Platelet Adhesion Induced by Fibrinogen Adsorbed onto Glass* (33866)

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Platelets in the platelet-rich plasma (PRP) of subjects with congenital afibrinogenemia adhere to glass only if fibrinogen is added *in vivo* or *in vitro* (1–3). Vroman and Adams (4, 5), using an ellipsometer, found that wettable oxidized silicon crystals or anodyzed tantalum slides adsorbed fibrinogen from plasma within 2 sec. The adsorbed protein was identified by its ability to bind fibrinogen antiserum. About 10 sec later, avidity for antiserum began to decrease, and by 25 sec it was completely lost. This apparent loss of fibrinogen is unexplained; it did not represent loss of protein, since the film reached its peak thickness at 15 sec. The subsequent gradual decrease in film thickness required Hageman factor (factor XII) (4). These results suggested that fibrinogen, while it was bound to glass, might promote platelet adhesiveness. We therefore studied the adhesion of platelets to glass slides exposed for varying periods to solutions, some of which contained fibrinogen.

Materials and Methods. Plasma was obtained from blood collected into ⅛ vol of 0.105 *M* (3.1%) sodium citrate from subjects (i) with no known disorder, (ii) with afibrinogenemia (samples kindly provided by Dr. K. M. Brinkhous), or (iii) with severe von Willebrand's disease. Normal plasma was rendered fibrinogen-free by heating to 56° for 5 min. Serum was prepared from normal plasma recalcified in the presence of dilute thromboplastin and was incubated at 37° for 4 hr. Human, plasminogen-free fibrinogen or 7S gamma globulin (Mann Research Laboratories, New York, N.Y.) was prepared in 1% solution in buffered saline. These reagents were used fresh or after storage at −20°.

Ordinary glass microscope slides were marked on the back with one or two circles 5–10 mm in diameter. The other side was scrubbed with a solution of Sparkleen (Fisher Scientific Co., Springfield, N.J.), rinsed thoroughly with water, dried, and briefly flamed. Some slides, left uncoated, were rinsed with 10 ml of isotonic saline, buffered to pH 7.2–7.3 with 0.1 vol of 0.15 *M* Veronal or imidazole, followed by 10 ml of water, and then allowed to dry. Areas on other slides were coated either by placing a drop of fibrinogen over a circle for 3–5 min or a drop of plasma or serum over one circle and a second drop over the other circle 3 min later. The slides were immediately tilted, the drops were rinsed as described, and the slides were allowed to dry.

Some experiments were performed with fresh, citrated PRP and others with washed platelets prepared at room temperature from citrated PRP with addition of 0.04 vol 0.135 *M* (5%) disodium ethylenediaminetetraacetate. The platelets were centrifuged and resuspended three times in isotonic saline, suspended in a small volume of saline, and diluted with 9 vol of one of the preparations of plasma described above. A drop of PRP or platelet suspension was placed on an uncoated or coated area within 30 min of coating and 1.5 hr of venipuncture, and the slides were left in a moist chamber at room temperature for 30 min. They were tilted and washed under a stream of 5 ml of buffered saline, and then 5 ml of saline, flowing by gravity from a 5-ml pipette onto the slide above the coated areas. After brief fixation in methanol the slides were dried and observed with a phase microscope.

Results. Data obtained with washed normal platelets are shown in Table I. Platelets suspended in plasma adhered to areas coated by exposing the slide to fibrinogen or plasma, but often failed to adhere to areas exposed

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TABLE I. Effect of Platelet Suspension Medium and Type of Coating on Number of Platelets Adhering to Glass Slides.[a]

Expt.	Suspension medium for platelets	Type of coating and length of exposure to glass slide (sec)						
		Fibr.	Plasma		Afibrin. plasma[b]		Serum	
		180	5	180	5	180	5	180
1	Normal plasma	+++		++		+		+++
2		+++		++		+++		++
3			++	++	0	0		
4			++	++	+	0	±	+
5			++	0	0	0	0	0
6			+++	++	+++	+	0	±
1	Afibrinogenemic plasma[b]	+++		++		0		0
2		+++		+		0		0
3			0	+	0	0		
4			+	0			0	0
5			+++	0	0	0	0	0
6			+++	±	±	±	±	±
1	Normal serum	+++		++		0		0
2		+++		++		0		0
3								
4			++	+			0	0
5			++	+++	0	0	0	0
6			+++	+++	0	±	±	±

[a] Estimated number of platelets per high power (400×) field: +++ = 500; ++ = 50–500; + = 10–50; and ± = 1–10.

[b] In Expts. 1–3, obtained from a patient with congenital afibrinogenemia; in Expts. 4–6, obtained by heating normal plasma.

to afibrinogenemic plasma or serum. Platelets suspended in afibrinogenemic plasma or serum adhered to areas of glass which had been exposed to normal plasma or to fibrinogen—never, except in trace numbers, to areas exposed to afibrinogenemic plasma or serum. In some of these experiments, many more platelets adhered to an area exposed to plasma for 5 sec than for 3 min.

When washed normal platelets suspended in plasma were exposed to an uncoated glass surface, varying numbers adhered in different experiments and even in different areas on the same slide. Platelets in normal PRP also behaved unpredictably.

Platelets in citrated or heparinized PRP from a subject with afibrinogenemia (studied through the courtesy of Dr. Harvey Weiss) adhered only if the slide was first exposed to fibrinogen or for 5 sec to plasma from a normal subject or one with severe von Willebrand's disease (antihemophilic factor 7%) (Table II). Platelets in PRP from two thrombasthenic subjects failed to adhere even after the slide was exposed to plasma or to fibrinogen.

Discussion. Others have reported that washed platelets suspended in Tyrode's solution adhere to surfaces coated with purified gamma globulin or fibrinogen (6, 7). We found that platelets suspended in serum or in afibrinogenemic plasma adhered to glass only if it had been previously coated with a solution containing fibrinogen, indicating that gamma globulin in plasma does not promote adherence in the absence of fibrinogen. Exposing glass to fibrinogen solution or briefly to plasma from a von Willebrand patient or normal subject promoted adhesion of platelets from an afibrinogenemic subject; gamma globulin solution or fibrinogen-free plasma was ineffective. These results reemphasize the

TABLE II. Effect of Type and Duration of Coating on Number of Platelets in Citrated Afibrinogenemic Platelet-Rich Plasma Adhering to Glass Slides.

	Type of coating and length of exposure to glass slide (sec)							
	Fibr.	Gamma glob.	Normal plasma		von Willebrand plasma		Afibrin. plasma[a] or serum	
Expt.	180	180	5	180	5	180	5	180
1[b]	+++	0	+++					
2			+++	±	++	±	±	±

[a] Heated normal plasma.

[b] Heparinized platelet-rich plasma behaved similarly.

requirement of fibrinogen for platelet adhesion to glass. In some experiments, fewer platelets adhered to areas of glass exposed to plasma for 3 min than to areas exposed for 5 sec, suggesting that the fibrinogen deposited during the 5-sec exposure was not altered by subsequent 30-min exposure to the plasma in which the platelets were suspended. It seemed unlikely that this apparent stability of the adsorbed fibrinogen resulted from drying the film, since, in unpublished experiments with the ellipsometer (Vroman and Adams), drying areas of oxidized silicon after brief exposure to plasma did not prevent loss of reactivity to fibrinogen antiserum on reexposure to plasma.

Summary. Platelets suspended in serum or afibrinogenemic plasma (heated or congenital) adhered to glass slides previously exposed to normal plasma or to fibrinogen but not to slides exposed to serum or afibrinogenemic plasma. Platelets in platelet-rich plasma from a patient with congenital afibrinogenemia adhered to glass coated with plasma from a normal subject or von Willebrand patient but not to areas coated with afibrinogenemic plasma or serum. Platelets in platelet-rich plasma from two thrombasthenic patients failed to adhere even when the slide was coated with fibrinogen or plasma. At times, a much higher percentage of platelets adhered to areas exposed to plasma for 5 sec than to areas exposed for 3 min. According to other evidence, the protein film adsorbed from plasma onto a surface no longer reacts with antiserum to fibrinogen after 3 min. We conclude that a fibrinogen layer must first be adsorbed to glass for the platelets to adhere.

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