Von Willebrand factor and platelet adhesiveness

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SYNOPSIS A modification of Salzman's method has been used in an attempt to provide an assay *in vitro* for the von Willebrand factor. Platelet adhesiveness was increased in von Willebrand's disease by previously coating the beads with normal or haemophilic plasma or cryoprecipitate, whereas von Willebrand plasma had no corrective effect. Antihaemophilic factor (AHF) concentrates were studied in the same way and results compared with experiments *in vivo*.

Platelet adhesiveness to glass beads as described by Salzman (1963) is a valuable test in the diagnosis of von Willebrand's disease (Strauss and Bloom, 1965; Meyer, Larrieu, Maroteaux, and Caen, 1967b), although not specific for the disorder.

As suggested by the results of infusion *in vivo* of normal plasma or fraction I-0, both the decreased platelet adhesiveness (Salzman, 1963) and the long bleeding time (Nilsson, Blomback, Jorges, Blomback, and Johanson, 1957) may be related to the deficiency of an, as yet unidentified, plasma factor, called 'vascular factor' or 'Willebrand factor'. To examine this possibility further, a modification of Salzman's method has been used in an attempt to provide an *in-vitro* assay for the Willebrand factor. Data obtained were compared with results of trials *in vivo* in von Willebrand patients.

Material and Methods

Platelet adhesiveness was measured using Salzman's method (1963). The time taken for the blood to pass through the glass bead column was kept constant (45 seconds) and results were discarded if the volume of blood collected was not between 5.5 and 6.5 ml. Owing to the slight modifications of the original method (Meyer and Larrieu, 1967a), platelet adhesiveness in

the control varies from 20 to 50%. In 28 patients with von Willebrand's disease the index value was consistently abnormal, usually below 10% on repeated examinations.

The following modifications were used for the assay *in vitro*: buffer or test material (plasma, cryoprecipitate, or AHF concentrates) was drawn into the glass bead column at a constant rate (3 ml in 90 seconds). The coated glass bead column was left for 10 minutes at 20°C, after which the test material or buffer was removed by suction. Platelet adhesiveness was then assessed in a patient with von Willebrand's disease in the usual way, using the coated glass bead column. A control glass bead column prepared under the same conditions was used for the assay of the amount of protein remaining in the glass bead column by the Folin-Ciocalteu method (Lowry, Rosenbrough, Farr, and Randall, 1951). The material used, including glass beads, polyvinyl tube, and connectors, was rinsed in 10 ml of either distilled water or NaOH 1 N. The results were expressed as the final amount of protein per millilitre of blood passing through the glass beads during the test.

Platelet counts were performed in double-blind by phase-contrast microscopy, using 1% ammonium oxalate solution as diluent.

Citrated platelet-poor plasma (0.38% final concentration) was obtained from normal, haemophilic, or von Willebrand patients.

Received for publication 21 August 1969.

	Cryoprecipitate	AHF Concentrates	
		Intermediate Purity	High Purity
Protein (g/100 ml)	3.1	5.3	2.0
Fibrinogen (g/100 ml)	0.42	0.78	0.16
Factor VIII (%)	560	700	2,100

Table I Mean factor VIII, fibrinogen, and protein concentration of plasma fractions

	Platelet Adhesiveness Index (%)		
	Normal	Haemophilic	Von Willebrand
Plasma	18 ¹ (9-28) ²	15 (10-20)	2 (0-6)
Cryoprecipitate	25 (13-38)	21 (14-32)	1 (0-2)

Table II Effect of plasma and cryoprecipitate from normal and haemophilic or von Willebrand patients on platelet adhesiveness in von Willebrand's disease

¹Mean

²Extreme values

	Control (standard column)	Buffer	Control	Citrated Buffer	Control	Citrated Normal Plasma
Platelet adhesiveness index (%)	28	27	26	18.3	25.2	27.2

Table III Effect of citrate on platelet adhesiveness (mean of six experiments)

Plasmas for the assay *in vitro* of Willebrand factor were used either immediately after collection or quickly frozen in dry ice-alcohol and kept at -20°C .

Cryoprecipitate was prepared from control or patients' plasmas according to the method of Hershgold, Pool, and Pappenhagen (1966). The mean protein, fibrinogen, and factor VIII concentrations are listed in Table I.

Intermediate and high purity AHF concentrates (Johnson, Newman, Howell, and Puszkun, 1967) were kindly provided through the courtesy of Dr Alan Johnson (Table I). For the experiments *in vivo*, the amount of protein infused was 200 mg/kg body weight for the intermediate purity AHF and 20 mg/kg for the high purity concentrate.

Albumin and gamma globulin solutions were made up of NBC crystalline human albumin and NBC human fraction II.

Michaelis buffer was at pH 7.35.

Citrated buffer was prepared by adding 1 part of 3.8% sodium citrate to 5 parts of buffer.

Results

EFFECT OF NORMAL, HAEMOPHILIC, OR WILLEBRAND PLASMA

All the patients with von Willebrand's disease used for the assay *in vitro* had a platelet adhesiveness index below 5%.

A definite increase in platelet adhesiveness was observed with normal (mean 18%) or haemophilic (mean 15%) plasma, although the results were variable, but coating the beads with Willebrand plasma had no corrective effect (Table II).

The effect of citrate on platelet adhesiveness was elucidated in control subjects, using glass bead columns previously incubated with normal citrated plasma or citrated buffer. Platelet adhesiveness in the control was only slightly modified by normal citrated plasma (Table III) whereas it was consistently decreased in the presence of citrated buffer.

EFFECT OF NORMAL, HAEMOPHILIC, OR WILLEBRAND CRYOPRECIPITATE

Cryoprecipitates prepared from fresh normal or haemophilic plasma increased platelet adhesiveness to a mean of 25% and 21% (Table II) whereas cryoprecipitate from Willebrand plasma was ineffective.

EFFECT OF AHF CONCENTRATES

Intermediate and high purity AHF concentrates were tested in the same way and results were compared with purified albumin or gamma globulin solutions, using the same protein concentration. Both AHF concentrates completely normalized platelet adhesiveness which was not modified in the presence of albumin or gamma globulin (Table IV).

CONCENTRATION OF THE PROTEINS COATING THE GLASS BEADS

This concentration was measured for the different materials tested. The final protein concentration per millilitre of blood passing through the glass beads was compared with the mean result of platelet adhesiveness for each test material (Table V). It appears that there is a progressive increase in the platelet adhesiveness index in von Willebrand's disease with the different materials, despite a progressive decrease in the final protein concentration in the test system.

Case No.	Control (standard column)	Platelet Adhesiveness Index (%)			
		AHF Concentrates		Albumin (25 mg/ml)	γ Globulin (20 mg/ml)
		Intermediate Purity (50 mg/ml)	High Purity (20 mg/ml)		
1	3	53			3
2	1.5	39			
3	0	41			
4	2	17	37	4.5	
5	0			3	0
6	3	29	25		
7	4		24		
8	0		27		
9	4.5		24		

Table IV Effect of AHF concentrates on platelet adhesiveness in von Willebrand's disease

Test Material	Platelet Adhesiveness Index (mean) (%)	Final Protein Concentration (mean) (μ g/ml blood)
Normal plasma	18	832
Haemophilic plasma	15	772
Normal cryoprecipitate	25	361
Intermediate purity AHF	35.8	310
High purity AHF	27.4	74

Table V Comparison of platelet adhesiveness and protein concentration coating the glass bead column

AHF Concentrates				
Intermediate Purity		Time after Infusion	High Purity	
Ivy Bleeding Time (min)	Platelet Adhesiveness (%)		Ivy Bleeding Time (min)	Platelet Adhesiveness (%)
>30	1	Before	>30	2
>30	25	15 min	10	29
>30	23	1 hr	12	25
—	—	2 hr	>30	32
—	19	5 hr	—	36
—	9	24 hr	>30	25

Table VI Infusion of AHF concentrates in a case of a severe form of von Willebrand's disease

Time	Ivy Bleeding Time (min)	Platelet Adhesiveness (%)
Before	>30	4
15 min	29	17
1 hr	28	21
2 hr	>30	12

Table VII Infusion of high purity AHF concentrates to a case of a moderate form of von Willebrand's disease

CORRELATION WITH EXPERIMENTS IN VIVO

Antihaemophilic factor concentrates (intermediate and high purity material) were infused in five patients with von Willebrand's disease. Intermediate purity AHF resulted in a complete correction of the platelet adhesiveness index and of the Duke bleeding time, lasting for five hours but Ivy bleeding time was not modified (Table VI). The effect of high purity material was even better in the same patient with a very severe form of von Willebrand's disease (Table VI) as the increase in the platelet adhesiveness index lasted for 24 hours and the Ivy bleeding time was normalized for one hour. Results with the same material in another patient with a moderate form of von Willebrand's disease are reported in Table VII. Bleeding time according to Ivy's criteria was shortened (28 minutes) although not normalized, and platelet adhesiveness rose to 21% after one hour (Table VII).

With both AHF concentrates, factor VIII increased as previously described (Cornu, Larrieu, Caen, and Bernard, 1963).

Discussion

With respect to certain important technical conditions, platelet adhesiveness to glass beads on whole blood as measured by Salzman (1963) is a useful test in the diagnosis of patients with von Willebrand's disease and of their relatives (Larrieu, Caen, Meyer, Vainer, Sultan, and Bernard, 1968). Among those technical conditions, the transit speed of blood passing through the glass bead column is the most important to consider, as O'Brien and Heywood (1967) have shown that only a high flow rate will demonstrate an abnormality in von Willebrand's disease. Using a different method *in vitro* some authors (Zucker, 1963; Salzman and Britten, 1964) have proved the correction of platelet adhesiveness in von Willebrand's disease with normal citrated (Zucker, 1963) or heparinized (Salzman and Britten, 1964) plasma. In these methods, blood from patients with von Willebrand's disease was previously mixed with normal plasma and then passed through a glass bead column. The coating of glass beads with plasma before the test was suggested by Vigliano and Horowitz (1966) for the study of the inhibitory effect of IgA myeloma plasma on platelet adhesiveness.

Plasma proteins, and especially fibrinogen greatly influence the interaction of platelets with glass surfaces (Packham, Evans, Glynn, and Mustard, 1969) and adsorption of proteins on the glass beads may be an important factor in the measurement of platelet adhesiveness by Salzman's method. However, the effect of platelet adhesiveness in von Willebrand's disease

reported in our experiments does not seem to be explained by a non-specific coating of proteins on the glass beads, since Willebrand plasma or cryoprecipitate had no corrective effect. Besides, there is no correlation between the fibrinogen content of the plasma fractions and the increase in platelet adhesiveness. Moreover, our results obtained *in vitro* correlate well with experiments *in vivo* (Larrieu *et al*, 1968).

In summary, studies *in vitro* indicate the existence in normal and haemophilic plasma of a factor, transferable to glass beads and capable of inducing retention of Willebrand platelets by such beads. These results correlating experiments *in vivo* lend further support to the existence of a plasmatic factor deficiency which is responsible for the decreased platelet adhesiveness characteristic of this disorder.

This work was supported in part by a grant from INSERM. The assistance of B. Obert is gratefully acknowledged, and the courtesy of Dr Alan Johnson, American Red Cross Research Laboratory, New York, in providing AHF concentrates.

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