Prolonged Clot Lysis Time and Absence of Platelet γM-globulin in Patients with Thrombasthenia

THE presence of fibrinogen, γM-globulin, γG-globulin, albumin and plasminogen on or in normal washed platelets has been demonstrated by the use of specific antisera[1–4]. Taylor and Müller-Eberhard[4] observed that blocking either fibrinogen or γM-globulin with specific antibody prevented normal retraction and lysis of dilute whole-blood clots prepared by the technique of Fearnley *et al.*[5]. Because platelets are also necessary for a normal response[4], their studies suggested that the fibrinogen and γM-globulin adsorbed onto the platelets play an essential part in the process.

The finding of a prolonged dilute clot lysis time in a patient with thrombasthenia (M. M.)[4] lent support to the view. In this rare congenital disorder, the platelets fail to promote clot retraction or to aggregate with adenosine diphosphate (ADP) or thrombin[6–9], and most patients have a low platelet fibrinogen level[7,8,10–12]. The dilute whole-blood clot lysis test was performed on samples from M. M. and two other thrombasthenic patients[9]. The platelets of the three patients were also tested to determine whether they contained normal amounts of γM-globulin. Previous studies[7,12] on these patients had shown that platelet counts on whole blood were between 180,000 and 225,000/mm³, plasma fibrinogen concentrations were normal, and platelet fibrinogen levels were low.

Twenty millilitres of blood was drawn from a non-fasting patient and from a normal subject used as a control. For clot lysis studies, 2 ml. of blood was immediately diluted 1 : 10 in ice cold phosphate buffer (0·034 M, μ 0·072, *p*H 7·4). Aliquots, 2 ml. of the diluted blood, were placed in tubes and clotted at 4° C with one unit of bovine thrombin (Topical Thrombin, Parke Davis and Co., Detroit, Michigan). The tubes were transferred to a 37° C water bath after 30 min and observed for clot retraction and complete lysis[5].

The remaining 18 ml. of the patient's original blood sample provided platelets for identification of γM-globulin and other proteins by platelet agglutination. All procedures were carried out at room temperature. The blood, with 2 ml. of 3·2 per cent (0·11 M) sodium citrate added, was centrifuged slowly to prepare platelet-rich plasma (PRP). The PRP, with 1/25th volume of 5 per cent (0·135 M) EDTA added, was centrifuged to sediment the platelets. The supernatant was decanted, and the platelet button was resuspended in 7–10 ml. of 0·15 M NaCl. This centrifugation washing procedure was repeated twice with saline, and the final platelet button was resuspended in 1–2 ml. of phosphate buffer. The platelets were counted and the volume of the suspension adjusted to 500,000–600,000 platelets/mm³. Approximately 0·05 ml. of the platelet suspension was added to 0·05 ml. of rabbit antiserum or normal rabbit serum in each well of a haemagglutination plate which was placed on a shaker. Observers unaware of the composition of the samples assessed the degree of agglutination after 30 min with the aid of a phase microscope. Anti γM and G-globulin were obtained from Behringwerke, Marburg/Lahn, Germany, and anti-fibrinogen and albumin from Hyland Laboratories, Los Angeles, California.

The results in Table 1 confirm the preliminary finding of abnormal clot lysis in a thrombasthenic patient, and demonstrate that platelets in such patients do not agglutinate nearly as strongly with anti-γM-globulin and anti-fibrinogen as do normal platelets. Platelets from two of the three patients also showed less agglutination with anti-γG-globulin. The platelets of all three patients, unlike normal platelets, agglutinate with anti-albumin. The studies (1) reveal new abnormalities in the surface proteins of thrombasthenic platelets; (2) suggest that these platelets are unable to adsorb normal amounts of plasma proteins of high molecular weight; (3) introduce new evidence of the mediating role of platelet-bound γM-globulin and fibrinogen in the retraction and lysis of dilute whole-blood clots; and (4) raise the question of possible involvement of γM-globulin in ADP-induced aggregation of normal platelets.

Table 1. CLOT RETRACTION, CLOT LYSIS AND AGGLUTINATION OF PLATELETS WITH VARIOUS ANTISERA IN CONTROL SUBJECTS AND PATIENTS WITH THROMBASTHENIA

Subject	Clot retraction at 2 h (0−4+)	Clot lysis time* (h)	Platelet agglutination with antisera (0−4+) Anti-γM	Anti-fbgn.	Anti-γG	Anti-alb.
Pt. (M. M.)	1+	>6	1+	0	0	1+
Normal 1	4+	3	4+	3+	1+	0
Pt. (L. W.)	1+	>6	0	0	1+	2+
Normal 2	4+	3	3+	4+	1+	0
Pt. (M. C.)	0	6	1+	1+	0	2+
Normal 3	4+	3	4+	3+	1+	0

* Normal values: 3–4 h.

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Lateral Inhibition Explanation of Geometrical Illusions

GANZ[1] has suggested that figural distortions found with geometrical illusions are produced by lateral inhibition which results whenever the visual system signals information about spatially adjacent contours[2]. In explaining why two lines appear out of alignment when a further figure is next to one of the lines (Fig. 1) Ganz has assumed that apparent location is determined by the mean of the spatially distributed ridge of excitation which is the neural correlate of a contour. When one figure is near another the distribution of excitation from each figure is modified through lateral inhibition. Excitation is not, however, uniformly reduced; the sections of a distribution which are closer to the peak of the excitatory ridge of the other figure are inhibited more than sections farther away. The mean of each distribution will thus be shifted, and the figures will appear displaced from each other.