healthy volunteers who received up to 4 mg of beclomethasone dipropionate by mouth and 2 mg by inhalation for periods of 48 hours, their 24-hour urinary 17-oxogenic excretion rate remained within normal limits (Harris, 1972). The inhaled dose in the volunteer study was over six times the dose which in this trial successfully controlled the patients' asthma, as the FEV₁, VC, and PEFR measurements show. The patients' bronchodilator requirements were identical whether they were receiving oral or inhaled steroids and so was their preference for the two treatments. From the study the use of inhaled aerosol steroid seems to be preferable to oral prednisolone in the management of reversible diffuse airways obstruction requiring long-term, low-dose steroid therapy.

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Genetic Variants of von Willebrand's Disease

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Summary

A specific antiserum against an antihaemophilic factor (AHF)-related plasma protein was raised in rabbits. A quantitative immunochemical method was used to determine the amount of this protein present in the plasma of 33 patients with haemophilia A and 70 patients with von Willebrand's disease. The protein probably consisted of AHF residing in or complexed with the von Willebrand factor. The patients with von Willebrand's disease were shown to fall into two separate genetic groups, one with decreased and one with normal amounts of the AHF-related protein. The patients with haemophilia A had normal amounts of the protein in their plasma.

Introduction

von Willebrand's disease is a haemorrhagic disorder characterized by a low plasma content of antihaemophilic factor (AHF; factor VIII) and a prolonged bleeding time (Nilsson et al., 1957a). According to Salzman (1963) the platelet adhesiveness is often decreased, but the platelets themselves are normal (Nilsson et al., 1957b; Larrieu et al., 1968). The prolonged bleeding time in

von Willebrand's disease is due to the absence of a plasma factor, the von Willebrand factor. Infusion of this factor both corrects the bleeding time and stimulates synthesis of or activates AHF. The factor is present in the plasma of normal subjects and patients with haemophilia A and can be recovered in fraction I—O and cryoprecipitate as well as in other concentrates of plasma (Nilsson et al., 1959; Creveld and Mochtar, 1960; Cornu et al., 1961; Bennett and Dormandy, 1966).

The mode of inheritance of von Willebrand's disease has been regarded as autosomal and dominant, but with varying penetrance and with varying expressivity (Nilsson and Blombäck, 1962).

The presence of AHF-related antigenic material has recently been shown in plasma from patients with haemophilia A, but with varying frequency (Hoyer and Breckenridge, 1968; Bennett and Huehns, 1970; Meyer and Larrieu, 1971; Stites et al., 1971; Zimmerman et al., 1971). Stites et al. and Zimmerman et al. found little or no such immunologically reactive material in six and 11 patients with von Willebrand's disease respectively.

We have raised an antiserum against a high-molecular-weight protein, probably consisting of a complex between the von Willebrand factor and AHF. We found this AHF-related protein to be present in normal amounts in the plasma of 33 patients with haemophilia A, but 70 patients with von Willebrand's disease fell into two separate genetic groups, one with decreased and one with normal amounts of AHF-related protein.

Materials and Methods

Blood was collected with the silicone technique and citrated plasma was prepared as described by Nilsson et al. (1970). The plasma was stored at -60°C until used.

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The AHF activity of the patient's plasma was assessed from its normalizing effect on the recalcification time of platelet-rich haemophilia A plasma containing less than 1% of the normal AHF activity. The amount of AHF present was expressed as a percentage of that found for a normal standard consisting of pooled plasma from 10 healthy individuals (Nilsson *et al.*, 1970). The bleeding time was determined by the methods of Duke and Ivy (Nilsson *et al.*, 1963). Platelet adhesiveness was tested by the method of Salzman (1963).

To prepare cryoprecipitate 3 ml of citrated plasma was frozen at -60°C and thawed at +6°C for five hours and afterwards centrifuged at +4°C and at 4,000 g for 15 min. The precipitate was dissolved in 0.5 ml of 0.075 M barbitone buffer, pH 8.6, at 37°C. The supernatant plasma never contained more than 15%, generally 10% of the initial AHF activity. Cryoprecipitate of pooled plasma from 20 registered blood donors was always prepared simultaneously in the same way and used as 100% standard.

PREPARATION OF ANTISERUM AGAINST AHF

AHF was purified by the method of van Mourik and Mochtar (1970) with the use of gel chromatography on Sepharose 6B (Pharmacia, Sweden) with a buffer containing 3.3% Rheomacrodex. As starting material we used plasma fraction I—O (A.B. Kabi). The contents of one phial was dissolved in 100 ml of distilled water and 15 ml of the solution was applied to the column. Ultraviolet absorbing material appeared with the void volume, which was determined with blue dextran. After this material had been concentrated it had an AHF activity of 100%. It did not contain factors II, V, VII, IX, X, XI, or XII; neither could fibrinogen, immunoglobulin, or α_2 -macroglobulin be

shown immunologically with specific antisera. Rabbits were immunized by two injections, one three weeks after the other, of this active fraction emulsified in Freund's adjuvant. Electrophoresis in agarose gel containing antiserum produced only one precipitation line with plasma, fraction I—O, or cryoprecipitate. The antiserum could also neutralize the AHF activity of plasma.

DETERMINATION OF AHF-RELATED PROTEIN

AHF-related protein in plasma was determined immunologically by electrophoresis in agarose gel containing antibodies (Laurell, 1966). The buffer used was 0.075 M barbitone buffer, pH 8.6, and 0.5% antiserum was dissolved in 1% agarose in buffer. Plasma or cryoprecipitate, prepared as described above, was applied in 5- μ l quantities to the holes in the gel. The electrophoresis was run at 20 V/cm for four hours. The plates were stained with Coomassie brilliant blue. The determination was made on both plasma and cryoprecipitate, as the precipitation line sometimes tended to be less well-defined when plasma was used, probably owing to low antigen concentration. However, good agreement was always found between the determinations on plasma and cryoprecipitate. A pool of normal plasma from 20 registered blood donors and cryoprecipitate of this plasma were used as standards.

Results

Seventy patients with von Willebrand's disease were examined. The patients belonged to 23 Swedish families, all of which are registered in the official Swedish record of families with von Willebrand's disease. Most of the families have been described

TABLE 1—Blood Values in 15 Families with von Willebrand's Disease (Group 1: Members with Reduced Amount of Immunologically-determined AHF-related Protein)

Family and Case No.	Plasma AHF Activity (%)	Plasma AHF Related Protein (%)	Bleeding Time (min.)		Platelet Adhesiveness (Salzman) (%)	Symptoms
			Ivy	Duke		
Family A 1	20	0	—	>30	10	Severe
2	8	0	—	>30	7	Severe
Family B 3	23	29	30	9	9	Mild
4	28	38	21	16	6	Mild
Family C 5	60	36	19	5	3	Mild
6	6	7	>30	>30	10	Severe
Family D 7	10	<1	>30	>30	0	Severe
Family E 8	24	6	>30	>30	3	Severe
Family F 9	5	0	>30	>30	2	Severe
Family G 10	20	11	>30	—	0	Mild
11	19	8	>30	8	0	Mild
12	38	19	>30	6	0	Mild
13	39	8	>25	3	10	Mild
Family H 14	41	20	>30	2	61	Mild
Family I 15	26	5	>30	>30	13	Severe
Family J 16	43	18	>30	8	0	Mild
17	47	33	>30	2	0	Mild
18	42	27	25	4	5	Mild
19	29	27	>30	24	2	Mild
20	48	25	30	2	35	Mild
21	36	16	>30	5	7	Mild
22	29	10	>30	—	—	Mild
23	54	23	—	—	10	Mild
24	38	30	28	4	1	Mild
25	43	12	—	23	7	Mild
26	44	27	>30	2	6	Mild
27	58	50	17	1	0	Mild
28	55	53	>30	5	39	Mild
29	48	17	15	2	31	Mild
30	40	17	—	—	0	Mild
31	—	11	—	—	—	Mild
32	30	12	>30	4	—	Mild
33	37	27	>30	3	19	Mild
34	—	28	—	—	4	Mild
35	21	19	>25	8-15	34	Mild
36	53	23	>30	4	0	Mild
Family K 37	45	23	>30	8	11	Mild
38	45	13	>30	7	—	Mild
39	40	37	>30	5-30	0	Mild-severe
Family L 40	35	15	>30	8	—	Mild
41	48	17	23	2	18	Mild
42	63	25	30	6	—	Mild
43	30	7	>30	>30	—	Severe
44	62	33	15	5	3	Mild
45	33	21	21	—	14	Mild
Family M 46	53	15	>30	>30	—	Mild
Family N 47	35	19	24	17	—	Mild
Family O 48	30	20	>30	30	3	Mild
49	49	23	27	4	—	Mild
50	38	20	>30	>30	11	Mild-severe
51	88	35	>30	—	—	—

previously (Nilsson *et al.*, 1957a; Nilsson *et al.*, 1959; Silwer and Nilsson, 1964; Cronberg *et al.*, 1966).

Fifty-one patients, 29 males and 22 females, belonging to 15 families (group 1) had low values (0-53%) for the immunochemically determined AHF-related protein. These values, as well as the AHF activity values, the Duke and Ivy bleeding times, and the Salzman (platelet adhesiveness) values, are given in Table I. The AHF activity was also low (5-63%) in all but one of the 49 patients tested. All those tested had a prolonged Ivy bleeding time, but the Duke bleeding time was markedly prolonged only in patients with severe von Willebrand's disease. Nearly all (35 out of 40 tested) had decreased Salzman values.

All the 51 affected members of these 15 families had a low plasma content of AHF-related protein (0-53%), while all the unaffected members examined had normal values (80-150%).

Seven of the 51 patients were judged as having the severe form of the disease because of severe bleeding symptoms, AHF activity values around or below 20% and noticeable prolongation of the Duke bleeding time (Table I). These patients also had the smallest amounts of AHF-related protein (0-7%), while those with mild disease had moderately low values. Judging from Table I, however, there was no clear correlation between the amount of the immunologically reactive protein and the AHF activity or the bleeding time.

In contrast with the first group of 15 families, 19 patients with von Willebrand's disease from eight families (group 2) had values for the AHF-related protein ranging from 64 to 225% and not distinguishable from those in normal subjects. All affected members of these families showed the same pattern (Table II). The AHF activity was decreased in all, and the Ivy bleeding time was prolonged in all but three females. In two

FIG. 2—Pedigree of Family T, illustrating possible X-chromosomal inheritance. Subjects II 4 and II 11 had low AHF values and prolonged bleeding time, but had negligible symptoms. Subjects II 2 and II 9 were not examined. Subjects I 6, III 7, and III 15 had severe symptoms. Black symbols indicate affected subjects.

patients with severe disease the Duke bleeding time also was greatly prolonged. The Salzman values tended to be higher than in the families in group 1. The clinical picture was the same in the two groups, with bleeding from mucous membranes, haematomas, and bleeding after tooth extractions, but rarely bleeding into joints.

We also studied 33 patients with haemophilia A. All had normal amounts of the immunologically reactive protein (69-225%).

The number of healthy and affected offspring of affected men and women in the two groups of families with von Willebrand's disease are shown in Table III. In group 1 the proportions could well be compatible with autosomal dominant inheritance. As

FIG. 1—Pedigree of Family J illustrating autosomal and dominant inheritance of von Willebrand's disease. Shaded symbols indicate affected subjects.

TABLE II—Blood Values in 8 Families with von Willebrand's Disease (Group 2: Members with Normal Amount of AHF-related Protein)

Family and Case No.	Plasma AHF Activity (%)	Plasma AHF-related Protein	Bleeding Time (min.)		Platelet Adhesiveness (Salzman) (%)	Symptoms
			Ivy	Duke		
Family P	52	16	225			Severe
	53	38	110		15	Mild
	54	47	112		24	Mild
	55	27	75		47	Mild
	56	26	122		19	Mild
Family Q	57	25	87		6	Mild
	58	40	100		—	Mild
Family R	59	59	120		0	Mild
	60	52	122		—	Mild
	61	32	144		—	Mild
Family S	62	33	116		89	Mild
	63	33	71		—	—
Family T	64	35	72		30	Mild
	65	20	88		49	Severe
Family U	66	45	124		11	Mild
	67	77	91		—	Mild
Family V	68	57	100		21	Mild
	69	5	64		18	Severe
Family W	70	53	76		14	Mild

TABLE III—Offspring of Affected Men and Women from Families with von Willebrand's Disease

	Offspring of Affected Men				Offspring of Affected Women			
	Sons		Daughters		Sons		Daughters	
	Healthy	Affected	Healthy	Affected	Healthy	Affected	Healthy	Affected
Group 1 ..	10	23	11	10	18	24	15	21
Group 2 ..	8	0	0	6	11	10	11	3

an example, the pedigree of a family in group 1 is shown in Fig. 1.

In group 2 the affected men had no affected sons and no healthy daughters, while the affected women had both healthy and affected sons and daughters. An X-chromosomal inheritance was therefore possible. The pedigree of a family in group 2 is shown in Fig. 2.

Discussion

An antiserum has been raised against an apparently homogeneous plasma protein fraction containing AHF activity. The majority of patients with von Willebrand's disease had a decreased amount of this protein in their plasma. All the 33 patients with haemophilia A had normal amounts of the AHF-related protein, which is in accordance with the findings in the 22 patients of Zimmerman *et al.* (1971). It is conceivably the von Willebrand factor that is detected by the antiserum. AHF may reside in, or be complexed with, the von Willebrand factor and be functionally active only in this form. Bouma *et al.* (1971) also suggested that both activities are located on the same molecule. The exclusion of AHF activity from a Sepharose column shows that it is linked to a high-molecular-weight compound, probably an aggregate of smaller units. Ratnoff *et al.* (1969) estimated the molecular weight of their AHF to be at least 2,000,000.

In haemophilia A the X-chromosomally induced part of the complex may be missing or malformed, but the von Willebrand factor will still be immunologically detectable. In most patients with von Willebrand's disease an autosomal gene mutation (regulatory gene?) results in a suppression of the von Willebrand factor and consequently also of the complex and of AHF activity in the plasma.

The second type of von Willebrand's disease was apparently a separate entity. The clinical manifestations and the common laboratory characteristics resembled those of the first type, but the plasma content of AHF-related protein was normal, as in haemophilia A. The pedigrees suggested X-chromosomal inheritance, with the exception that a few of the presumably heterozygous females had bleeding symptoms of the same degree as the presumably hemizygous males. However, the most severely affected girl belonged to a family with coexisting haemophilia A (Holmberg and Nilsson, 1972). She might

therefore not only have had von Willebrand's disease but also have been a carrier of haemophilia A.

In this second type of the disease the X-chromosomally induced part of the complex may be malformed, as it may be in haemophilia A, but then in such a way as to interfere with the ability of the complex to keep the bleeding time normal as well as to suppress the procoagulant activity.

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Observations on Blood Glucose and Insulin Determinations

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Summary

The relation of capillary to venous blood glucose concentration, whole blood to plasma values, and, where possible, the effect of pregnancy on the variables have been studied. We have not been able to show constant

relations between capillary and venous blood glucose concentrations as have been implied by a W.H.O. expert committee on diabetes mellitus and the British Diabetic Association.

The choice of anticoagulant was shown to have a negligible effect on the results of a single antibody-charcoal separation insulin assay method.

Introduction

In an earlier study of changes occurring in oral glucose tolerance tests during pregnancy we used capillary blood samples for glucose determinations (Lind *et al.*, 1968). The use of capillary whole blood for this purpose is traditional and technically convenient, but the glucose concentration in extracellular fluid as represented by plasma or serum, is probably more meaningful from the point of view of physiology. In later studies plasma for insulin determinations was needed in amounts greater

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