

Biological findings in Von Willebrand's pedigrees: implications for inheritance

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SYNOPSIS Thirty-one subjects from three families affected by Von Willebrand's disease have been investigated with the following tests: Ivy's bleeding time; platelet adhesiveness according to Salzman; two-stage factor VIII assay. Twelve patients have a complete form of the disease, *i.e.*, a prolonged bleeding time with low platelet adhesiveness, and a reduced factor VIII level. Eight subjects have an isolated low platelet adhesiveness associated in three cases with a prolonged bleeding time. The low platelet adhesiveness in these subjects was corrected, as in Von Willebrand's disease, by infusion of haemophilia A plasma. The dominant autosomal mode of inheritance appears to be due to a pleiotropic gene, expressed in a variety of ways

Von Willebrand's disease is classically defined as a prolonged bleeding time associated with factor VIII deficiency and an autosomal dominant mode of inheritance, the presence of the disorder being expressed in a variety of ways. The long bleeding time has been related to the lack of a plasma factor named the vascular factor (Nilsson, Blombäck, and Von Francken, 1957a; Nilsson, Blombäck, Jorpes, Blombäck, and Johansson, 1957b) or anti-Willebrand factor, and defect in factor VIII to the lack of a precursor of factor VIII, the anomaly of synthesis being different from that in haemophilia as suggested by experiments *in vivo* (Nilsson, Blombäck, and Blombäck, 1959; Cornu, Larrieu, Caen, and Bernard, 1963). Von Willebrand's disease would therefore appear to result from a double plasma deficiency or a single one if the vascular factor and the precursor of factor VIII are identical.

Thus, the study of the correlation of both abnormalities in Willebrand's relatives appeared important. We have therefore studied the members of three families affected by Von Willebrand's disease, using the following tests: Ivy's bleeding time (more sensitive than Duke's); platelet adhesiveness according to Salzman's method (1963) instead of the photometric assay previously described by Vainer and Caen (1964); factor VIII activity by a two-stage sensitivity method and factor VIII survival after infusion of fresh haemophilic plasma.

MATERIAL AND METHODS

MATERIAL Thirty-one subjects (14 females and 17 males) from three families have been twice investigated at three-month intervals. Special inquiries have been made concerning any personal or family bleeding tendency.

Blood for experiments *in vivo* was collected from haemophilic donors who had a factor VIII level below 1% and no circulating anticoagulant.

METHODS Bleeding time was estimated by Ivy's method, modified by Borchgrevink (1960): control: 4–8 minutes.

Platelet adhesiveness was measured according to Salzman's method (1963), with the following modifications: venipuncture was performed using a siliconized, 20 gauge needle; the blood was allowed to flow through a 4.7 in. plastic tube containing 1.5 g. of glass beads,¹ and subsequently collected in a 7 ml. Vacutainer tube² containing Na₂-E.D.T.A. The time taken for the blood to pass through the column of glass beads was constant, *i.e.*, 40–45 sec., and the volume collected between 5 and 7 ml. A control sample was collected in another tube from the same vein or from the opposite arm, without passing through the glass column. Platelet counts were performed in duplicate on both samples, using a 3% novocaine solution (Piette and Piette, 1959) as diluent and a phase contrast microscope. The difference between the platelet counts in the test and the control samples was expressed as a percentage of the control count and the value obtained measured platelet adhesiveness to glass beads. The results using our apparatus as well as

¹Reflex Perlen 31/7—Dragon-Werk, Bayreuth, Germany.

²Becton-Dickinson and Co, Rutherford, N.J. (3 204 Q).

those of Salzman (1963) were compared and found to be similar (control range = 20-50 %).

Factor VIII assay was performed according to the method of Biggs, Eveling, and Richard (1955), modified by Cornu and Larrieu (1959). Results were expressed as a percentage of a standard human plasma stored at -60°C. for two weeks. The range of human control samples tested in comparison with this standard varied from 65 to 160%.

Plasma for experiments *in vivo* was collected from haemophilic donors into siliconized glassware, using 0.4% sodium citrate as anticoagulant. Blood was centrifuged at 2,700 r.p.m. for 15 minutes at + 4°C. The maximum interval between collection and infusion was two-and-a-half hours.

RESULTS

FAMILY STUDIES The three pedigrees of the families studied are shown in Fig. 1, 2, and 3 and the results of the biological investigations are detailed in Tables I, II, and III. They are summarized and illustrated in Figure 4. A prolonged bleeding time with low platelet adhesiveness and factor VIII deficiency has been found in 12 patients out of 31. Reduced platelet adhesiveness was present in eight cases without factor VIII deficiency. Among these

TABLE III
BLEEDING SYNDROME AND HAEMOSTATIC DATA
IN FAMILY BUH

Subject	Bleeding Syndrome	Bleeding Time Ivy (min.)	Glass Adhesiveness (Salzman) (%)	Factor VIII (%)
III ₁	—	6	37	115
2	—	7	5	140
III ₃	—	7	53	140
4	—	21	3	102
6	—	10	14	120
7	+	>30	0	1
9	—	7	12	125
10	—	6	4	115
IV ₃	—	7	19	83

eight patients, five have a normal bleeding time, three a prolonged bleeding time. Isolated factor VIII deficiency has never been found in the 31 cases studied. There is no evident correlation between the bleeding syndrome, the low platelet adhesiveness, and the bleeding time, but all the patients with factor VIII deficiency except one (I₂, family PEI) have a bleeding history.

The mode of inheritance is indicated by our results in these three families. In family PEI. (Fig. 1), the father is normal but the mother has a complete form of the disease as well as three of the children (II₆, II₇, and II₉). In family BOC. (Fig. 2), patient III₁ has one of her sons affected, as well as five of her brothers and sisters. Parents could not be investigated but the father, who is now dead, had a positive bleeding history. One of her brothers (III₅), who is not affected, has a son (IV₆) with an isolated low platelet adhesiveness. In family BUH. (Fig. 3), there is only one patient affected (III₇), with a most severe form of the disease, but laboratory investigation reveals in the mother and four of her 10 children an isolated low platelet adhesiveness.

The expression of the disease thus appears to be variable when one considers the level of factor VIII. In family PEI. (Fig. 1), patient II₅ has a normal

TABLE I
BLEEDING SYNDROME AND HAEMOSTATIC DATA
IN FAMILY PEI

Subject	Bleeding Syndrome	Bleeding Time Ivy (min.)	Glass Adhesiveness (Salzman) (%)	Factor VIII (%)
I ₁	—	6	53	108
2	—	23	5	48
II ₃	—	8	21	140
4	—	7	31	150
5	—	17	6	115
6	+	>30	0	12
7	+	>30	7	55
8	—	9	25	130
9	+	>30	6	12

TABLE II
BLEEDING SYNDROME AND HAEMOSTATIC DATA
IN FAMILY BOC

Subject	Bleeding Syndrome	Bleeding Time Ivy (min.)	Glass Adhesiveness (Salzman) (%)	Factor VIII (%)
III ₁	+	>15	5	34
4	+	14-30	5	100
5	—	3-30	28	95
6	+	25	2	55
7	+	>30	16	42
8	+	>30	0	38
9	+	>30	3	20
10	+	>30	0	33
IV ₁	—	5	18	96
2	+	>30	2	18
4	—	4-30	27	95
5	—	6-30	32	67
6	—	6-30	9	73

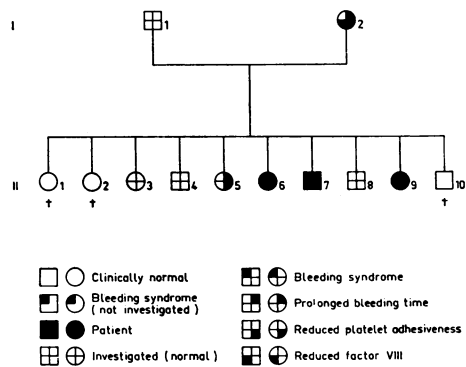


FIG. 1. Pedigree of family PEI.

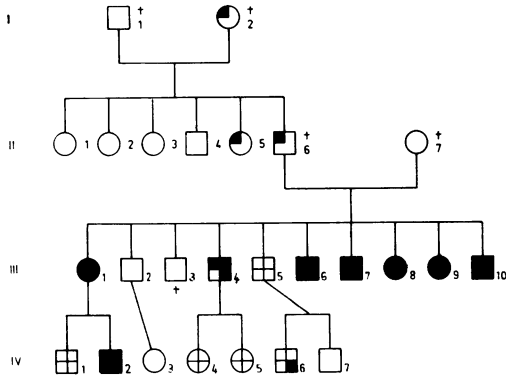


FIG. 2. Pedigree of family BOC. (key as in Figure 1).

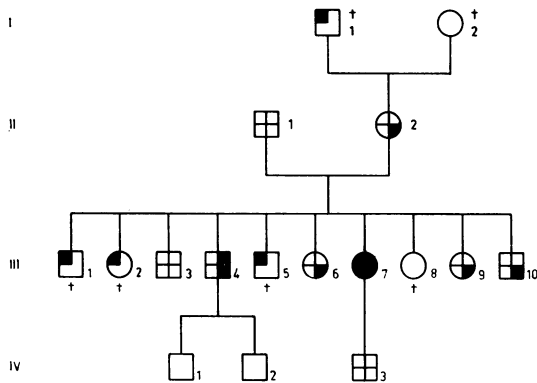


FIG. 3. Pedigree of family BUH. (key as in Figure 1).

factor VIII but a prolonged bleeding time with low platelet adhesiveness. The same findings were noticed in family BOC. (Fig. 2) in patient III₄, as well as in patient IV₆, although the latter's father is normal. In family BUH. (Fig. 3), the mother and four brothers and sisters of the patient (III₇) have an isolated low platelet adhesiveness. Among these cases, only one (III₄) has a prolonged bleeding time.

EXPERIMENTS IN VIVO During infusion *in vivo* of fresh normal blood and fraction I-A in a severe form of the disease (patient III₇, family BUH) the following changes were observed (Fig. 5): a partial correction of Ivy's bleeding time, a complete correction of platelet adhesiveness and photometric assay, and a delayed increase in factor VIII. Another experiment was carried out *in vivo* by infusing haemophilic fresh plasma into a patient suffering from an incomplete form of the disease (patient II₅, family PEI), *i.e.*, with a prolonged bleeding time, a low platelet adhesiveness, and a normal factor VIII.

The same results were obtained (Fig. 6): partial and transient correction of Ivy's bleeding time; immediate and complete, but brief correction of platelet adhesiveness; the increase of factor VIII, although starting from a normal level, showed all the characteristics previously described in Von Willebrand's disease (Cornu *et al.*, 1963).

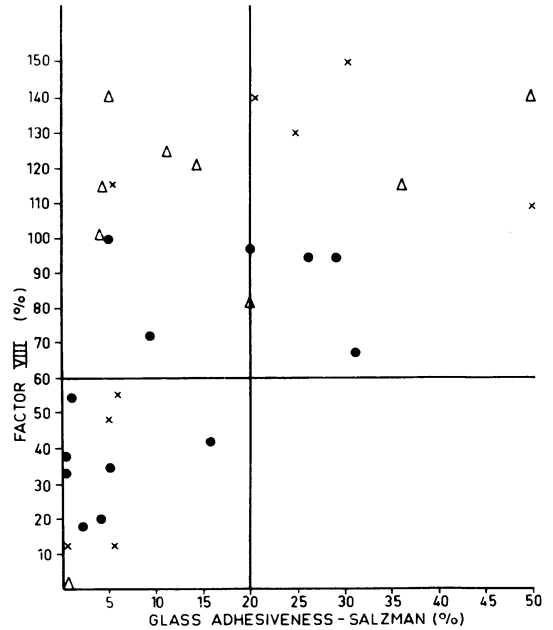


FIG. 4. Platelet adhesiveness and factor VIII in patients and relatives of three pedigrees. x = family PEI ● = family BOC; Δ = family BUH.

DISCUSSION

Among 31 subjects tested in three families, 12 patients have a complete form of the disease: prolonged bleeding time and low factor VIII activity, always associated with a low platelet adhesiveness. Eight other subjects have a reduced platelet adhesiveness with a normal level of factor VIII. Platelet adhesiveness thus appears to be the most sensitive test in the diagnosis of the disease, as already mentioned by Salzman (1963) and by our own experiments (Fig. 7). It seems above all more important in the detection of relatives, although it is not specific for Von Willebrand's disease. Our results confirm the findings of Strauss and Bloom (1965) and support the autosomal dominant mode of inheritance (Nilsson *et al.*, 1957a; Barrow and Graham, 1964), but with a variable expression: in

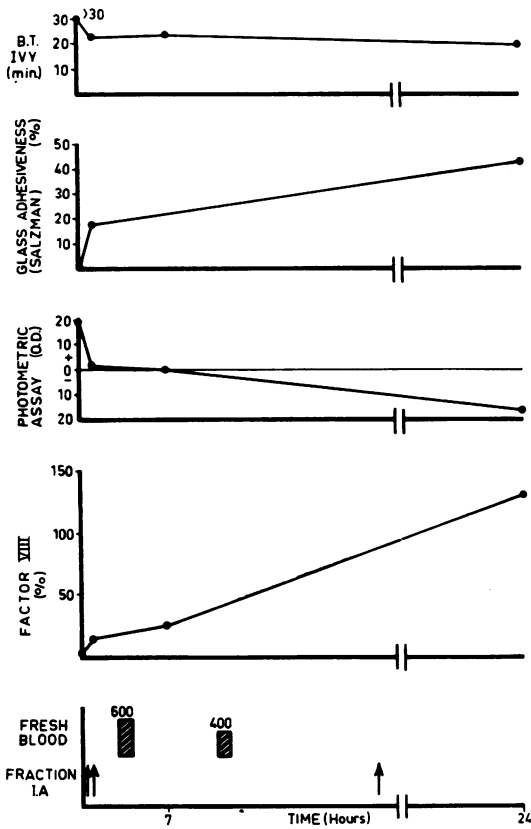


FIG. 5.

FIG. 5. Effect of infusion of fresh normal blood and of fraction I-A on bleeding time, platelet adhesiveness, photometric assay, and factor VIII in a severe case of Von Willebrand's disease (III₇, family BUH).

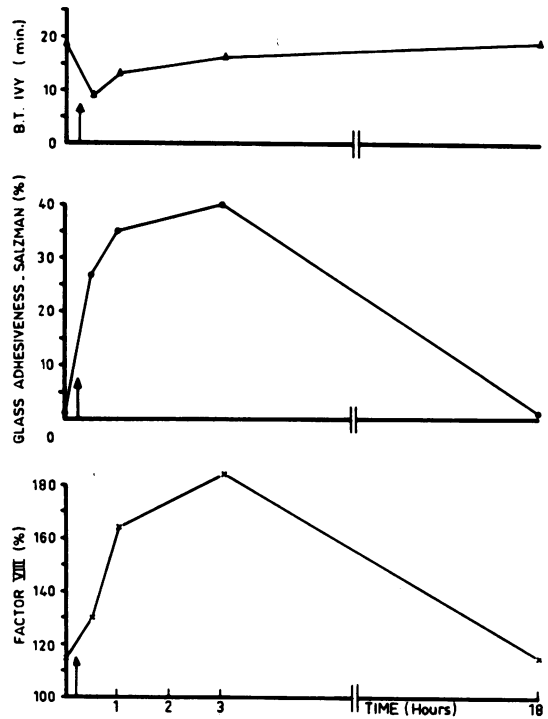


FIG. 6.

FIG. 6. Effect of infusion of 370 ml. of fresh platelet-poor haemophilic plasma (factor VIII below 1%) on bleeding time, platelet adhesiveness, and factor VIII in an incomplete form (II₅, family PEI).

FIG. 7. Platelet adhesiveness to glass beads (Salzman): results obtained in 30 controls and 23 patients with Von Willebrand's disease.

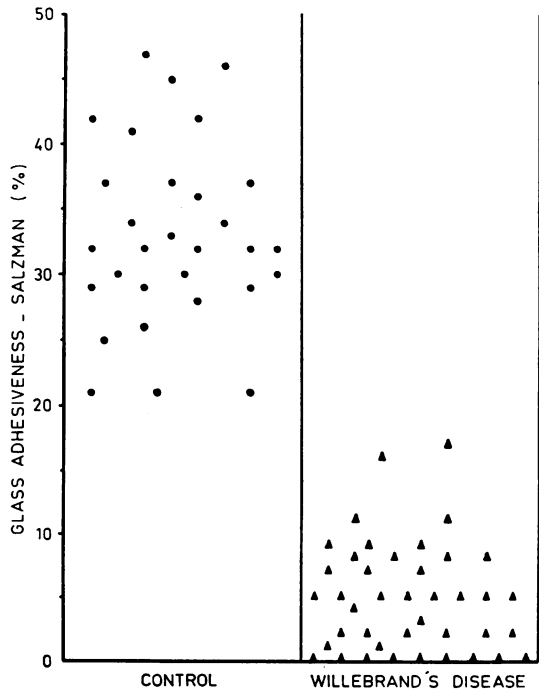


FIG. 7.

family BUH (Fig. 3), the mother (II_2) of one of our most severely affected patients (III_7) exhibits only a partial defect, as well as four brothers and sisters of the patient. This observation is a definite proof that only one pleiotropic gene is responsible for the disease, but that the expression is variable, and especially the incidence on the factor VIII level; therefore the patients in Von Willebrand families, suffering from an incomplete form of the disease, can be considered as cases of Von Willebrand's disease, even if factor VIII is normal. The expression of the disease appears so variable that it can even be unnoticeable in some cases: subject III_5 (family BOC), who is the brother of six patients affected with Von Willebrand's disease, is normal, but one of his sons has an isolated reduced platelet adhesiveness.

Three different hypotheses may be postulated to explain the variable expression of the disease:

1 The gene responsible for the disease would result in a double plasma abnormality, an inconstant one affecting factor VIII, and another platelet adhesiveness and bleeding time.

2 The gene would result in a single plasma abnormality, but with a variable incidence on factor VIII level.

3 In both hypotheses, it may be possible that our techniques are incapable and insufficient to detect minor or functional abnormalities of factor VIII.

We thought that the characteristic synthesis of factor VIII *in vivo* after infusion of haemophilia A

plasma in Von Willebrand's disease would help in understanding this problem. Thus, after infusion of haemophilic plasma into a patient suffering from an incomplete form of the disease, we indeed observed, besides the correction of platelet adhesiveness, a paradoxical and delayed increase in factor VIII which initially was at a normal level, but in preliminary experiments, we obtained similar results in the normal. This experiment *in vivo* therefore does not permit the diagnosis of Von Willebrand's disease, in the absence of family history, in patients with a long bleeding time, low platelet adhesiveness, and normal factor VIII.

We wish to thank Dr. Goudemand who kindly let us examine three of his patients with Von Willebrand's disease; Dr. E. Salzman who willingly gave us some of his equipment for the measurement of platelet adhesiveness; Dr. Y. Sultan, who helped us with *in vivo* experiments, and J. Rossi, N. Verheecke, and C. Davis for technical assistance.

REFERENCES

- Barrow, E. M., and Graham, J. B. (1964). *Progr. Hemat.*, **4**, 203
 Biggs, R., Eveling, J., and Richards, G. (1955). *Brit. J. Haemat.*, **1**, 20.
 Borchgrevink, C. F. (1960). *Acta med. scand.*, **168**, 157.
 Cornu, P., and Larrieu, M. J. (1959). *Path. et Biol.*, **7**, 2595.
 —, —, Caen, J., and Bernard, J. (1963). *Brit. J. Haemat.*, **9**, 189.
 Nilsson, I. M., Blombäck, M., and Von Francken, I. (1957a). *Acta med. scand.*, **159**, 35.
 —, —, Jorppe, E., Blombäck, B., and Johansson, S. A. (1957b). *Ibid.*, **159**, 179.
 —, —, and Blombäck, B. (1959). *Ibid.*, **164**, 263.
 Piette, M., and Piette, C. (1959). *Sang.*, **30**, 144.
 Salzman, E. W. (1963). *J. Lab. clin. Med.*, **62**, 724.
 Strauss, H. S., and Bloom, G. E. (1965). *New Engl. J. Med.*, **273**, 171.
 Vainer, H., and Caen, J. (1964). *J. clin. Path.*, **17**, 191.