MATERIAL AND METHODS

MATERIAL

The material consisted of all patients with a firm or highly probable diagnosis of von Willebrand's disease and seen at the Coagulation laboratories in Malmö, Stockholm or Gothenburg in the years 1956–1967. Inquiries were made into all of the families of the patients included in the investigation. When possible, laboratory studies were made of the closest relatives of the probands, particularly their parents, siblings and children. The material presumably includes all known cases of von Willebrand's disease in Sweden up to the end of 1967. The diagnosis requires special laboratory facilities, at present available only in Malmö, Stockholm and Gothenburg.

The investigation included a careful inquiry into the probands history regarding bleeding symptoms and the information obtained was, as a rule, supplemented by data from earlier hospital records in those cases in which the patient had sought medical advice or had been admitted to hospital because of their hemorrhagic symptoms.

In most cases, and particularly in probands, a complete investigation was made of the patient's bleeding and coagulation status.

Examination of relatives of the probands was often limited to determinations of the bleeding time according to Duke and Ivy and of the AHF.

The probands were all examined several times and at least on one occasion during a period when they were not bleeding. Relatives were also often examined more than once, especially in doubtful cases.

METHODS

Interest was focused mainly on determinations of:

AHF

Bleeding time according to Duke and Ivy
Platelet adhesiveness according to Salzman

AHF-determinations

Blood sampling. — Venipuncture was performed with sharp, wide needles with a polished bore (caliber 1.2–1.6). The first few millilitres were discarded, and the blood was allowed to flow directly through the needles into the tubes.

The blood was collected with the silicone technique. Citrated plasma (one part 3.8% trisodium citrate solution x 2 H2O + 9 parts blood) and serum were prepared by methods, described previously by Nilsson et al. (Nilsson, Blombäck & von Francken 1957, Nilsson, Blombäck, Thilén & von Francken 1959 and Paraskevas, Nilsson & Martinsson 1962). The blood was immediately centrifuged at 2,300 g for 25 minutes at room temperature. Plasma was withdrawn by means of siliconised pipettes and immediately frozen in plastic tubes at −20°–−60°C.

The plasma antihemophilic factor (AHF or factor VIII) was assayed on platelet-rich haemophilia A-plasma (AHF-content < 1%) in a recalcification system, in which the ability of the citrated control plasma and of the test plasma to correct the prolonged recalcification time of citrated haemophilia A-plasma was compared. The haemophilia A-blood was drawn with the silicone technique and centrifuged without delay at about 700–1,000 g for 10 minutes. It was checked that haemophilic plasma contained at least 200,000 platelets per cu.mm. The plasma was then distributed in plastic tubes and stored at −20°–−60° C (it was usually used within 2 weeks). This method has been described and commented upon by Nilsson et al. (Nilsson, Blombäck & von Francken 1957, Nilsson, Blombäck, Thilén & von Francken 1959). The plasma of patients with von Willebrand's disease was assayed at dilutions 1:20, 1:50 and 1:100.

As 100% standard for the assay of AHF in the patients use was made of mixed citrated plasma from 10–20 normal subjects collected at about the same time (at most an interval of 5 days) as the test specimen.

Normal values in 20 healthy men and women aged 20–40 years ranged from 60 to 160%, with a mean of 100 ± 17.5%. The AHF-content in a group of 10 healthy women past the menopause ranged from 76 to 199%, with a mean of 139%.

The mean error of the method at different AHF-levels had been determined:
Table I
Mean error at different AHF-levels

| AHF-level (%) | Standard error |
|---------------|----------------|
| 0.1 - 1       | ± 0.1 ± 0.3    |
| 2 - 3         | ± 0.6          |
| 4 - 6         | ± 1.4          |
| 15 - 25       | ± 4.5          |
| 30 - 40       | ± 5.2          |

Bleeding time

This was determined by
1) The method of Duke using standardised haemolets (Dade Reagent, Inc. Miami, Florida, U.S.A.). Determinations were mostly performed on both ears. Normal range 1 to 5 minutes.
2) The method of Ivy (Ivy, Nelson & Bucher 1941), as modified by Borchgrevink and Waaler (1958) and also described by Nilsson, Magnusson and Borchgrevink (1963). An arm cuff was wrapped round the upper arm and inflated to 40 mm Hg. Three transverse incisions, 1 mm deep and 10-14 mm long, were made on the volar side of the forearm with a surgical blade (Gillette Surgical Blade E). The blood shed was gently and carefully absorbed at about 15 second intervals with a filter paper until the bleeding stopped. The mean of tride determinations in 35 volunteers examined at the coagulation laboratory in Malmö was 9.5 minutes (range 5-15.5 minutes) and in a later investigation (Cronberg 1968) of 70 volunteers 10.0 minutes (range 5-20 min.), S.D. ± 3.1 and standard error ± 0.37. The bleeding time exceeded 15 minutes in 2 out of the 70 persons.

The corresponding mean found in Stockholm was somewhat lower, and the bleeding time in normal persons did not exceed 12 minutes.

Platelet adhesiveness

The original method of Salzman (1963) was used with a thin needle (caliber 0.14–0.2). Fresh blood was drawn directly from a vein through a short column (11–13 cm) of glass beads into a vacuum tube with EDTA. The tube should be filled with about 5 ml in 40–50 seconds. Normal value according to Salzman 20–60%. The normal value at the coagulation laboratory in Malmö based on 34 volunteers, aged 20–30 years, is 33% with S.D. ± 17.0 and standard error ± 2.9.

At estimation of platelet adhesiveness the platelets were counted with macro-method. For this purpose Hellem’s modification (Hellem 1960) of Nygaard’s method (Nygaard 1933) or Björkman’s method (1959) were used.

Other tests

Other tests concerning bleeding and coagulation-status on persons included in the present investigation are listed below:

Coagulation time in glass tubes. Modified method of Hedenius (1936).
Coagulation time in plastic tubes, measured in the way described by Cronberg (1968).
Recalcification time of plasma (Nilsson et al. 1962).
Platelet counts (Kristensson 1928–29 or Björkman 1959).
Haemophilia B – factor ( = fIX) (Nilsson et al. 1962).
Prothrombin consumption test (Biggs and Macfarlane 1957).
Prothrombin + factor VII + factor X. The P & P method of Owren and Aas (1951).
Factor V (Wolf 1953).
Fibrinogen (Blombäck and Blombäck 1956 or Nilsson and Olow 1962).
Fibrinolysis (Blombäck and Blombäck 1956, Bergström et al. 1960 or Nilsson and Olow 1962).
Platelet adhesiveness according to Hellem (Hellem 1960).
Anticoagulants (Lewis, Ferguson and Arends 1956, Laurell and Nilsson 1957 or Hedner and Nilsson 1971).
Capillary fragility test, as described by Hedner and Nilsson 1971.
Platelet aggregation, measured in the way described by Karaca and Nilsson 1972.
Platelet factor 3, measured in the way described by Karaca and Nilsson 1972.

AHF-CONCENTRATES FOR TREATMENT

For treatment with AHF-concentrates human Fraction I–O, containing AHF, prepared by the glycine method of Blombäck, B. and Blombäck, M., was used.

The preparations were originally made at Karolinska Institutet, and every batch was made from 1,400–1,600 ml of fresh plasma, obtained from 8 blood donors. This yielded about 3 g. of Fraction I–O, which was dissolved in two bottles of 100 ml (100 ml = 1 dose of AHF). This preparation had an activity of 5–8 times that in normal plasma. The purification of AHF per mg protein in Fraction I–O...
compared with fresh human plasma was about 20 times. The yield of AHF in Fraction I-O constituted 70–100% of the AHF-content of the original plasma (method described in detail by Blombäck & Blombäck 1956, Blombäck, Blombäck, Jorpes & Nilsson 1960 and Jorpes et al. 1962). A good correlation exists between in vitro and in vivo yield.

Since 1967 AB Kabi has taken over the production of AHF-containing Fraction I-O (F I-O). They use for each batch about 25 liters of blood from about 125 blood donors. AB Kabi use mainly frozen plasma as starting material. Larger batches, sterile filtering and deep freezing have resulted in a somewhat less good yield. 100 ml F I-O (1 dose) now has, as a rule, the AHF-activity of only 250–300 ml fresh plasma. Such large scale manufacture of the preparation has also resulted in a weakening of its effect on the bleeding time.