BLEEDING

Ages	Factor VIII Deficiency (percent of normal)		
	0-1	2-5	6-20
<3	14	4	0
4-9	7	2	2
10-17	11	0	0
≥18	15	0	1

Replacement Therapy

In 1964 Pool described the preparation of a factor VIII-rich fraction of plasma by cryoprecipitation. This technique began an era in which effective replacement therapy for hemophilia could be offered. Therapy with factor VIII preparations are administered for the control of bleeding episodes, prior to surgery or prophylactically in some situations. The amount and frequency of administration are dependent on the nature of the problem being managed. For mild hemorrhages, such as early hemarthrosis or hematoma, a single infusion sufficient

to raise the factor VIII level to 30% is administered (26,28). As the infused factor VIII has a half-life of between 8 and 12 hours, a single infusion is usually sufficient to halt the bleeding in mild hemorrhagic lesions. For more advanced joint or muscle bleeding, a level of 50% factor VIII is desired. Often several days of therapy will be required for such lesions to resolve. For surgery or life-threatening situations, such as intracranial bleeding, an infusion of factor VIII sufficient to raise the level to 80-100% is initially given and the level is maintained above 30-50% by the administration of factor VIII preparations every 8 to 12 hours. In such situations, the response to factor VIII infusions should be monitored by measuring the factor VIII procoagulant activity after infusion of the concentrate. Because of the significant risk of complications from intracranial bleeding and the delay in onset of symptoms, all episodes of head trauma or symptoms suggestive of CNS bleeding should be treated aggressively for at least 48 hours.

Factor VIII replacement therapy is calculated in units, where 1 unit is the amount of factor VIII in 1 ml of pooled human plasma. The amount of factor VIII which needs to be administered is based on the plasma volume of the patient and the desired level of factor VIII. A simple formula is that each unit of factor VIII infused per kg body weight yields a 2% rise in the plasma factor VIII level. For a 50-kg man with an early hemarthroses, 750 units of factor VIII would yield a 30% factor VIII level.

Two types of factor VIII replacement products are primarily used for treatment (26,28). Cyroprecipitate is made from fresh-frozen plasma after slow thawing at 4°C. Each bag of cyroprecipitate comes from a single unit of plasma and contains about 70-100 units of factor VIII. Because of the difficulty in storing cyroprecipitate and the variability of the factor VIII concentration it is not used in the treatment of most hemophiliacs. Because of the lower risks of infectious complications, primarily because it is derived from single donors, it is mainly used for thre treatment of mild hemophiliacs who require infrequent infusions of factor VIII. It is also used for the management of patients with von Willebrand's disease because it has a higher concentration of von Willebrand's factor than factor VIII concentrates.

Factor VIII concentrates are lyophilized preparations of factor VIII prepared from large pools (>1000 donors) of normal plasma. Each vial contains a defined amount of factor VIII activity (usually 250-1500 units) that can be reconstituted in a small volume. The lyophilized preparation is stable in the refrigerator for months to years and at room temperature for weeks to months. The availability of such preparations has made home therapy possible.

The most important aspect of replacement therapy is to provide access to factor VIII therapy as soon as possible after a bleeding complication has developed. For most severe and moderately severe hemophiliacs this has been accomplished by instituting programs of home-based self therapy (35). The patient and has family receive intensive education and a thorough evaluation in hemophilia centers. At home the patient administers the appropriate amount of factor VIII concentrate at the first sign of a hemarthrosis, hematoma or other minor bleeding complication. Usually prompt therapy prevents further progression of the bleeding and no further factor VIII therapy is required. For more serious bleeding, surgery or other problems the patient goes to the hemophilia center. Most centers have hematologists, orthopedic surgeons, oral surgeons, physical therapists, social workers and other personnel to assist with the problems of the hemophiliac. As Table IV shows, they can lead to a more productive life at a lower cost (26).

TABLE IV. EFFECT OF COMPREHENSIVE CARE PROGRAM ON 220 MASSACHUSETTS HEMOPHILIACS

	Before	After
Loss of time (in days) from work or school	12.2	5.5
Number of admissions to hospitals	2.1	0.3
Number of days spent in hospital	6.4	0.9
Annual costs per patient	$ 8,850	$ 5,810

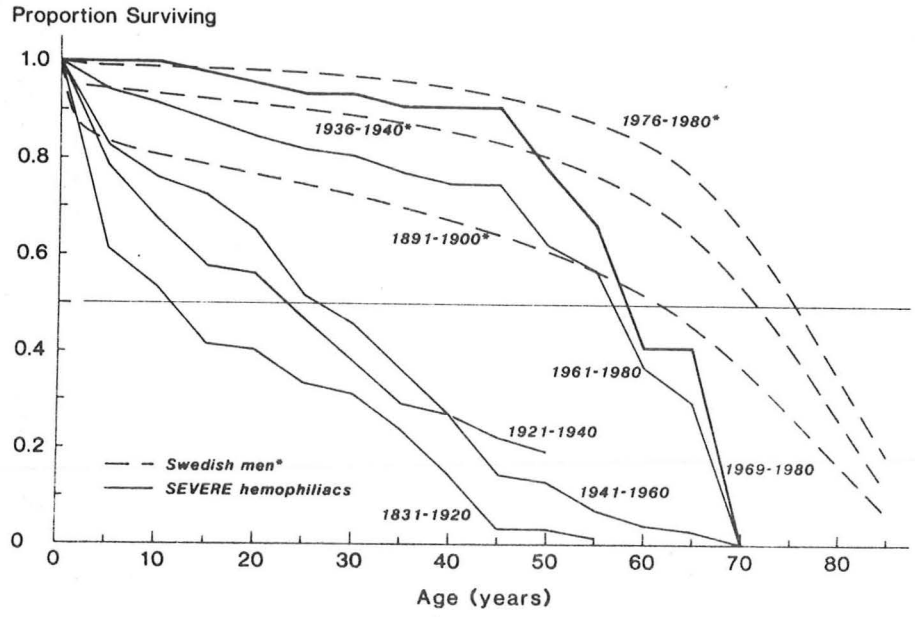
The ready availability of such factor VIII preparations has made a significant impact on the care of patients with hemophilia. Together with better medical care and recognition of associated problems, the increased mortality associated with this disease is declining. In a study by Larsson, the life expectancy of Swedish hemophiliacs was estimated for the period 1831-1980 (36). Among severe hemophiliacs median life expectancy increased five-fold, from 11 years during the period 1831-1920 to 56.8 years during 1961-1980. For moderate hemophilia, life expectancy increased from 27.5 to 71.5 years for the same periods. Over the last 12 years of the study (1969-1980), the death rates for patients with severe hemophilia under the age of 45 were very close to that of the normal population. The life expectancy curves for severe hemophiliacs are shown in Fig. 7 and compared with those of the normal Swedish population for these same periods.

Complications of Therapy

There are several potential problems associated with the use of factor VIII. Allergic reactions, liver disease, acquired immunodeficiency syndrome (AIDS) and inhibitors of factor VIII have been encountered. Mild allergic reactions or rarely bronchospasm or anaphylactoid reactions are seen after the administration of cryoprecipitate. With the use of factor VIII concentrates, such reactions are much more rare (26).

Hepatitis B and non-A non-B hepatitis frequently occur in hemophiliacs (37,38). Antibody to hepatitis B surface antigen is found in 80-95% of of hemophiliacs (37). Anti-HBsAg was found more frequently in those patients that had received frequent transfusions with factor VIII products. The frequency of HBs antigenemia is between 5-20%. Among those patients with HBs antigenemia, 14 out of 49 patients had antibody to the hepatitis-B virus associated delta (δ) agent (39). These viral agents probably account for the high frequency (greater than 50%) of abnormal liver functions that are found in hemophiliacs.

One of the greatest concerns among patients with hemophilia is the occurrence through 1984 of over 50 cases within the United States of the acquired immunodeficiency syndrome (AIDS) (40,41). The incidence rate for AIDS is estimated to be 3.6 cases per 1000 hemophilia A patients by the Center for Disease Control (41). Both viral isolation and epidemiologic studies of serology indicate that AIDS is caused by the T-cell lymphotropic retrovirus known as human T lymphotropic virus type III (HTLV-III) or lymphadenopathy-associated virus (LAV). Several studies have examined the plasma of assymptomatic hemophiliac patients and a very high frequency of antibodies to HTLV-III has been detected. Among patients in Britain, 34% of hemophiliacs receiving pooled clotting factors were positive (42) whereas studies from the United States and Europe have detected antibodies to HTLV-III in over 70% of hemophiliacs (43-46). This frequency is much higher than that found in healthy homosexual men, which is around 17%. Anti-HTLV-III in one study was found in all hemophiliacs in 1984 that had received factor VIII concentrate, however



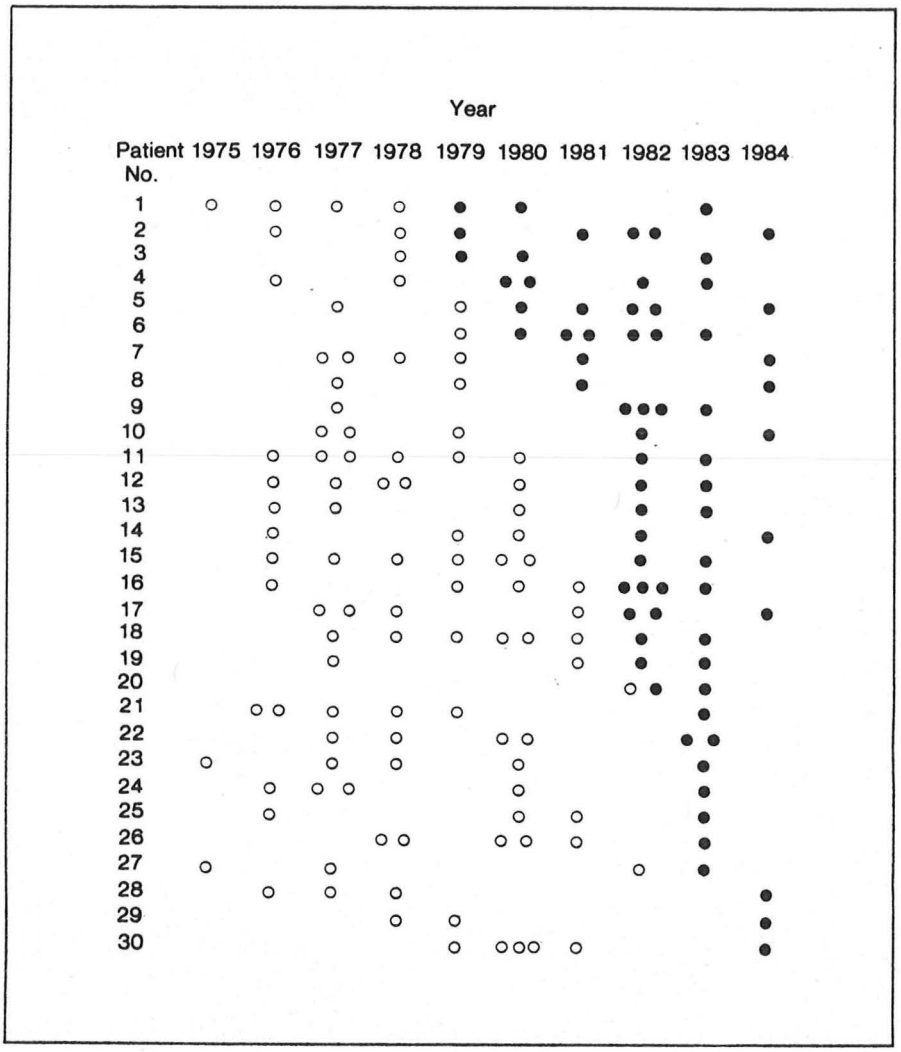
from ref. 36

Fig. 7. The proportion of surviving hemophiliacs from 1831-1980 (solid lines). The corresponding curves for Swedish men is indicated (dashed lines).

anti-HTLV-III was also found in 2 out of 6 patients that had just received cryoprecipitate (>300 units/kg/year) (45).

In addition to the high prevalence of antibodies to HTLV-III, many assymptomatic patients with hemophilia have been noted to have abnormalities in T-lymphocyte populations (47-50). Before the availability of tests to determine the anti-HTLV-III status of hemophiliacs, several groups reported a relative decrease of helper T cells. This was found in over 50% of hemophiliacs that used factor VIII concentrates. The early natural history of HTLV-III infection has been recently reported by Eyster, et al., who describe serial antibody studies in a group of patients that were discovered to be anti-HTLV-III postitive in 1983-1984 (46). As shown in Fig. 8, none of the seropositive patients had detectable antibody until 1979. Two of these patients, No. 4 and No. 18, who seroconverted in 1980 and 1982, respectively, developed AIDS or an AIDS-like illness. As listed in Table V, when examined in 1984 lymphadenopathy and a decrease in helper T cells were noted in those patients who seroconverted before 1981. It is unknown whether these responses are part of the immune response to the HTLV-III antigens or result from chronic active HTLV-III infection.

The source of the HTLV-III virus exposure is from the factor VIII concentrates. These products are derived from a large pool of donors. Levy has shown that virus added to plasma is stable after cryoprecipitation and remains infectious through the process used to prepare factor VIII concentrates (51). In the lyophilized state, the retrovirus is sensitive to heat treatment. Heating factor VIII concentrates that had been supplemented with retroviruses prior to lyophilization to 68°C for several hours resulted in a substantial decline in the retrovirus titer. Recently the results of a two year treatment period of heat-treated factor VIII concentrate virus non-heated concentrate were reported (52). Eighteen previously untreated hemophiliacs were given the heat-treated product and compared to a matched group of previously untreated patients that had been treated with equivalent doses of non-heated products. The results are shown



from ref. 46

Fig. 8. Seroconversion of 30 recipients of factor VIII concentrate who had antibodies to HTLV-III in 1983-1984.

TABLE V

Clinical, Hematologic, and Immunologic Parameters During 1984 in 30 Hemophiliacs by Year of Seroconversion to HTLV-III* Antibodies

Midpoint in Time for HTLV-III Seroconversion	No. of Patients Evaluated	No. (%) of Patients With Each Condition in 1984				Mean SE Phenotypic T Cells/cu mm†	
		Lymphadenopathy	Splenomegaly	Lymphocytopenia, <1,000 Cells/cu mm	Thrombocytopenia, <100,000 Cells/cu mm	Helper	Suppressor
Before 1981	10	7 (70)	3 (30)	4 (40)	3 (30)	307 ± 64	417 ± 142
During 1981	11	1 (9)	0 (0)	1 (9)	1 (9)	520 ± 88	699 ± 135
After 1981	9	1 (11)	3 (33)	1 (11)	1 (11)	641 ± 94	625 ± 100
P value‡	. . .	.004	.91	.11	.27	.008	.24

from ref. 46

in Table VI. None of the patients treated with heated factor VIII developed antibodies to HTLV-III whereas in the control group 17% of the patients had detectable antibody.

TABLE VI

CHARACTERISTICS OF TWO GROUPS OF PATIENTS TREATED WITH HEATED FACTOR VIII CONCENTRATE VERSUS NON-HEATED AND PREVALENCE OF ANTIBODIES TO LAV

–	Test group (n = 18)	Control group (n = 29)
Mean age (yr) (range)	9 (0·25–58)	13 (2–50)
Factor VIII concentrate	Heated (US)	Non-heated (US)
Period of treatment	Dec 1982–June 1984	1982–1984
Mean total dose (IU) (range)	9711 (240–66 720)	7700 (1000–83 540)
Antibodies to LAV	0/18	5/29 (17%)

from ref. 52

Acquired inhibitors of factor VIII occur in about 8-14% of patients with hemophilia A (53,54). These are antibodies that can neutralize the factor VIII procoagulant activity and thus interfere with factor VIII replacement therapy. Such inhibitors are quantitated in Bethesda units/ml. (This is measured by mixing a test plasma with normal plasma and measuring the residual factor VIII activity after incubation at 37°C for 2 hours.) Two patterns of immune response to factor VIII have been observed (55,56). In more than half of those patients with inhibitors, antibody titers are very high and persist for years even in the absence of transfusion. In these high-responders, an anamnestic response is often seen following an infusion with factor VIII concentrate. In contrast, a low-responder group has low levels of antibody (<5 Bethesda units/ml) that do not rise following transfusion. Often the antibodies spontaneously disappear. For factor VIII replacement therapy, the low responders do not create a serious problem. Factor VIII concentrates can be administered as needed to control bleeding episodes. In contrast, those patients with high antibody titers are a difficult problem. Alternative forms of therapy can be used for mild bleeding episodes. In more critical situations, high levels of factor VIII can be given for 5-7 days until an anamnestic response reaches its maximum. At this point other forms of therapy can be given. The most common alternative form of therapy is prothrombin-complex concentrate (PCC) (57). Such preparations contain "bypassing activity" that is capable of promoting hemostasis in patients with inhibitors. For major bleeding episodes that are poorly controlled with PCC, activated preparations of PCC can be used. Other approaches to hemophiliacs with inhibitors include the continuous administration of factor VIII to induce immune tolerance, the use of purified porcine factor VIII and plasmapheresis (58,59).

Alternative Therapy for Hemophilia

For some surgical procedures or bleeding episodes, therapy with agents other than factor VIII can be used successfully. For dental work, the antifibrinolytic agent, epsilon-aminocaproic acid (EACA) can be administered either together with factor VIII therapy or by itself. Treatment with EACA alone is usually sufficient to control bleeding during dental work in patients with mild to moderate hemophilia (26).

Another approach would be to use an agent that could increase the endogenous level of factor VIII, perhaps by the release of material stored in body cells. It appears that the agent 1-deamino-8-D-arginine vasopressin (DDAVP) is capable of stimulating the release of factor VIII and von Willebrand factor in normal individuals and patients with hemophilia (60-63). This approach is primarily

useful in those patients with mild to moderate hemophilia that have measurable levels of factor VIII, however it is below the level necessary for hemostasis. The intravenous administration of 0.4 μg/kg can result in a significant rise in the factor VIII level sufficient to perform dental work or minor surgery (60). The half-life of the released material is similar to that of infused factor VIII so that DDAVP should be administered every 12 hours to maintain the level of factor VIII procoagulant activity. Fig. 9 shows the factor VIII response in four patients with mild hemophilia who received DDAVP. Administration of DDAVP by the intranasal route also resulted in a rise in factor VIII activity, however the response was suboptimal and not as predictable as intravenous administration (63). No problems with hyponatremia have been encountered in hemophiliacs given this drug. Another agent which has been shown to result in increased factor VIII levels is the androgen derivative, danazol (64).

Factor VIII Production by Genetic Engineering

As a result of the risk of hepatitis and AIDS associated with factor VIII concentrates, a major effort is underway to try to synthesize factor VIII by

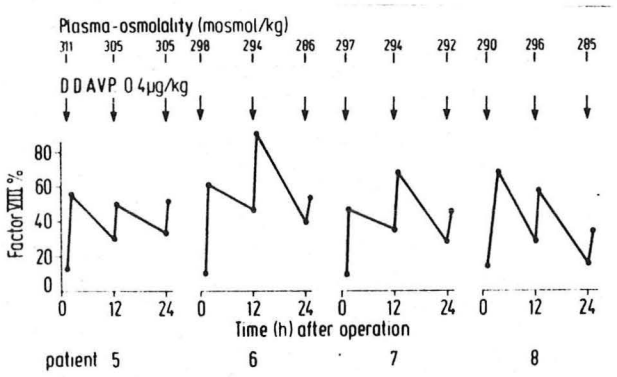


Fig. 9. Factor VIII Procoagulant Activity after DDAVP infusion in four patients with mild hemophilia.

genetic engineering. This approach has attracted attention not only in the scientific community, but also in business circles. In the initial reports of the cloning of factor VIII both Genentech in San Francisco and Genetics Institute in Boston showed that when DNA encoding the factor VIII messenger RNA was introduced into cultured mammalian cells under the appropriate conditions, factor VIII activity could be detected in the culture medium (10-13). As shown in Table VII the amount of factor VIII produced was low, but was inactivated by antibodies directed against the factor VIII molecule. This material was also shown to bind quantitatively to von Willebrand factor protein and could be dissociated from this complex by 0.25 M $CaCl_2$. Similar to plasma factor VIII, the material derived from the media was initially activated and subsequently inactivated by incubation with thrombin. These studies indicate that it is possible to produce factor VIII in genetically engineered cells. Further efforts are now underway to increase the production of the factor VIII material so it can be tested in animals and ultimately in humans. Trials in hemophiliacs could begin within two years. Such a product would eliminate many of the serious complications arising from therapy with factor VIII concentrate derived from pooled plasma.

Carrier Detection and Prenatal Diagnosis

Accurate identification of the assymptomatic carrier for hemophilia is important both for the daughter of a known hemophilia carrier who has a 50% chance of inheriting the hemophilic gene from her mother or the mother of a sporadic case of hemophilia. In this instance, many of the cases are actually the result of a

TABLE VII

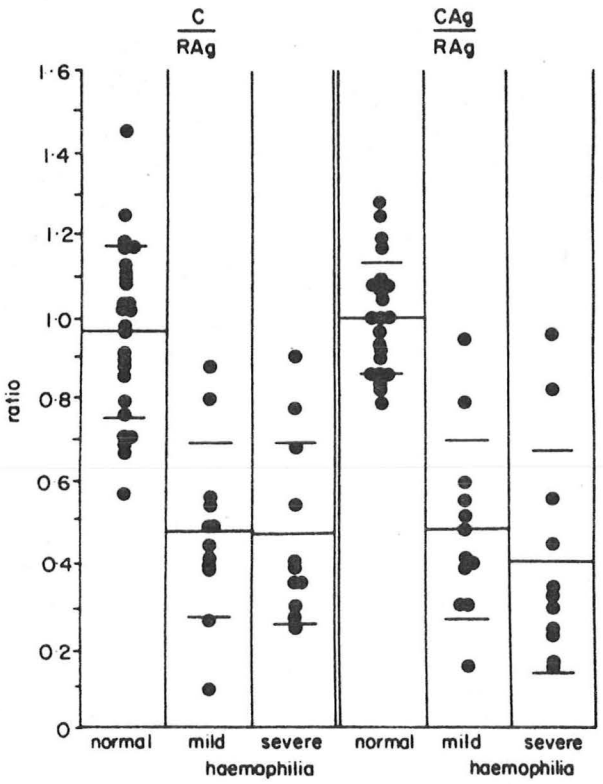
Assays of recombinant factor VIII activity			
Sample	Coatest assay (units ml^{-1})	Radioimmune assay (units ml^{-1}) C7F7 Ab	C10 Ab
62.2 supernatant	0.07	0.28	0.34
62.2 supernatant +C7F7	<0.01	—	—
62.2 supernatant +Synbiotic	<0.01	—	—
Control supernatant	<0.01	<0.001	<0.001
62.2 cell lysate	—	0.1	0.1

from ref. 11

new mutation in the mother rather than her son. She would thus be at risk of having additional sons with hemophilia and her daughters might be carriers. Initially factor VIII activity was used to assess the carrier status of females and less than 50% of the carriers could be detected. With the inclusion of immunologic tests of factor VIII and von Willebrand factor, carriers can be detected with about 90% accuracy (65-73). At first factor VIII procoagulant activity was compared to the factor VIII-related antigen, the material corresponding to vWF protein. A decreased ratio was used to predict the carrier status. With the use of human antibodies to the factor VIII molecule, it became feasible to measure immunologically the factor VIII protein and the vWF protein. This was of particular benefit because of the stability of the factor VIII:CAg compared to the coagulant activity. With this technique carriers can be detected with an accuracy of greater than 90% from plasma samples that have been obtained, frozen and sent to a central laboratory for analysis. Fig. 10 shows the results using factor VIII coagulant activity or factor VIII:CAg relative to the factor VIII-related antigen. The use of these measurements has been shown to be valid in pregnant women such that sufficient information can be gained for genetic counseling, if necessary (73). In most patients with hemophilia the factor VIII procoagulant activity is proportional to the factor VIII:CAg. The presence of an immunologically cross-reactive but functionally inactive protein is uncommon. If the family is known to have immunologically detectable material, the factor VIII procoagulant activity should be used for diagnosis and carrier detection.

Hemophilia A can be diagnosed prenatally by applying the same tests to fetal blood samples obtained via fetoscopy. Factor VIII procoagulant activity can be measured in fetal blood in which there is minimal contamination with amniotic fluid (74). The ratio of the factor VIII:CAg and Factor VIII-related antigen are accurate predictors of affected male children.

Polymorphisms of the factor VIII gene or closely linked genetic markers can also potentially be used for carrier detection and prenatal diagnosis. Restriction fragment length polymorphisms (RFLP) are variations in the structure of the genome that result from insertions, deletions or even single nucleotide changes. The change of a single nucleotide can result in the alteration of the recognition sequence for a restriction enzyme such that the enzyme will no longer cleave the DNA at that site. This results in a different size fragment when DNA is analyzed with a cloned DNA probe. RFLPs are detected as a shift in the size of the DNA fragment that hybridizes to the DNA probe. A DNA probe (St14) that detects a highly polymorphic region on the X chromosome has been found to be closely linked to hemophilia A (75). Among 12 families with hemophilia no recombination was observed between this genetic marker on the X chromosome and the hemophilia gene.



from ref. 72

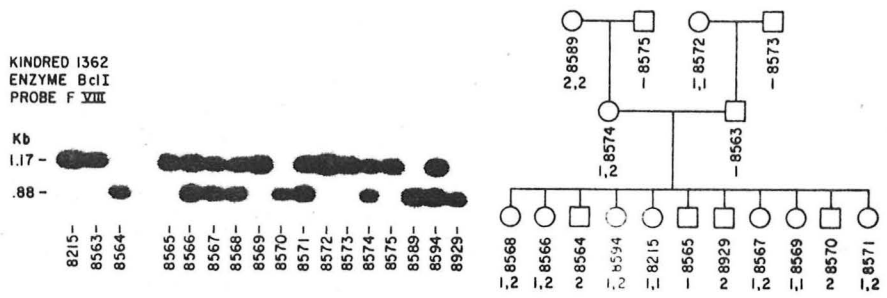
Fig. 10. Ratios of Factor VIII procoagulant activity/Factor VIII-Related Antigen and Factor VIII:CAg/Factor VIII-Related Antigen for 26 normal plasmas and 23 carriers of hemophilia.

This DNA probe is informative in about 90% of families and when combined with assays of factor VIII and von Willebrand factor could increase the accuracy of prediction to greater than 95%. Using a DNA probe from the factor VIII gene another restriction fragment length polymorphism has also been detected (76). An example of this DNA polymorphism is shown in Fig. 11. This change in the DNA sequence of the gene is not related to the molecular abnormality responsible for hemophilia, in that it commonly occurs in normal individuals, however can serve as a marker for the abnormal hemophilia gene. Based on the frequency of the observed alleles this polymorphism would be informative in 42% of families.

Factor VIII Gene Structure and Molecular Basis for Hemophilia

The factor VIII gene is 186,000 base pairs in length and represents one of the largest genes in the human genome known to date. It occupies about 0.1% of the total X chromosome (10). This gene is composed of 26 exons separated by introns, sequences within the RNA transcript that are spliced out to yield the mature messenger RNA species. Sequences that are translated to produce the factor VIII protein are present in all the exons of the gene.

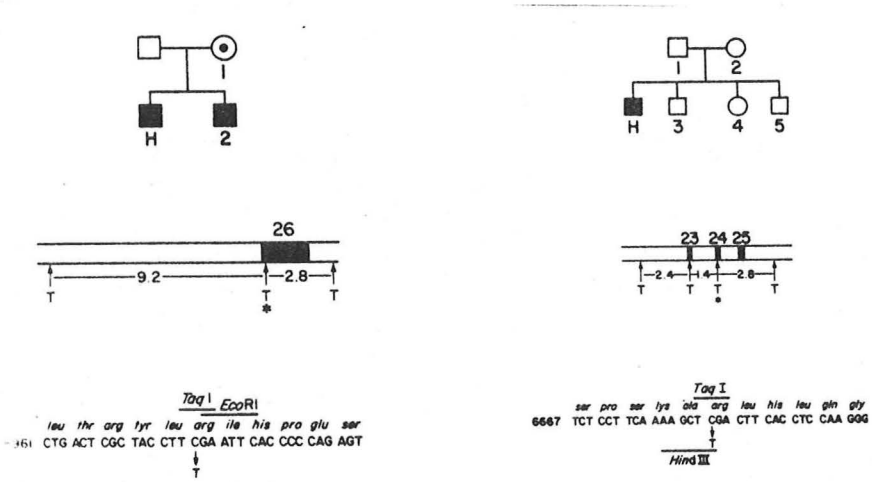
Analysis of the gene structure in 92 patients with hemophilia has revealed four abnormalities that may account for abnormal factor VIII expression (77). In two patients, an abnormal pattern of restriction fragments was detected when DNA was analyzed with the restriction enzyme, Taq I. The factor VIII gene was cloned from the DNA of these patients and the region of interest sequenced to determine the change in the DNA structure. As shown in Fig. 12, in one patient a C has been substituted with a T. This nucleotide substitution caused the Taq I enzyme to no longer cleave this site in the DNA and also resulted in the change of an arginine



from ref. 76

Fig. 11. Inheritance of the factor VIII BcI I polymorphism.

codon (CGA) to an in-frame stop codon (TGA). This would result in a protein that terminates 124 amino acids before the normal chain termination. In a second patient, also shown in Fig. 12, a similar C to T substitution occurred in the last exon of the factor VIII gene. This would result in a protein that is only 26 amino acids shorter than normal factor VIII, a protein 2332 amino acids in length. It is possible that such a protein would be inactive as this region constitutes one of the duplicated C domains of the factor VIII protein and is part of the 80,000 dalton peptide that is part of the catalytic complex.



from ref. 77

Fig. 12. Altered Factor VIII Gene Structure in two patients with Hemophilia.

Two patients that had deletions rather than point mutations were also detected among this group of hemophiliacs. The region of the factor VIII gene that is deleted in these patients is shown in Fig. 13 and also corresponds to a deletion at the carboxy-terminal end of the factor VIII molecule. In the patients with the point mutations resulting in premature termination or deletions, it is unknown whether factor VIII messenger RNA is present or if an abnormal protein is present. No correlation of the factor VIII mutations with the inhibitor status of the patients was found.

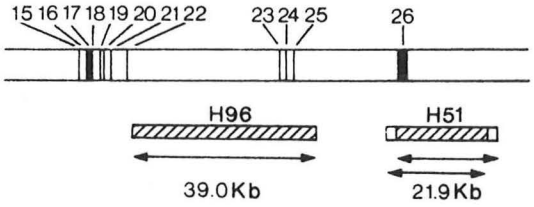


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Fig. 13. Map of the Deletions in the Factor VIII Genes in two patients with Hemophilia.

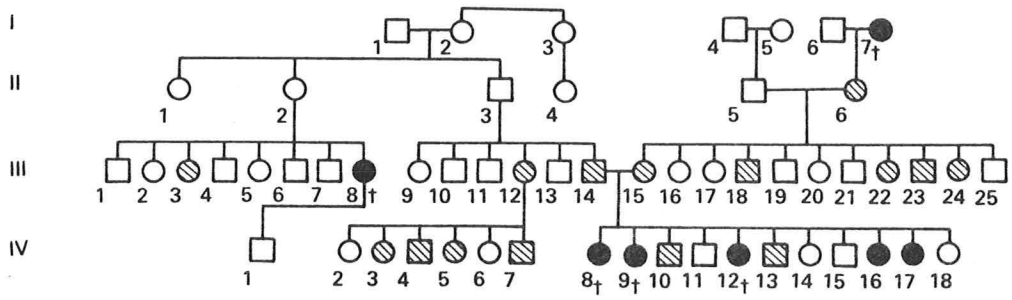
von Willebrand's Disease

Erik von Willebrand first described this disorder in 1926 among a family living on the small Aaland island (78). The hemorrhagic tendency was inherited in an autosomal dominant pattern as shown in Fig. 14. Individuals in this kindred that have severe bleeding problems appear to be homozygous for the defective allele.

In most patients with von Willebrand's disease (VWD) the bleeding manifestations are relatively mild and often do not require replacement therapy (79-80). Epistaxis, superficial bruising, and menorrhagia are relatively common. In severely affected patients, epistaxis, hemarthroses, trauma or surgical procedures can require replacement therapy to control the bleeding.

The response to cryoprecipitate infusion in patients with von Willebrand's disease is different from that observed in hemophilia. Factor VIII procoagulant levels initially rise, however a plateau level is maintained for 12-24 hours (18,78,81). In this time the factor VIII:C can rise even further. Thereafter factor VIII:C levels fall. A similar response is observed following the infusion of material containing vWF protein but no factor VIII procoagulant activity, such as cryoprecipitate from a patient with hemophilia. Despite the correction of factor VIII:C levels, bleeding times are only transiently corrected after the infusion. This prolonged increase of factor VIII:C appears to be due to stabilization of the factor VIII molecule by the vWF multimers. When highly purified factor VIII free of vWF protein is given to VWD patients, only a transient rise in the factor VIII:C levels is observed. The larger multimers are the most effective agent to correct the bleeding abnormalities. Cryoprecipitate contains larger multimers of vWF than factor VIII concentrates and is more effective in correcting the bleeding time of VWD patients.

Diagnosis of von Willebrand's disease is based on measuring the functional and protein level of the vWF protein (79,80). Although crude, the bleeding time (preferably done by a standardized template method) best reflects the functional activity of vWF protein. The factor VIII-related antigen and ristocetin cofactor level should be measured, however due to the heterogeneity of this disease these measurements will not always be low. The factor VIII procoagulant level should also be measured. For more definitive studies, the SDS-agarose gel electrophoresis pattern of VIII:RAg should be analyzed. Using these techniques, it has become apparent that von Willebrand's disease is actually a quite heterogeneous disorder at a molecular level. The subtypes of von Willebrand's disease have been divided according to the pattern of VIII:RAg multimers in the plasma and the response to ristocetin.



from ref. 78

Fig. 14. Kindreds studied by von Willebrand. Shaded figures represent individuals with a mild bleeding tendency. Solid figures are those with a severe bleeding tendency.

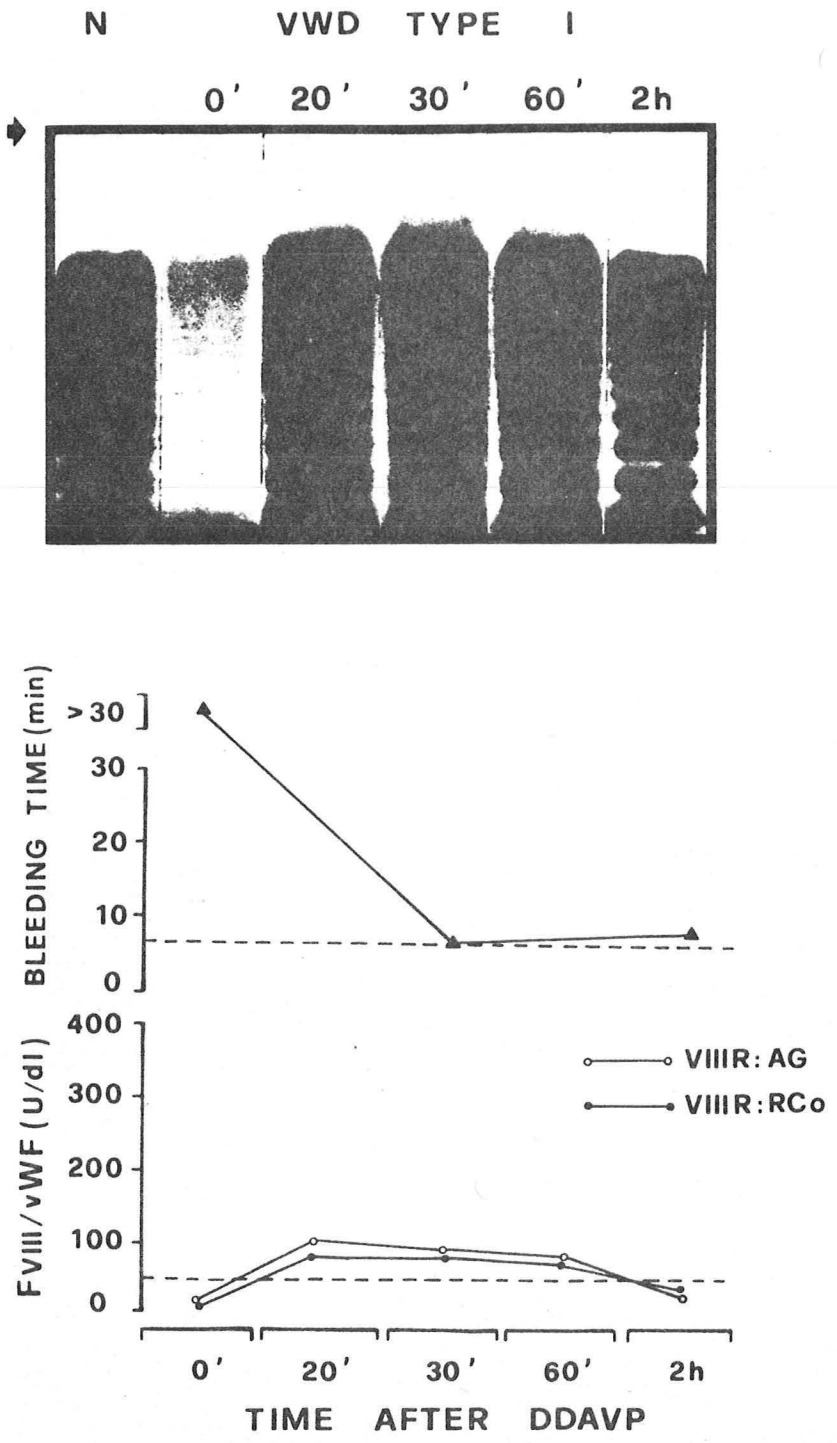
Type I von Willebrand's disease accounts for about 70% of patients (82,83). In this group the concentrations of VIII:C, VIIIR:Ag and VIIIR:RCo are all decreased to the same degree. Within the platelets, the concentration of VIIIR:Ag is normal. Administration of DDAVP results in a rapid increase in all the multimers of vWF in plasma. Associated with this increase in VIIIR:Ag, the bleeding time is shortened. Hoyer has further subdivided this group into types Ia and Ib (83), with the latter having a relatively reduced content of the large multimers although all forms are present.

Type IIa von Willebrand's disease is seen in less than 10% of patients (82-84). The concentration of VIIIR:Ag is variable, however the ristocetin cofactor is very low. Platelets contain VIIIR:Ag, however both the platelet and plasma forms lack the large and intermediate forms of vWF protein. Following the administration of DDAVP, only the small multimers increase with little effect on the bleeding time.

Type IIb is characterized by a heightened responsiveness to low concentration of ristocetin, below the level that induces platelet aggregation in normals. Multimeric composition of platelet vWF is normal, however large multimers are absent in the plasma (28-85). Within this group of patients, platelet abnormalities may account for the apparent von Willebrand's disease in some of the patients (87-89), whereas in other patients the defect appears to be related to the vWF protein from the patient (89). Among some patients with the platelet-type of VWD abnormal structure of platelet glycoprotein Ib, the presumed receptor for vWF protein, has been observed (88). In both instances, the larger multimers of vWF appear to be bound to platelets and cleared much quicker than the smaller multimers.

All of the above types of von Willebrand's disease are inherited in an autosomal dominant pattern. Rare cases of VWD have been reported and classified as types IIc and IId (90,91). vWF multimers in these patients have an abnormal pattern of small multimers. In type IIC the abnormality was recessive, whereas the IID defect was inherited in an autosomal dominant pattern. The double heterozygote for VWD can have a very severe bleeding disorder with essentially no vWF protein detectable.

The heterogeneity of the vWF expression in von Willebrand's disease shows that this disorder is the end result of many abnormalities of von Willebrand factor



from ref. 82

Fig. 15. Response of Type I VWD patient to DDAVP.

and platelets. The relative concentration of the large multimers of the vWF in plasma seems to correlate with the effect on hemostasis. As the pathway of vWF synthesis, storage and secretion is better understood together with the molecular structure of the vWF protein, the molecular defects responsible for the varied expression in von Willebrand's disease will become apparent.

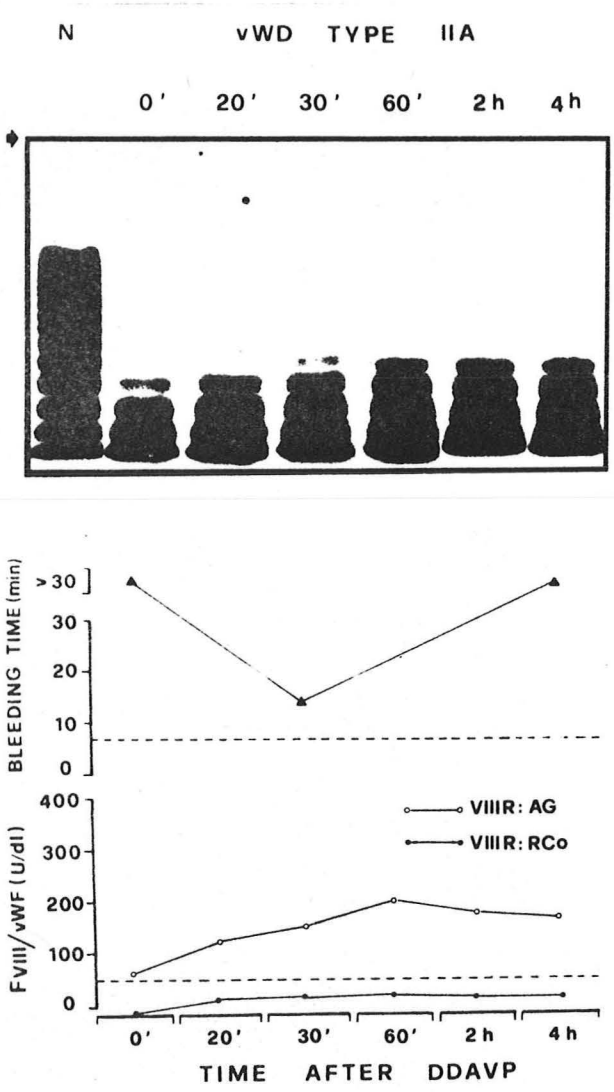


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Fig. 16. Response of Type IIA VWD patient to DDAVP.

Conclusion

Within the last decade our understanding of factor VIII deficiency states and the molecular complex of factor VIII and von Willebrand factor protein has advanced a great deal. With the advent of newer techniques to study the proteins and genes involved in this system, a more complete understanding of the underlying pathogenesis of these diseases should evolve over the next decade. Expression of cloned DNA to produce factor VIII should resolve many of the complications now associated with replacement therapy. When coupled with the production of von Willebrand factor protein, a stable and effective product should be available.

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