Effect of Fibrinogen Concentration on Platelet Adhesion to Glass[1] (35645)

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Adhesion of platelets to glass requires the presence of fibrinogen. This has been demonstrated with washed platelets (1), platelets in citrated plasma (1), and platelets in native whole blood (2). An understanding of the role of fibrinogen in the adhesion of platelets to glass and possibly other surfaces such as collagen and plastics appears essential to development of athrombogenic vascular prostheses and even antithrombotic agents. The development of a simple test for quantitation of platelet adhesion (2) and the availability of a patient congenitally deficient in fibrinogen made possible a quantitative investigation of the need for fibrinogen in the platelet adhesion phenomenon.

Materials and Methods. In the quantitative test for platelet adhesion to glass (2), 4.5 ml of native human blood were drawn into each of several syringes (Monoject, Brunswick Laboratories, St. Louis, Mo.) containing 0.5 ml of a solution of varying concentrations of purified human fibrinogen (300 mg/100 ml, 95 to 96% clottable) (3) in a 0.05 *M* tris(hydroxymethyl)aminomethane buffer (Tris buffer), pH 7.4. An acid-washed glass rectangle measuring 75 × 10 × 1 mm was quickly immersed for 2 min in the blood–fibrinogen mixture following removal of the syringe plunger. The glass rectangle was then twice immersed gently in 0.154 *M* NaCl, fixed in buffered 1.3% glutaraldehyde for 45 min, rinsed in Tyrode's solution and then in water, and air dried. Platelets adhering to the glass surface were counted by incident phase contrast microscopy in 11 (1000×, 61,575 μ^2) fields along the central longitudinal axis of the rectangle; all platelets in each microscopic field were counted. Tests were performed on the afibrinogenemic patient on 3 separate occasions with 2 different fibrinogen preparations; representative data are presented.

The patient, congenitally deficient in fibrinogen (4, 5), had received no blood, plasma, or fibrinogen for at least 3 months prior to each study and had a hematocrit of 46%.

Results. The effect of fibrinogen concentration on the adhesion to glass of platelets from an afibrinogenemic patient is shown in Fig. 1. In the absence of added fibrinogen, an average of 7 platelets adhered to glass/1000× field. This "background" adhesiveness value was subtracted from each of the values plotted in Fig. 1. Fibrinogen concentrations of 0.2 mg/100 ml had no effect on platelet adhesion to glass. Increasing concentrations of fibrinogen from 0.5 to 14.0 mg/100 ml produced progressively increasing platelet adhesion. Increasing the fibrinogen concentration above 14.0 mg/100 ml produced only a slight increase in platelet adhesion.

Discussion. That fibrinogen plays a role in the reaction of blood with foreign surfaces is now well known. Vroman and Adams (6) found that fibrinogen rapidly adsorbed to certain materials and later underwent partial desorption. Baier and Dutton (7) presented indirect evidence for the adsorption of fibrinogen to surfaces in contact with blood. In addition, Dutton *et al.* (8) and Scarborough *et al.* (9) have shown that platelets adhere to the layer of adsorbed plasma pro-

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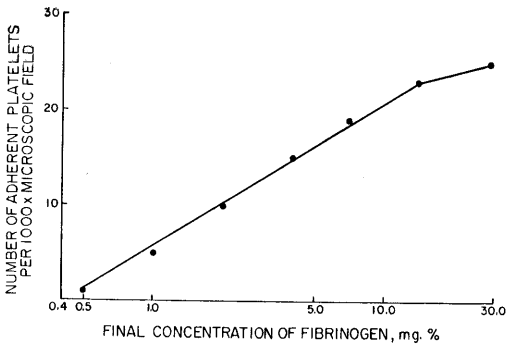

FIG. 1. Effect of fibrinogen concentration on adhesion of afibrinogenemic platelets to glass. The concentration of fibrinogen given is the calculated final concentration in blood in the syringe in which the test was performed.

tein which coats most materials in contact with blood rather than to the uncoated surface of the materials themselves. At present it is unclear whether fibrinogen forms part of the adsorbed protein layer or is required for reaction of platelets with the layer or both.

Fibrinogen has been found to influence the adhesion of platelets to glass. Mason and Gilkey (2) reported markedly decreased adhesion of afibrinogenemic platelets to glass. Zucker and Vroman (1) and Packham *et al.* (10) found that more platelets adhere to glass coated with fibrinogen than to uncoated glass. On the other hand, Lyman *et al.* (11), using a somewhat different test system, reported that coating of Teflon with fibrinogen produced decreased adhesion of platelets. In the present study a direct relationship was demonstrated between the concentration of added fibrinogen in the range 0.5 to 14.0 mg/100 ml and the number of platelets adherent to glass.

The current report emphasizes that relatively small quantities of fibrinogen are sufficient to "normalize" the defective adhesion of afibrinogenemic platelets to glass as measured in our test system. The normal value for this adhesiveness test determined by study of 36 subjects is 33.29 $\pm$ 3.08. (2). Deviation of the curve in Fig. 1. from linearity at higher values for platelet adhesiveness is of interest, since these higher values are near the normal range for platelet adhesion to glass. It can be calculated that in the volume of normal blood in the 3-μ thick zone adjacent to the glass, there are approximately 55 platelets available to adhere/1000$\times$ microscopic field, assuming a platelet count of 300,000/mm^3. The value of 3 μ was chosen since it is the approximate diameter of a platelet. The finding that relatively small amounts of fibrinogen can serve to essentially normalize the adhesion to glass of afibrinogenemic platelets may give some insight to the observation that afibrinogenemic patients can achieve hemostasis with strikingly low circulating levels of fibrinogen.

It has been demonstrated (2) that decreased adhesion of platelets to glass, as measured by the present test system, correlates well with prolongation of bleeding time. With this platelet adhesion test (2), afibrinogenemic and thrombasthenic patients, who had markedly prolonged bleeding times, also had greatly decreased platelet adhesiveness values. With a modification of this test (2), it has been shown that patients with von Willebrand's disease and long bleeding times had decreased platelet adhesiveness values, while those with normal bleeding times had normal adhesiveness values.

Added fibrinogen does not appear necessary for adhesion of all afibrinogenemic platelets to glass. In the absence of added fibrinogen, an average of 7 of the patient's platelets/1000$\times$ field still adhered. This finding may indicate the presence of a small population of platelets which do not require fibrinogen for adhesion or perhaps which carry sufficient fibrinogen associated with the plasma membrane to permit adhesion to glass. Another possibility is that a small number of the patient's platelets can, in the proximity of a glass surface, release sufficient of the fibrinogen known to be associated with their granules (5) to permit adhesion to occur. Also, the small amount of fibrinogen (less than 1 mg/100 ml) present in the patient's plasma may support adhesion of a limited number of platelets.

Since fibrinogen is required not only for clot formation but also for platelet adhesion and stable platelet aggregates (4), one approach for development of antithrombotic agents would be through drugs destroying or

masking fibrinogen. Similarly, surfaces which do not absorb fibrinogen may prove to be thromboresistant.

Summary. The extent to which platelets adhere to glass is dependent on fibrinogen concentration. Platelet adhesion increased progressively in a linear fashion when increasing concentrations of purified fibrinogen (0.5–14.0 mg/100 ml) were added to the blood of a patient with congenital afibrinogenemia.

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