Role of Shear Rate and Platelets in Promoting Fibrin Formation on Rabbit Subendothelium

Studies Utilizing Patients with Quantitative and Qualitative Platelet Defects

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Abstract

The deposition of platelets on subendothelium of rabbit aortic segments exposed to non-anticoagulated human blood increased progressively with increasing wall shear rates (50–2,600 s^{-1}), whereas fibrin deposition decreased. Studies in normal subjects and patients with platelet disorders suggested that, under the conditions used, platelets were essential for fibrin deposition at intermediate (650 s^{-1}) but not low (50 s^{-1}) shear rates. Fibrin deposition was markedly diminished in a patient with Scott syndrome whose platelets have a diminished capacity to bind Factor X_a and activate Factors IX and II. In glycoprotein IIb-IIIa deficiency, fibrin deposition was normal (or somewhat increased), whereas in glycoprotein Ib deficiency the association of fibrin with platelets, but not subendothelium, was decreased. The findings indicate that platelets, perhaps through surface localization of coagulation proteins, promote fibrin deposition on subendothelium at arterial shear rates and suggest that agents directed against platelet coagulant properties could be antithrombotic.

Introduction

The properties of platelets that permit them to adhere and aggregate at sites of blood vessel injury account, in part, for their role in the primary arrest of bleeding, and explain the prolonged bleeding time in patients with disorders of platelet adhesion/aggregation (cohesion) such as von Willebrand's disease and thrombasthenia (1–3). These properties of adhesion and cohesion also contribute to the formation of occlusive platelet thrombi in arteries where local shear conditions, through convective and diffusion mechanisms, favor the deposition of platelets on the vessel wall (4, 5). Extensive laboratory studies, generally performed in test tubes under poorly defined shear conditions (probably low), have also established that platelets provide a surface which can greatly accelerate the coagulation mechanism (6–8) and, hence, could play an important role in the permanent arrest of bleeding. Platelets could also contribute to the formation of the fibrin component of intravascular thrombi, but the extent to which the coagulant properties of platelets may be influenced by flow conditions in various parts of the circulatory system has not been studied. It is generally held, for example, that platelets play a relatively minor role in the development of thrombi in large veins (shear rate of 100 s^{-1} or less) because fibrin and red cells account for most of the mass of such thrombi (9, 10). However, the nidus of a venous thrombus may contain a small but significant number of platelets (11–13) which could, in theory, accelerate the formation of fibrin, even though they do not account for the bulk of the thrombus (14). Whether or not this small mass of platelets is of any significance in the initiation and extension of a venous thrombus is still an open question (10). Even where platelets clearly contribute to the mass of a mixed platelet–fibrin thrombus, as in the major epicardial coronary vessels (15, 16), (shear rates 200–800 s^{-1} [17]), it is not clear to what extent the potential platelet coagulant activity is actually utilized in fibrin formation. Finally, and almost paradoxically, the increasing contribution of platelets to a thrombotic mass, or a primary hemostatic plug, at even higher shear rates (2,000 s^{-1}, or more), such as those found in arterioles (17), is generally associated with a decrease in fibrin formation (2, 18).

This study was undertaken to evaluate the procoagulant properties of platelets on vascular surfaces throughout a wide range of shear rates. Utilizing an annular perfusion chamber, we exposed everted segments of rabbit aorta, from which the endothelium has been removed by balloon catheter, to human blood at shear rates ranging from 50–2,600 s^{-1} and measured the deposition of platelets and fibrin on the subendothelium, and the blood fibrinopeptide A levels in postchamber blood. We studied normal and thrombocytopenic subjects to establish the flow conditions at which platelets contribute to fibrin formation, and utilized patients with well-characterized platelet defects to identify platelet properties which may contribute to this process.

Methods

Perfusion chamber containing vessel segments

The perfusion chamber is an annular device on the core of which is mounted an everted segment of rabbit aorta whose endothelium has been completely removed by balloon catheter (19–22). Chambers of two different sizes, designated original and small (20), were used in the study. Non-anticoagulated blood entering the chamber passes through the annular space formed by the subendothelial surface and the outer cylinder wall, thereby permitting fibrin and platelet deposition on the exposed subendothelial surface. The flow parameters, dimensions, and temperature (37°C) control for the chamber have been previously described (20, 23). The wall shear rate for each flow rate used was determined as previously described (20, 23). For flow rates of 10, 20, and 40 ml/min in the small chamber, the corresponding wall shear rates are 650, 1,300, and 2,600 s^{-1}. For the original chamber, flow rates of 10 and 20 ml/min correspond to wall shear rates of 50 and 100 s^{-1}. Vessel segments 20 mm in length were stored in 0.2 M Tris buffer, pH 7.4, at 4°C for periods of 7–28 d prior to use.

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Perfusion procedure

The basic technique for exposing everted vessel segments to non-anticoagulated blood at varying shear rates, and for obtaining pre- and post-chamber samples for fibrinopeptide A levels (24), has been described in detail previously (20, 24–26). Basically, blood is drawn directly from the antecubital vein through an infusion set (Butterfly, Abbott Laboratories, Irving, TX) into the perfusion chamber and is not recirculated (24–27). Flow rate is controlled by a Holter roller pump (Extracorporeal Medical Specialties, Inc., King of Prussia, PA) placed distal to the perfusion chamber and is accurately determined during each run by measuring the actual amount of blood collected at 30-s intervals (21, 24). The perfusion is terminated by stopping the pump, and blood proximal and distal to the perfusion chamber (designated prechamber and postchamber blood) is collected for measurement of fibrinopeptide A (FPA)[1] (24) as follows: proximal to the chamber the infusion tubing used for the venipuncture is clamped with two hemostats and severed with scissors. Blood (0.2 ml) from the venipuncture site is collected through the tubing by gravity into a predetermined volume (1.8 ml) of inhibitor solution (24). Distal to the chamber, the silastic tubing that connects the perfusion chamber to the pump is removed and the blood (0.2 ml) contained within is drained into inhibitor solution (1.8 ml). The period of time required to sample blood for FPA measurements, during which blood remained in contact with the vessel segment under zero flow conditions, is recorded and varied from 30 to 45 s. After obtaining blood samples for FPA, the distal tubing is replaced and the vessel segment is washed free of blood and fixed with glutaraldehyde as follows: the severed end of the infusion tubing that is connected to the perfusion chamber is first immersed into phosphate-buffered saline (0.02 M phosphate, 0.1 M NaCl, pH 7.4), and the pump flow is reinitiated at the original flow rate for variable periods of time (see below) to wash out the blood in the chamber. The pump is then stopped, the infusion tubing is switched to 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, and the flow is restarted to fix the segment on the chamber rod. Wash and fixation times of the perfusion system were adjusted for the various flow conditions and approximated a constant volume of fluid (7–10 ml) passing through the chamber. At a shear rate of 2,600 s^{-1}, washout times were 5 and 10 s with buffer and fixative respectively; at 1,300 s^{-1}, 5 and 15 s; at 650 s^{-1}, 10 and 30 s; at 100 s^{-1}, 15 and 15 s; and at 50 s^{-1}, 30 and 30 s. The perfusion rod with vessel attached was then removed from the chamber and immersed in buffered glutaraldehyde solution (as above) for up to 1 h, then transferred to 7% sucrose solution, and kept at 4°C until embedded in epoxy.

Evaluation of vessel segments

After perfusion, vessel segments were embedded in epoxy resin in an oriented manner. Vessel cross-sections, ~0.8 μm in thickness, were stained and evaluated morphometrically by light microscopy for platelet interactions and fibrin deposition (19–21, 24). All evaluations were by technologists who were unaware of the sources of the material. For each vessel segment, one section, located 10–13 mm distally from the proximal end of the vessel segment, was evaluated for platelet and fibrin deposition.

Fibrin (presence or absence) on the subendothelium (F_{SE}) and on platelets (F_{plt}) was evaluated systematically at 10-μm intervals (~1,000 intersections) and total fibrin ($F_T = F_{SE} + F_{plt}$) expressed as a percentage of the total number of evaluations (20, 24–26). Identity of fibrin was demonstrated by electron microscopy showing apparent banding of approximately 230 Å of longitudinally cut fibrin strands. Recent studies (Inauen, Baumgartner, Haeberli, and Straub, manuscript in preparation) have demonstrated an excellent correlation ($r = 0.92$, $n = 48$, $P < 0.001$) between surface fibrin estimated morphometrically and also measured by a previously described method (28) from fragment E and (thrombin-induced) FPA levels in plasmin digests of surface thrombi. In brief, the thrombus on the center piece of subendothelium is proteolytically degraded by plasmin and the total (fibrin + fibrinogen) in the thrombus is calculated from measurement (by radioimmunoassay) of the late soluble fragment E. The plasmin lysates are then incubated with an excess of thrombin and, from the resulting FPA (measured by radioimmunoassay), the amount of fibrinogen deposited in platelets and nonspecifically fixed in the thrombus can be calculated. From the above, the amount of fibrin in the original thrombus is determined by subtraction.

For determining platelet adhesion, the presence of platelets on subendothelium was evaluated at 10-μm intervals and platelet–subendothelial interaction was evaluated as either (*a*) contact (C)—platelets are attached, but not spread on the surface or (*b*) spread (S)—platelets are spread on the surface and may, in addition, have superimposed aggregates of platelets (thrombi) extending for varying distances into the lumen. Platelet adhesion is defined as C + S, expressed as a percentage of the total number of evaluations (~1,000) per vessel segment. Platelet thrombus volume and thrombus height were determined planimetrically using a manual optical picture analysis system (MOP) (24, 26). The thrombus volume per unit surface area is calculated from the total cross-sectional area of all platelet aggregates in a section and normalized by the cross-sectional length (circumference) of the subendothelial surface (26). The maximum thrombus height is the average peak-to-base distance for the three tallest thrombi in a section. At shear rates ≥ 650 s^{-1}, all platelet thrombi present in a section are connected to the subendothelial surface and measurements of maximum thrombus height refer to such thrombi. At shear rates of 50 and 100 s^{-1}, many platelet thrombi form within, or on top of, the fibrin meshwork. Inasmuch as there is no evidence that these platelet aggregates are connected to the subendothelial surface, the measured maximal thrombus heights (30–40 μm) at these very low shear rates considerably overestimate the maximal heights of thrombi that are directly attached to subendothelium which, although not systematically measured, are of the order of 5–10 μm. Further details of the manual optical picture technique have been previously published (26, 27).

Calculation of platelet-bound fibrin

Calculation of total surface coverage with fibrin (F_T) takes into account fibrin that is deposited directly on the subendothelial surface (F_{SE}) as well as fibrin that is observed on platelets that are attached to the surface (F_{plt}). In order to account for the different levels of surface coverage with platelets observed in various patient groups, we calculated, for each subject, a parameter $F_{plt}/(C + S)$, and designated this normalized value as platelet-bound fibrin.

Determination of FPA levels

Aliquots of the prechamber and postchamber blood samples were mixed with 0.1 vol of 0.15 M NaCl containing Trasylol (Bayer, Leverkusen, Federal Republic of Germany), 50 U/ml, and heparin (Liquemin sodium, Organon Diagnostics, West Orange, NJ), 1,000 U/ml, and centrifuged at 1,500 *g* at 4°C for 20 min. The supernatant plasma was kept at −70°C prior to assay. Radioimmunoassay of FPA was performed by a modification (29) of the method of Nossel et al. (30) as previously described (24). We have previously shown that, in studies on three normal subjects, at a shear rate of 650 s^{-1}, the postchamber increase in FPA levels over the average prechamber values is almost entirely attributable to the vessel segment (24). To determine whether this was also the case at lower shear rates, we measured postchamber FPA levels in the absence of a vessel segment in six normal subjects at a shear rate of 50 s^{-1} and, in addition, extended the number of "empty chamber" studies at 650 s^{-1}, to include eight normal subjects. Results are shown in Table I and demonstrate that, at both shear rates, the increase in FPA levels across the chamber is due almost entirely to the vessel segment. Thus, at 50 s^{-1}, FPA levels across the chamber increased from 12±4 to 595±81 ng/ml when the perfusion chamber contained a vessel segment compared with a postchamber value of 38±16 ng in the absence of a vessel segment. The results obtained at 650 s^{-1} were similar (Table I).

Subjects

Thrombasthenia. Three patients (M.C., L.M., and C.G.) had platelet abnormalities characteristic of Glanzmann's thrombasthenia (1, 3, 31).

1. *Abbreviations used in this paper:* FPA, fibrinopeptide A; GP, glycoprotein.

Table I. Fibrinopeptide A Levels in Blood

Shear rate	Perfusion time	Blood FPA values* Prechamber	Postchamber Vessel absent	Postchamber Vessel present
s^{-1}	*min*	*ng/ml*	*ng/ml*	*ng/ml*
650	5	14±2 (24)‡	36±10 (8)	456±34 (24)
50	5	12±4 (6)	38±16 (6)	595±81 (6)

* Mean±SEM on blood collected at termination of perfusion (see Methods).
‡ Number of normal subjects studied.

The platelets of these patients do not aggregate with 5×10^{-5} M ADP or epinephrine, or with collagen (20 μg/ml). One previously reported (32, 33) patient (M.C.) and another subject C.G. (kindly studied by Dr. Graham Jamieson) have decreased amounts of glycoproteins (GP) IIb and IIIa detected by periodic acid-Schiff staining after separation of platelet membrane proteins by polyacrylamide gel electrophoresis (33). Patient L.M. was kindly referred for study by Dr. Margaret Johnson and had been shown previously to have decreased amounts of GPIIb-IIIa (34). Other studies on the thrombasthenic patients have shown decreased amounts (C.G. 5%, L.M. 10%, and M.C. 2% of normal) of GPIIIa (Dr. Perumai Thiagaragan, personal communication), and markedly reduced or absent amounts of the GPIIb-IIIa complex (M.C., L.M., and C.G.), detectable by crossed immunoelectrophoresis of Triton X, solubilized whole platelets against a polyclonal rabbit anti-human platelet antibody (kindly performed by Dr. Ira Sussman).

Scott syndrome. Patient M.S. (Scott syndrome) is a 45-yr-old woman with a moderate bleeding disorder who has been previously reported to have an isolated defect in platelet procoagulant activity, reflected by impaired kaolin-induced activation of platelet factor 3 in platelet-rich plasma and a serum prothrombin time (11–12 s) similar to that observed in hemophilia (35). Other studies on this patient have shown that the Factor Xa binding capacity of her isolated platelets is about one-third normal, and is not corrected by addition of Factor Va (36). These abnormalities are associated with an impaired capacity of her platelets to convert prothrombin to thrombin in the presence of Factors Va and Xa (36). More recently, an impairment in the capacity to convert Factor X to Xa in the presence of Factors VIII and IXa has also been demonstrated (37). The phospholipid content of her platelets is normal (35) but the expression of acidic phospholipids, such as phosphatidyl serine, on the platelet surface after collagen plus thrombin is decreased (37). No morphologic abnormalities in her platelets have been detected (35), and her platelets aggregate and secrete normally in response to ADP, collagen, and epinephrine (35).

Bernard-Soulier syndrome. Two previously reported (20, 21) patients (A.J. and T.H.) whose findings are typical of those in Bernard-Soulier syndrome (3, 31) were studied. Previous studies on these patients have demonstrated absent platelet aggregation with ristocetin (21), impaired platelet adhesion to subendothelium in citrated blood (shear rates 800 [21] and 1,300 [20] s^{-1}) and non-anticoagulated blood (1,300 s^{-1}) (20), and decreased amounts of platelet GPIb (33).

Thrombocytopenia. Ten patients (seven females and three males) with either drug-induced thrombocytopenia (three patients) or idiopathic thrombocytopenic purpura (seven patients) were studied. The hemoglobin level, white blood cell count, prothrombin time, and partial thromboplastin time were normal in each patient. No evidence for an underlying disease which might have accounted for isolated thrombocytopenia was found in any of these patients.

Normal subjects. Control values were obtained by studying normal hospital personnel, ages 25–50.

Blood values

Platelets were counted on blood collected into EDTA by an electronic counting device (model S, Coulter Electronics, Inc., Hialeah, FL) or by phase microscopy. The average values and ranges for platelet counts (per microliter) in patients and normal subjects were as follows: thrombasthenia (n = 3), studied once at 650 s^{-1}, 224,000 (162,000–328,000); Bernard-Soulier syndrome (n = 2), studied once at 50 and 1,300 s^{-1}, and twice at 650 s^{-1}, 91,000 and 115,000; Scott syndrome, studied at 50 (n = 2), 100 (n = 1), 650 (n = 5), and 1,300 (n = 2) s^{-1}, 367,000 (range 304,000–403,000); thrombocytopenia (n = 10), 24,000 (1,000–68,000). A total of 36 normal subjects (14 males and 22 females) were studied, some on several occasions, at shear rates ranging from 50 to 2,600 s^{-1}. The average platelet count was 288,000 (201,000–370,000). Normal subjects with platelet counts > 370,000/μl were excluded from the study.

Results

Effect of shear rate on platelet and fibrin deposition on subendothelium in normal subjects

The everted de-endothelialized vessel segment was exposed to directly sampled (non-anticoagulated) venous blood from normal subjects for 5 min at shear rates of 50, 100, 650, 1,300, and 2,600 s^{-1}. After perfusion, a postchamber blood sample was obtained for determination of FPA and the vessel segments were removed for evaluation of platelet and fibrin deposition. Fig. 1 *A* shows the relationship between subendothelial coverage with adherent platelets (C + S) and fibrin deposition on the subendothelium (F_{SE}) and Fig. 1 *B* shows the dimensions of the platelet thrombi. At the lowest shear rate (50 s^{-1}), only 5.3±0.5% of the vessel surface was covered with adherent (C + S) platelets (Fig. 1 *A*). With increasing shear rate, platelet adhesion, as well as thrombus volume and average maximum thrombus height (Fig. 1 *B*), also increased. The average maximum height, for example, increased from 36±8 μm at 50 s^{-1} to 130±22 μm at 2,600 s^{-1}. Thus, the effect of increasing shear rate was to enhance both the attachment (C + S) of platelets to the surface as well as the size of the platelet thrombi that formed over adherent platelets. The opposite was true for fibrin deposition on the subendothelium, which was maximum (F_{SE} = 85.3±3.1% surface coverage) at 50 s^{-1}, and decreased sharply at shear rates > 650 s^{-1}. The latter was also the case for blood FPA levels, shown in Fig. 2 in relation to the total fibrin deposition on the surface ($F_{plt} + F_{SE}$).

From the above studies, a shear rate of 650 s^{-1} seemed appropriate for examining a possible role of platelets, and platelet properties, on fibrin deposition.

Studies performed at a shear rate of 650 s^{-1} and a perfusion time of 5 min

Normal subjects. In 24 normal subjects, the value of platelet adhesion (C + S) after 5 min of perfusion was 28.3±1.6% surface coverage. Table II shows the values obtained for surface-bound fibrin. The total fibrin (F_T = 76±4%) is the sum of fibrin on subendothelium (F_{SE} = 56±3%) + fibrin on platelets (F_{plt} = 20±2%). The calculated value of platelet-bound fibrin (F_{plt}/(C + S), see Methods) was 0.72±0.04. The FPA value in blood distal to the segment was 456±34 ng/ml.

Effect of platelet number on fibrin deposition. In order to evaluate a possible role of platelets in promoting fibrin deposition at a shear rate of 650 s^{-1}, we studied 10 patients with immune thrombocytopenia whose platelet count varied from 1,000 to

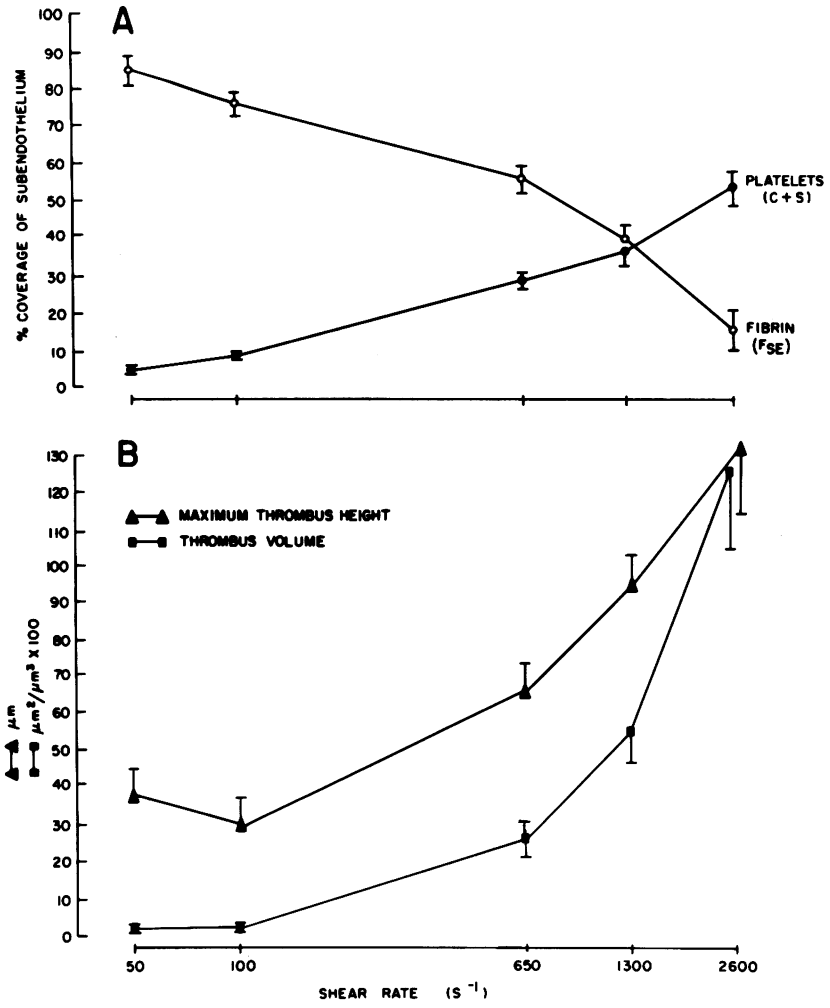

Figure 1. Fibrin and platelet deposition on subendothelium in normal subjects. Everted vessel segments were exposed to non-anticoagulated (directly sampled) venous blood from normal subjects for 5 min at the shear rates shown. (*A*) Subendothelial coverage with adherent platelets (C + S) and fibrin (F_{SE}). (*B*) Dimensions of platelet thrombi, shown as (*a*) the average height (μm) of the three tallest thrombi (see explanation in Methods pertaining to measurements of thrombus height at very low [50 and 100 s^{-1}] shear rates) (*b*) platelet thrombus volume (μm^3) per square micrometer of vessel surface. All values are mean±SEM.

63,000/μl. The range of values for fibrin deposition in these patients was 3.4 to 99.3%. Fibrin deposition was clearly related ($r = 0.67$, $P < 0.05$) to surface coverage with platelets adherent to the subendothelium (Fig. 3 *B*). (Not surprisingly, the surface coverage with platelets also correlated [$r = 0.80$, $P < 0.01$] with the number of platelets in the blood, Fig. 3 *A*.) Thus, the lowest values for fibrin deposition (3.4 to 19.6%) were in four patients whose adhesion (C + S) values were <4%, whereas adhesion values > 4% in the six other patients were associated with fibrin deposition values that were, with one exception, >79% (one value was 40.5%). These findings suggest that platelets are necessary to support normal levels of fibrin deposition on subendothelium at a shear rate of 650 s^{-1}, and that only a relatively small number are required. Interestingly, although fibrin deposition on the

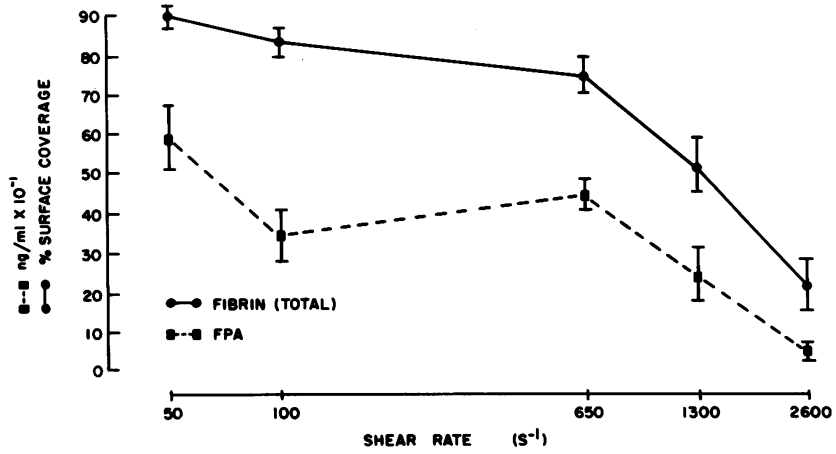

Figure 2. Total fibrin deposition and FPA levels in normal subjects. Values shown (mean±SEM) are the total surface coverage with fibrin, which is the sum of the fibrin deposited on the subendothelium (F_{SE}) and on platelets (F_{plt}), and the FPA levels in postchamber blood immediately after completion of the 5-min perfusion.

Table II. Fibrin and Platelet Deposition on Subendothelium: 5-min Perfusion*

Subject	n	Fibrin deposition—fibrin (F)			FPA	Platelet deposition			
		Total (F_T)	On SE (F_{SE})	On plts (F_{plt})		Adhesion (C + S)	Thrombus volume	Maximum thrombus height	Platelet-bound fibrin‡—$F_{plt}/(C + S)$
		% coverage			*ng/ml*	*% coverage*	*$\mu m^3/\mu m^2$*	*μm*	
Normal	24	76±4§	56±3	20±2	456±34	28±2	2.6±0.6	68±7	0.72±0.04
Thrombocytopenia									
(C + S) < 4%	4	12±4	12±4	0	498±76	1±1	0	0	*0.11±0.05*
(C + S) > 4%	6	82±8	72±3	10±2	479±87	12±2	0.12±0.04	5±2	0.79±0.09
Scott syndrome‖	1	14	12	2	196	39	0.6	28	0.05
Bernard-Soulier	2	50	47	3	274	12	0.4	25	0.29
Thrombasthenia	3	99±1	74±6	25±5	620±124	27±9	0	0	1.04±0.20

Abbreviations: C, contact; F, fibrin; plt, platelet; S, spread; SE, subendothelium. * Everted vessel segments exposed to blood for 5 min at a shear rate of 650 s^{-1}. ‡ Ratio [$F_{plt}/(C + S)$] calculated for each subject and values averaged to obtain group values. § All group values for measured parameters (except thrombus volume) are rounded to the nearest integer and are shown as mean±SEM. ‖ Average of five studies.

surface was clearly related to the number of adherent platelets, FPA values in the blood distal to the vessel were not. As seen in Table II, similar FPA values were observed in the four patients with the lowest fibrin deposition, in the six other patients with normal fibrin deposition, and in the normal subjects.

Scott syndrome. Studies in the patient with Scott syndrome were carried out on five separate occasions. Fig. 4 is a photomicrograph showing representative findings in this patient (Fig. 4 *B*). The data for all parameters of coagulation and fibrin on subendothelium are shown in Table II. Platelet adhesion (C + S) values in this patient were normal and, in fact, were slightly increased. Despite the normal values of platelet adhesion, total fibrin deposition (Fig. 4 *B* and Table II) was markedly decreased (F_T = 14%), and resulted from a marked decrease in both fibrin on subendothelium (F_{SE} = 12%) and fibrin on platelets (F_{plt} = 2%). The normalized platelet fibrin value [$(F_{plt}/(C + S)$] was also markedly decreased (0.05 vs. 0.72±0.04 in control subjects). Somewhat decreased values were also obtained for FPA (196 ng/ml) [although this value was not outside the mean±2 SD].

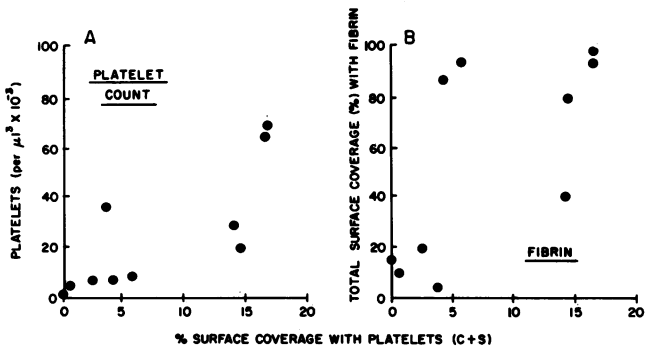

Figure 3. Platelet and fibrin deposition on subendothelium in patients with thrombocytopenia. Vessel segments were exposed for 5 min to blood from 10 patients with thrombocytopenia (idiopathic or drug-induced) at a shear rate of 650 s^{-1}. The figure depicts the relationship between percent surface coverage with platelets (adhesion, C + S, *abscissa*) and (*A*) platelet count (r = 0.80, $P < 0.01$); (*B*) total fibrin deposition (r = 0.67, $P < 0.05$).

Thrombasthenia. Thrombasthenic platelets adhered normally as a monolayer on the subendothelium. Platelet-to-platelet interaction was absent (Table II) at this shear rate, as has been observed previously at all other shear rates studied (38), demonstrating the complete lack of platelet-to-platelet cohesion in patients whose platelets are deficient in GPIIb-IIIa. In striking constrast to the patient with Scott syndrome, all parameters of fibrin deposition (total, subendothelial, and platelets) were normal and, in fact, were somewhat increased (Table II and Fig. 4 *C*).

Bernard-Soulier syndrome. As shown in Table II, platelet adhesion values (C + S) in two patients with the Bernard-Soulier syndrome were decreased to a level (12%) that was similar to values (12±4%) in the group of thrombocytopenic patients with normal fibrin deposition. However, a moderate decrease (F_T = 50%) in total surface coverage with fibrin was observed and was almost entirely due to a decreased value (3%) of F_{plt}. The decreased amount of platelet-bound fibrin is evident even when normalized for the somewhat decreased adhesion values [$F_{plt}/(C + S)$ = 0.29].

Studies performed at a shear rate of 650 s^{-1} and a perfusion time of 10 min

In addition to the 5-min perfusion studies described above, studies utilizing a perfusion time of 10 min were also performed on most patients (two of the 10 thrombocytopenic patients were not studied at this condition). The results, shown in Table III, are in essential agreement with those obtained with the 5-min perfusion, perhaps demonstrating to a somewhat greater degree, the marked decrease in platelet-bound fibrin in the Bernard-Soulier syndrome. In addition, the fibrin and FPA defects in the patient with Scott syndrome (studied on three occasions) are even more evident than at the 5-min perfusion time. With the longer perfusion time, platelet adhesion increased markedly (73% surface coverage) in this patient, and was associated with decreased values of fibrin deposition (4% vs. 75±2%, control), and FPA values (41 ng/ml) which are now unequivocally (>2 SD) less than controls (742±72 ng/ml). In contrast, FPA values in the blood of thrombocytopenic patients were normal, even in the one patient with an adhesion (C + S) value < 4% in whom

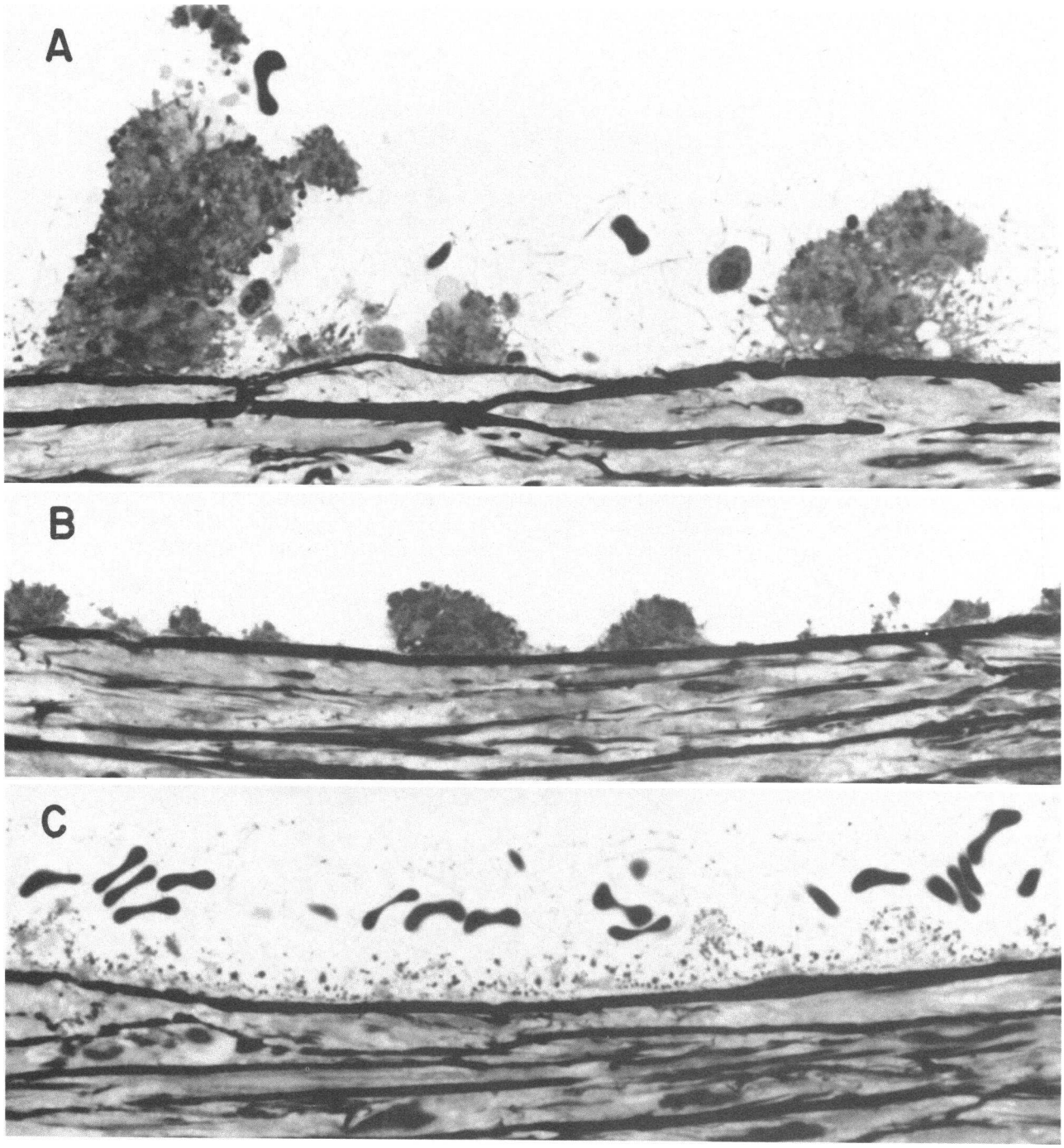

Figure 4. Blood–vessel wall interaction in Scott syndrome and thrombasthenia. De-endothelialized vessel segments were exposed to blood from various subjects at a shear rate of 650 s^{-1} for 5 min. (*A*) Normal subject. (*B*) Scott syndrome. (*C*) Thrombasthenia (× 1,300).

fibrin deposition on the surface (11%) was markedly decreased. Finally, the platelet thrombus height was less (>2 SD) than control values in Scott syndrome, probably owing to impaired thrombin generation, as previously observed in hemophilic patients at this shear rate (24).

Shear rate dependent deposition of fibrin in patients with platelet disorders

The above studies demonstrated a requirement for platelets [(C + S) > 4%] and for some specific platelet properties (deficient in Scott syndrome and, to a lesser extent in the Bernard-Soulier syndrome) in mediating fibrin deposition on subendothelium and the platelet surface at a shear rate of 650 s^{-1}. To assess further a possible role for platelets at other shear conditions, we studied selected patients at shear rates ranging from 50 to 1,300 s^{-1} and a perfusion time of 5 min. The results obtained for platelet adhesion (C + S) and for total fibrin (F_{SE} + F_{plt}) are shown in Fig. 5.

As noted previously, total fibrin deposition (F_{SE} + F_{plt} = 89.7±3.0% surface coverage), and FPA values (595±81 ng/ml, Fig. 2) in normal subjects were maximum at a shear rate of 50 s^{-1}, despite the lowest adhesion (C + S) values (5.3±0.5%).

Table III. Fibrin and Platelet Deposition on Subendothelium: 10-min Perfusion*

Subject	n	Fibrin deposition—fibrin (F) Total (F_T)	On SE (F_{SE})	On plts (F_{plt})	FPA	Platelet deposition Adhesion (C + S)	Thrombus volume	Maximum thrombus height	Platelet-bound fibrin‡— $F_{plt}/(C + S)$
		% coverage			ng/ml	% coverage	$\mu m^3/\mu m^2$	μm	
Normal	18	75±2§	54±5	21±2	742±72	38±3	9.9±1.1	149±11	0.58±0.05
Thrombocytopenia‖									
(C + S) < 4%	1	11	11	0	589	3	<0.1	3	—
(C + S) > 4%	7	78±7	63±7	15±2	327±18	18±3	0.5±0.1	17±2	0.84±0.03
Scott syndrome¶	1	4	3	1	41	73	1.5	30	0.01
Bernard-Soulier	2	36	34	2	452	22	0.5	19	0.08
Thrombasthenia	3	100	78±5	22±5	907±88	27±4	0	0	0.78±0.15

Abbreviations as in Table II. * Everted vessel segments exposed to blood for 10 min at a shear rate of 650 s^{-1}. ‡ Ratio [$F_{plt}/(C + S)$] calculated for each subject and values averaged to obtain group values. § All group values for measured parameters (except thrombus volume) are rounded to the nearest integer and are shown as mean±SEM. ‖ Two patients were not studied. ¶ Average of three studies.

At this low shear condition, fibrin deposition (88.7%, Fig. 5) and FPA levels (706 ng/ml) were normal in a patient with severe thrombocytopenia (1,000/μl), despite the absence of detectable platelets adherent to subendothelium (C + S = 0). Normal values for subendothelial fibrin deposition and FPA levels at 50 s^{-1} were also obtained in the patient with Scott syndrome (82% and

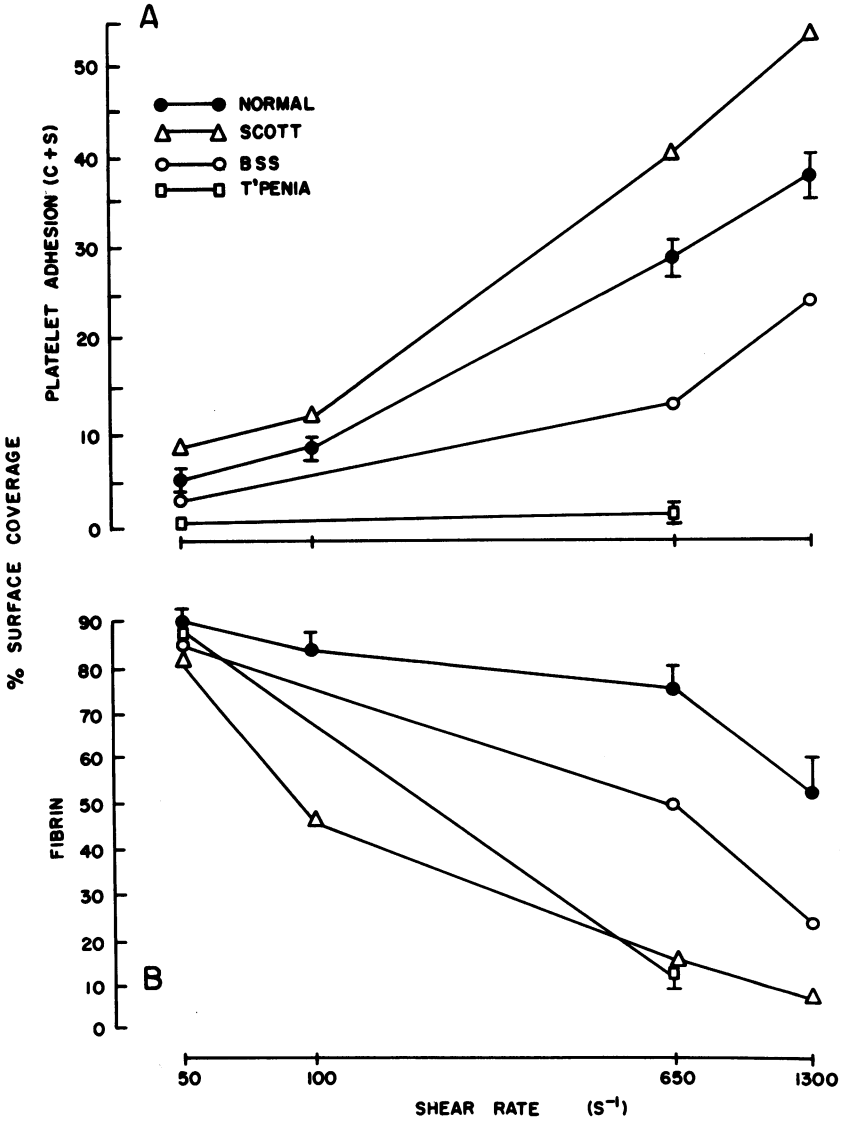

Figure 5. Effect of shear rate on platelet and fibrin deposition on subendothelium in patients with platelet disorders. Vessel segments were perfused for 5 min at the shear rates indicated with blood from normal subjects and patients with Scott syndrome, Bernard-Soulier syndrome (BSS), and patients (n = 4) with thrombocytopenia (t'penia) in whom values for platelet adhesion at 650 s^{-1} were <4%. One of these patients was also studied at a shear rate of 50 s^{-1} (see text). (*A*) Platelet adhesion (C + S) on subendothelium. (*B*) Total surface coverage with fibrin ($F_{Se} + F_{plt}$).

461 ng/ml) and in a patient with the Bernard-Soulier syndrome (82% and 408 ng/ml). Thus, at the shear rate of 50 s^{-1} fibrin deposition approached surface saturation despite low (0–6.5%) values of platelet deposition in both normal subjects and in patients with platelet defects in whom impaired fibrin formation is observed at the higher shear rate of 650 s^{-1}. These observations on patients, as well as the findings in normal subjects, indicate that fibrin deposition is strongly dependent on the shear rate. In addition, the patient studies at 650 s^{-1}, as well as the continued abnormality of fibrin deposition in Scott syndrome and the Bernard-Soulier syndrome at 1,300 s^{-1} (Fig. 5), demonstrate that platelets contribute to fibrin deposition at intermediate shear rates.

Discussion

Previous studies have established that the procoagulant properties of platelets are due, in part, to their capacity to bind activated coagulation factors and to accelerate the conversion of the zymogen forms of Factors X and II (prothrombin) to their active enzymes (6–8, 39–44). Impairment of these properties may result in a mild bleeding disorder (Scott syndrome) (35–37). In addition, a possible role for platelet glycoproteins has been implied by studies on patients with the Bernard-Soulier syndrome (45–47) (GPIb deficiency), and thrombasthenia (48–50) (GPIIb-IIIa deficiency). The above studies have, in general, been performed at relatively low shear conditions and in the absence of vascular surfaces where alternative mechanisms (such as tissue factor [51, 52]) for activating the coagulation pathway could be important. A major purpose of this study was to evaluate the procoagulant properties of platelets on a de-endothelialized vascular surface exposed in vivo to blood from normal subjects and patients with platelet disorders at shear rates ranging from 50 to 2,600 s^{-1}.

The present studies performed with normal non-anticoagulated blood clearly demonstrate the strong, and opposite, effect, of blood flow on platelet and fibrin deposition and suggest that, under the conditions utilized in the study, platelets influence fibrin formation at shear rates of 650–1,300 s^{-1}. Evidence that platelets do, in fact, influence fibrin deposition in this intermediate shear range was obtained in patients with thrombocytopenia. At 650 s^{-1}, fibrin deposition on the subendothelium after a 5-min perfusion of the vessel segments was decreased in the four patients with the lowest adhesion values, and was normal in the six others (Table II). Although the number of patients studied was small, the findings suggest that relatively few adherent platelets (in this study a surface coverage of 4% or more corresponding to platelet counts > 5,000/μl) were sufficient to support normal levels of fibrin deposition on the subendothelium. In contrast to the findings at the shear rate of 650 s^{-1}, platelets appeared to be less important for fibrin deposition at 50 s^{-1}. Thus, fibrin deposition was maximal at this shear rate, despite minimum values (5.3±0.5%) for platelet adhesion, and one patient with severe thrombocytopenia (platelet count 1,000/μl) was found to have normal (89% surface coverage) fibrin deposition despite the virtual absence of platelets on the subendothelium. An inverse relationship between fibrin deposition and shear rate was observed at shear rates above 650 s^{-1}. Thus, fibrin deposition decreased dramatically at 2,600 s^{-1} despite much higher values of platelet adhesion and larger platelet thrombi than at lower shear conditions (Fig. 1).

Studies performed in patients with functional platelet disorders at 650 s^{-1} provide additional information on the specific properties of platelets that contribute to fibrin deposition at this shear rate. Decreased fibrin deposition was observed in the patient with Scott syndrome with impaired platelet procoagulant activities (see Methods) despite normal (in fact, slightly increased) values of platelet adhesion. The findings in the present study demonstrate that the platelet properties deficient in Scott syndrome are important in promoting fibrin deposition on a vascular surface under flow conditions comparable to those in large arteries. In addition, studies performed at varying shear rates in this patient further support the relative unimportance of platelets at low shear rates (normal values for fibrin deposition at 50 s^{-1}), and demonstrate that the defect in fibrin deposition in Scott syndrome is strongly dependent on flow conditions throughout the entire range of shear rates studied (Fig. 5). The findings in patients with thrombasthenia, whose platelets are deficient in GPIIb-IIIa, were in marked contrast to those with Scott syndrome. In three patients with this disorder, fibrin deposition was normal (in fact, somewhat increased) at 650 s^{-1} despite the complete absence of platelet thrombi. The findings indicate that a monolayer of platelets (even those deficient in GPIIb-IIIa) is sufficient to promote the deposition of fibrin on the subendothelium under the conditions studied. We also found an abnormality of fibrin deposition in the Bernard-Soulier syndrome, but of a somewhat different nature than in Scott syndrome. Unlike the latter, which was associated with reduced fibrin deposition on both the subendothelium and on platelets, only the latter was strikingly decreased in the Bernard-Soulier syndrome (Tables II and III). The marked reduction in fibrin associated with platelets could be due to an impaired binding of polymerizing fibrin to these platelets, as suggested by a recent report that the interaction of platelets with polymerizing fibrin (which is normal in thrombasthenia [53]) is mediated by von Willebrand factor through a GPIb-dependent mechanism (54). Other mechanisms pertaining to impaired binding of thrombin by Bernard-Soulier platelets (55), due to deficiencies of GPIb and V (56–59) are also possible.

From the above observations, a plausible explanation for the various findings in this study must take into account the strong, and inverse, relationship of fibrin deposition with shear rate, the relative lack of a platelet requirement at low shear rates (where fibrin deposition can occur despite little or no platelet deposition), the progressive decrease in fibrin deposition at shear rates > 650 s^{-1} (despite increasing platelet deposition), and the findings in patients with various types of platelet disorders. A likely explanation is that fibrin deposition on the surface is influenced (as indicated above) by procoagulant properties of both the subendothelial surface and the deposited platelets, and by rheological factors which determine the concentrations of activated coagulation proteins either in the boundary layer or immobilized by surface binding to platelets on the subendothelial surface. As discussed extensively by Leonard (60), the concentration of proteins activated, but not bound, at the surface through the coagulation mechanism will be strongly influenced by flow factors, and the net concentration will be determined both by the biochemical reactions producing activated coagulation factors and by convective-diffusive factors that serve to moderate the concentrations of unactivated and activated coagulation proteins. In general, because the boundary layer for activated species (products) is diluted as wall shear rate increases, it follows that the effective concentration of activated coagulation factors in the boundary layer will also decrease. This might explain the inverse relationship between fibrin deposition and shear rate observed with normal blood (Figs. 1 and 2). In that platelets have been shown to bind activated coagulation factors, such as

Factor Xa (43, 44) and Va (61, 62), to their surface, the presence of adherent platelets on the subendothelium could, with increasing shear rates, serve to maintain activated coagulation proteins in the boundary layer at a concentration that would otherwise be reduced through convective diffusion in their absence. Thus, at low shear rates (50 s^{-1}), the concentration of activated coagulation factors in the boundary layer might be sufficient to support fibrin deposition despite the absence of platelets, whereas, at very high shear rates (2,600 s^{-1} and above), even the presence of platelets is insufficient to maintain the required concentration. The shear-dependent defect of fibrin formation in a patient with Scott syndrome, a disorder in which impaired binding of Factor Xa has been previously demonstrated (36), is consistent with such a theory.

In addition to measuring fibrin deposition on the vessel surface, we also measured FPA levels in the blood at the termination of the run. With blood from normal subjects, there was a good correlation between fibrin deposition on the surface and blood FPA levels; both decreased in parallel with increasing shear rate (Fig. 2). This was also the case in the patient with Scott syndrome. Decreased fibrin deposition was associated with decreased FPA levels, the latter becoming particularly striking at 650 s^{-1} with the longer (10 min) perfusion time during which the subendothelial surface became saturated (73% coverage) with her platelets (Table III). In contrast, FPA levels were consistently normal in patients with thrombocytopenia, even when (as in patients with platelet adheson values < 4%) surface coverage with fibrin was markedly reduced (Tables II and III). Several explanations are consistent with the above findings. First, the generation of blood FPA levels is dependent primarily on the surface and flow conditions, regardless of the presence of platelets. This is not inconsistent with previous findings demonstrating the procoagulant properties of platelets in systems utilizing washed platelets and purified coagulation proteins (6–8, 61, 62). Rather, it demonstrates the complexity introduced by the presence of a vessel surface which, itself, has procoagulant properties. A second conclusion is that a vessel surface which has become saturated with platelets whose defect is that observed in Scott syndrome has an impaired ability to generate FPA. Finally, the major role of platelets in normal blood exposed to a procoagulant vessel surface may be to localize specifically the deposition of fibrin on the vascular surface, as discussed above.

The findings in the present study emphasize the importance of considering flow in evaluating the properties of platelets which contribute to the localization of fibrin on a potentially coagulant vessel surface. These considerations are important in view of evidence, such as the results from recent studies utilizing fibrinolytic agents in patients with acute myocardial infarction (63), that fibrin is a major component in some types of arterial thrombi. We realize that the stimulus for activating the coagulation mechanism provided by a ruptured atherosclerotic plaque may not be indentical to that provided in our ex vivo model utilizing a de-endothelialized vessel that has been stored for varying periods of time prior to use. Fibrin deposition has not been observed in vessels ballooned in vivo where blood flow is allowed to reestablish normally (22, 64–66). However, even under these conditions, the deposition of fibrin appears to be influenced by both the local flow conditions and vascular factors. For example, if blood flow in the vessel is reduced by constriction to the ballooned area, fibrin deposition can be readily observed (22). In addition, fibrin deposition occurs more readily after reinjury of the neointimal surface (67–69) and this could be the case for vessels injured by atherosclerosis, or at sites where hemodynamic forces would be expected to cause repeated vessel injury (70) as well. Thus, the vessels used in our studies, perhaps injured during 7–28 d of storage, could more closely approximate the thrombogenicity of diseased vessels than those exposed to blood after a single balloon cathether injury in vivo. If our model has clinical validity, the results of our study could have therapeutic implications in developing antiplatelet agents which would inhibit fibrin deposition. Thus, fibrin deposition might be reduced on a surface covered with platelets equivalent to those found in Scott syndrome. This might be particularly relevant in vessels such as the major epicardial arteries, where shear rates in the range of 200–800 s^{-1} are characteristically observed (17). In addition, as seen in the patient with Scott syndrome (Tables II and III), by inhibiting thrombin formation, the size of the platelet thrombi which form over the adherent platelets would also be decreased. Thus, by inhibiting both fibrin formation and the size of platelet thrombi, a strategy designed to inhibit platelet procoagulant activity could be potentially useful in preventing thrombosis in clinically important blood vessels.

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