J. clin. Path. (1964), **17**, 191

A useful photometric test for the diagnosis of Von Willebrand's disease

H. VAINER AND J. P. CAEN
with the technical assistance of D. CHATELAIN and H. MICHEL

From Institut de Recherches sur les Leucémies de la Faculté de Médecine, Centre de Recherches sur les Leucémies de l'Association Claude Bernard, Hôpital Saint-Louis, Paris

SYNOPSIS When small amounts of adenosine diphosphate are added to citrated platelet-rich plasma, the consequent modification of platelets leads to a decrease in optical density. In Von Willebrand's disease the optical density at 610 mμ increases instead of decreasing. This finding is used to study the effects of treatment on this disorder.

Adenosine diphosphate (A.D.P.) potentiates platelet aggregation (Gaarder, Jonsen, Laland, Hellem, and Owren, 1961) and this action can be evalutated *in vitro* by microscopical, macroscopical, and photometric methods (Born, 1962; O'Brien, 1962). Using small amounts of A.D.P. in a photometric test we have been able to find a constant and immediate increase in optical density (O.D.) with citrated platelet-rich plasma taken from patients with Von Willebrand's disease: this differs from the decrease noted when normal platelet-rich plasma or that taken from patients with disorders of blood coagulation is similarly studied (Vainer and Caen, 1963). Our present paper deals with further results obtained by the use of this test in the study of the effects of various treatments *in vivo* using fresh human blood or plasma and prednisone. The effects of mixing *in vitro* normal or Von Willebrand's platelet-poor plasma or serum or their fractions are also recorded.

MATERIALS AND METHODS

Thirteen patients with established Von Willebrand's disease (Table I), shown by prolonged bleeding time, antihaemophilic globulin activity deficiency, diminished platelet consumption (Borchgrevink, 1961; Cornu, Larrieu, Caen, and Bernard, 1963) were studied together with 14 normal controls, two mild haemophiliacs (factor VIII deficiency), one case of congenital proconvertin (factor VII deficiency, and one case of Christmas disease (factor IX deficiency) as shown in Table II. Six patients with Von Willebrand's disease were treated on nine occasions, three using fresh siliconed platelet-rich blood and six using prednisone (Caen and Parquet-Gernez, 1963).

Received for publication 11 June 1963.

On eight occasions a test *in vitro* was performed to assess possible correction by adding various blood fractions to five samples of Von Willebrand's plasma. All blood samples were collected by venepuncture into siliconed glassware containing 3·8% trisodium citrate (1:9). Citrated platelet-rich plasma was obtained by centrifuging this blood at 1,000 r.p.m. (100 *g*) for four minutes at 4°C. and platelet-poor plasma by further centrifuging at 4,000 r.p.m. (1,600 *g*) for 30 minutes at 4°C.

Fraction I-O, prepared by Blombäck's method, was kindly given to us by J. P. Soulier and D. Ménaché. Cohn's fraction I was prepared by the National Centre for Blood Transfusion (J. P. Soulier).

Serum used for correction *in vitro* was obtained 10 minutes after coagulating platelet-poor plasma by the addition of thrombin (5 N.I.H. units/ml.), the fibrin being removed five minutes after adding the thrombin.

Platelet counts (novocain method) were performed using a phase-contrast microscope.

TABLE I

CORRELATION IN VON WILLEBRAND'S DISEASE BETWEEN BLEEDING TIME (IVY), PLATELET ADHESIVENESS, (BORCHGREVINK METHOD), A.H.G. ACTIVITY, AND INCREASE IN O.D.

Case	*Bleeding Time (min.)*	*Platelet Adhesiveness* in vivo (*no. of adhesive platelets* × 10^3)	*A.H.G. Activity* (%)	*Modification of O.D. with 0·05 λ of A.D.P.*
Pan	>10	10	1	+ 4
Tes	>15	22	4	+ 8
Buh	>20	0	8	+13
Tru	12	25	11	+ 8
Roi	>15	25	20	+ 5
Bat	>15	10	22	+35
Far	>20	0	25	+25
Dre	8	30	25	+35
Far	>20	25	29	+ 5
Yvo	>15	0	35	+ 8
Che	5-10	0	45	+30
Bug	12	35	50	+10
Mat	>15	11		+20

TABLE II

STUDY OF BLEEDING TIME (IVY), PLATELET ADHESIVENESS *IN VIVO* (BORCHGREVINK), A.H.G. ACTIVITY, AND O.D. IN 14 CONTROLS AND FOUR PATIENTS WITH BLOOD COAGULATION DISORDERS

Diagnosis	*Bleeding Time (min.)*	*Platelet Adhesiveness in vivo (no. of adhesive platelets × 10³)*	*A.H.G. Activity (%)*	*Modification of O.D. with 0·05 λ of A.D.P.*
Control 1	5	90	130	0
Control 2	8·30	53	110	0
Control 3	10·30	45	85	− 5
Control 4	6	83	115	−13
Control 5	6	48	130	− 2
Control 6	11	97	120	− 3
Control 7	11	50	145	− 3
Control 8	7	114	165	− 4
Control 9	9	64	140	− 7
Control 10	5·30	72	80	− 5
Control 11	5·30	115	100	− 5
Control 12	6	80	170	−10
Control 13	9	60	75	−13
Control 14	6·30	90	120	−25
Congenital hypopro-convert-inaemia 15	12	70	120	− 7
Christmas disease 16	12		120	−18
Mild A.H.G. deficiency 17	9-11	74	3-4	− 8
Mild A.H.G. deficiency 18	5·30	105	25	−10

RESULTS

This photometric test shows a paradoxical immediate (within 60 seconds) increase in optical density in platelet-rich plasma from Von Willebrand patients when small amounts of A.D.P. (0·05γ/0·8 ml. plasma) are added (Table III); with larger amounts of A.D.P., however, the reaction is the same as in normal controls (Vainer and Caen, 1963). This test is recommended for the diagnosis of Von Willebrand's disease although only a qualitative correlation is possible between increases in optical density, the Ivy bleeding time, and the so-called *in vivo* platelet adhesiveness test (Borchgrevink, 1961) (Table I).

This increase in optical density has never been demonstrated with normal (control) plasma or citrated plasma from patients with established coagulation factor deficiency (Table II) nor with platelet-rich plasma taken from patients with thrombasthenia (Vainer and Caen, 1963).

TABLE IV

CORRECTION *IN VIVO* OF O.D. IN SIX CASES OF VON WILLEBRAND'S DISEASE AFTER TREATMENT WITH FRESH NORMAL BLOOD AND PREDNISONE

Case	*Modification of O.D.*		
	Before Treatment	*After Treatment*	
		Fresh Normal Blood[1]	*Prednisone*[2]
Buh	+19	—26	—
Pan	+ 4	—	−18
			− 5
Dre	+35	—	−33
Tru	+ 8	—	0
Bug	+10	—	0
Bat	+20	−10	—
	+20	—	0
	+35	—	+ 3
	+35	—	−20
	+35	−45	—
	+35	—	−10
	+27	—	—

[1]600 ml. [2]0·8 to 2 mg./kg./day for two to 10 days.
In each case a partial or total correction of the bleeding time (Ivy) and platelet adhesiveness *in vivo* (Borchgrevink) was obtained. All determinations were made according to the general scheme (Table III).

TABLE III

SCHEME OF PHOTOMETRIC TEST FOR DIAGNOSIS AND CORRECTION OF VON WILLEBRAND ANOMALY

Cuvette No.	*Normal Platelet-poor Plasma (ml.)*	*Normal Platelet-rich Plasma (ml.)*	*Von Willebrand Platelet-poor Plasma (ml.)*	*Von Willebrand Platelet-rich Plasma (ml.)*	*Buffer Solution (ml.)*	*A.D.P.*		*Correcting Fraction (ml.)*	*Results in O.D.*	
						200 γ/ml. (ml.)	*0·2γ/ml. (ml.)*		*Normal*	*Von Willebrand*
1	0·80	—	—	—	0·40	—	—	—	Blank	—
2	—	0·80	—	—	0·40	—	—	—	Basal	—
3	—	0·80	—	—	0·15	0·25	—	—	Maximal decrease	—
4	—	0·80	—	—	0·15	—	0·25	—	Decrease	—
5	—	—	0·80	—	0·40	—	—	—	—	Blank
6	—	—	—	0·80	0·40	—	—	—	—	Basal
7	—	—	—	0·80	0·15	0·25	—	—	—	Maximal decrease
8	—	—	—	0·80	0·15	—	0·25	—	—	Increase
9	—	—	—	0·80	0·25	—	—	0·15	—	Basal (correction test)
10	—	—	—	0·80	—	—	0·25	0·15	—	Decrease

Buffer (veronal-acetate *p*H 7·4) is added to the plasma (substrate): A.D.P. (in buffered solution is then added: after 10 seconds of agitation with a plastic rod, the photometric readings are made at λ = 610 mμ after 1·15 and 60 minutes at 20°C.

TABLE V

CORRECTION *IN VITRO* OF O.D. IN VON WILLEBRAND'S DISEASE WITH NORMAL PLATELET-POOR PLASMA OR SERUM, VON WILLEBRAND PLATELET-POOR PLASMA OR SERUM, BLOMBÄCK FRACTION I.O, AND COHN FRACTION I

Case	*Modification of O.D.*						
	Before Correction	*After Correction with*					
		Normal Platelet-poor Plasma	*Normal Platelet-poor Serum*	*Von Willebrand Platelet-poor Plasma*	*Von Willebrand Platelet-poor Serum*	*Fraction IO (Blombäck)*	*Fraction I (Cohn)*
Far	+25	−110	−140	—	—	—	—
	+12	− 30	− 2	+10	—	—	—
	+24	−182	− 90	+ 4	+20	—	—
Tes	+ 8	—	—	—	—	+10	−5
	+ 5	− 6	—	+18	—	+25	−5
Pan	+20	− 5	− 2	+10	+ 7	—	−8
Yvo	+15	0	—	+30	—	—	—
Dre	+ 8	− 6	− 2	+ 3	+ 3	—	—

All determinations made according to the general scheme (Table III)

The influence of giving fresh blood or prednisone is summarized in Table IV. As assessed by this test, correction has been observed to occur simultaneously with correction of the prolonged bleeding time. Correction *in vitro* (Table V) is possible by the addition of either fresh normal platelet-poor plasma or serum but not with the use of Von Willebrand platelet-poor plasma or serum. Cohn fraction I corrected the anomaly but Blombäck's fraction I-O did not.

DISCUSSION

In Von Willebrand's disease, the prolonged bleeding time is thought to result from a local failure of platelet utilization due to platelets not adhering to the vessel wall (Borchgrevink, 1961; Caen and Cousin, 1962) and is associated with antihaemophilic globulin (factor VIII) activity deficiency (Cornu *et al.*, 1963). The level of activity of antihaemophilic globulin is corrected by transfusion of whole blood or platelet-poor plasma taken from normal or haemophilic patients but not by plasma transfusions from patients with Von Willebrand's disease (Nilsson, Blombäck, Jorpes, Blombäck, and Johansson, 1957; Cornu *et al.*, 1963). It would seem, therefore, that a plasma factor, different from that lacking in haemophilia, is deficient in patients with Von Willebrand's disease.

As the bleeding time is also rapidly but transiently corrected by transfusion of platelet-poor normal or haemophilic plasma, it is assumed that deficiency of the same or another plasma factor is responsible for its prolongation.

Until recently, no test *in vitro* has been capable of demonstrating this anomaly in Von Willebrand's disease, the adherence to glass and aggregation of platelets being normal in every instance (Inceman, 1963; Spaet, 1963). Recently, Salzmann (1963) and Zucker (1963) have claimed that platelet adherence to glass beads seems to be abnormal in this disease but their results are not always reproducible.

The significance of this paradoxical increase in optical density (Von Willebrand effect) in the presence of small amounts of A.D.P. is still unknown; it does not seem to be linked to A.H.G. deficiency and can be related to the results obtained in the measurement of A.T.P. in this disease (Caen and Cousin, 1962). This finding led us to believe that this simple method could represent an assay of the bleeding plasma factor (Caen, 1964).

This anomaly has also been found by us in some cases of dysproteinaemia, a finding which led us to pursue this study which is still in progress.

REFERENCES

Borchgrevink, C. F. (1961). *Acta med. scand.*, **170**, 231.
Born, G. V. R. (1962). *Nature (Lond.)*, **194**, 927.
Caen, J., and Cousin, C. (1962). *Nouv. Rev. franç. Hemat.*, **2**, 685.
——, and Parquet-Gernez, A. (1963). *Ibid.*, **3**, 355.
——, (1964). In Proceedings of International Conference in Haemophilia. Washington 1963, edited by K. Brinkhous.
Cornu, P., Larrieu, M. J., Caen, J., and Bernard, J. (1963). *Brit. J. Haemat.*, **9**, 189.
Gaarder, A., Jonsen, J., Laland, S., Hellem, A., and Owren, P. A. (1961). *Nature (Lond.)*, **192**, 531.
Inceman, S. (1963). Personal communication.
Nilsson, I. M., Blombäck, M., Jorpes, E., Blombäck, B., and Johansson, S. A. (1957). *Acta med. scand.*, **159**, 179.
O'Brien, J. R. (1962). *J. clin. Path.*, **15**, 446.
Salzmann, E. R. (1963). *J. lab. clin. Med.*, **62**, 724.
Spaet, T. H. (1963). Personal communication.
Vainer, H., and Caen, J. (1963). *Nouv. Rev. franç. Hemat.*, **3**, 149.
Zucker, M. B. (1963). *Nature (Lond.)*, **197**, 601.