Studies of Thromboxane B$_2$, Platelet Factor 4, and Fibrinopeptide A in Bleeding-Time Blood of Patients Deficient in von Willebrand Factor, Platelet Glycoproteins Ib and IIb-IIIa, and Storage Granules

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The blood volumes and concentrations of thromboxane B$_2$ (TxB$_2$), platelet factor 4 (PF4), and fibrinopeptide A (FPA) were measured every 30 seconds in bleeding-time blood in normal subjects and in patients with idiopathic thrombocytopenic purpura (ITP), β and αδ storage pool deficiency (SPD), Bernard-Soulier Syndrome (BSS), thrombasthenia (TSA), and von Willebrand's disease (vWD). Data were fitted to second-order (TxB$_2$, PF4, and FPA) or third-order (volumes) polynomials. Average values for various parameters over fixed-time intervals were determined by numerical methods. The bleeding time was greater than 15 minutes in all patient groups and the initial bleeding, as reflected by the initial slope of the fitted blood volume curves, was increased in ITP, BSS, and SPD (β-SPD in particular), but not in vWD and TSA. The increased values for both the initial slope and the volume of blood collected after 2 minutes in SPD suggest that vascular tone may be modulated, in part, by dense granule substances such as adenosine triphosphate (ATP) or serotonin. In TSA, uniquely, both platelet (TxB$_2$ and PF4) and coagulation (FPA) values were increased in early bleeding samples (initial slope). In vitro studies of TxB$_2$ production, together with previous flow studies of fibrin formation, also suggest enhanced activation and coagulant properties of thrombasthenic platelets. In other patients, reduced values of all substances at later times may reflect impaired platelet-fibrin plug formation in the high-shear regions at the ends of transected blood vessels. However, the initial slopes of the fitted curves for both TxB$_2$ and PF4 were normal in vWD, suggesting that the early appearance of these substances may typically be from platelets that are adherent to collagen within the lower shear environment of the wound surface. The finding that FPA values were not decreased initially in any patient group, including ITP, but were decreased at later times (except for TSA), suggests that early fibrin formation occurs independently of platelets in the low-shear environment of the wound surface, whereas at later times fibrin is formed in a platelet-dependent manner in the high-shear regions at the ends of transected vessels.

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ILLIa deficiency), the Bernard-Soulier Syndrome (BSS; GPIb deficiency), and storage pool deficiency (SPD) associated with either specific deficiencies in dense granule substances (α-SPD) or deficiencies in both dense granule and α-granule substances (αβ-SPD).12,23

MATERIALS AND METHODS

Bleeding Time and Collection of Incision Blood For Sequential Measurement of Blood Loss and Radioimmunoassay

The procedures for measuring the volume or TxB2, PF4, and FPA concentrations sequentially in samples of bleeding-time blood were those described previously.16 Briefly, after compressing the upper arm with a sphygmomanometer cuff to a pressure of 40 mm Hg, two simultaneous longitudinal incisions were made on the volar surface of the forearm using a Simplette II bleeding-time device (Organon-Teknica Corp, Durham, NC). At 30-second intervals until bleeding stopped (normal subjects) or for 15 minutes (patients), blood from each of the two wounds was collected into heparinized microcentrifuge tubes of known diameter (Fisher Scientific Co, Pittsburgh PA) and, after measuring the height of blood columns, the blood was expressed into microcentrifuge tubes containing microhematocrit capillary tubes of known diameter (Fisher Scientific Co, Pittsburgh PA) and, after measuring the height of the blood column, the blood was expressed into microcentrifuge tubes containing buffered saline plus either indomethacin and EDTA (for measurement of TxB2 in blood from the proximal wound), or heparin, trisylol (aprotinin), EDTA, indomethacin, adenosine, and theophylline (for measurement of PF4 and FPA levels in blood from the distal wound). The diluted blood samples were then centrifuged, and aliquots of the supernatants were processed for FPA radioimmunoassay before freezing at −80°C.16 All aliquots were stored at −80°C before determination of PF4, TxB2, or FPA by radioimmunoassay, as described in detail previously.16

All studies were performed under a protocol approved by the Institutional Review Board of the St Luke’s-Roosevelt Institute for Health Sciences.

TxB2 Formation in Gel-Filtered Platelets (GFP)

Platelet-rich plasma was prepared from acid-citrate-dextrose (ACD)-anticoagulated blood by centrifugation for 3 minutes at 1,000 g and gel-filtered into Ca-free Tyrode’s buffer containing 5 mmol/L HEPES, 0.1% glucose, and 0.2% bovine albumin as described previously.24 Aliquots (0.9 mL) of GFP adjusted to a platelet count of 200,000/mL were incubated at 37°C for 1 minute with the addition of elution buffer or with stirring for 2 minutes with the addition of 88 µg/mL LP-CP8, a monoclonal antibody (MoAb) specific for the GPIb-IIa complex,28 kindly provided by Dr Zaverio Ruggeri (The Scripps Research Institute, La Jolla, CA). Thrombin was then added in final concentrations varying from 0.05 to 5 U/mL for a period of 5 minutes, after which 100 µL of the platelet suspension was added to 500 µL of iced-cold phosphate-buffered saline (PBS)+5 mmol/L EDTA-12 µmol/L indomethacin and processed for measurement of TxB2 by radioimmunoassay as described previously.26

Serum TxB2 and PF4

Two-milliliter aliquots of nonanticoagulated venous blood to which bovine thrombin (Parke-Davis, Detroit, MI) was added to a final concentration of 2 U/mL were incubated at 37°C for 30 minutes. Serum was separated by centrifugation at 17,300g for 10 minutes at 4°C, and frozen at −80°C before assay of TxB2 and PF4.

Subjects

Normal subjects. Control subjects (n = 15) were normal hospital personnel, 25 to 50 years of age, who denied recent ingestion of drugs known to affect platelet function or hemostasis. Studies on the patients reported herein were performed both contemporaneously with those in the previously reported studies18 and subsequent to those studies. Hence, the control subjects comprise both those previously reported plus additional normal subjects studied during the latter part of the present studies. The average value for the platelet count was 319,000 ± 15,000/µL and for serum TxB2 was 1,251 ± 99 pmol/µL.

vWD. Three previously described27-28 patients (C.M., D.R., and W.D.) with severe type III vWD have undetectable levels of vWF antigen (vWF:Ag) by either Laurell electroimmunoassay (<0.06 U/mL) or immunoradiometric assay (<0.003 U/mL),29 and undetectable ristocetin cofactor activity (<0.03 U/mL). Factor VIII levels in C.M. and D.R., assayed on multiple occasions, ranged from less than 0.01 to 0.05 U/mL, and in patient W.D. were 0.05 to 0.08 U/mL, assayed as previously described.27 The average value for the platelet count was 296,000 ± 6,000/µL, and for serum TxB2 was 1,419 ± 290 pmol/µL. In these patients, platelet adhesion to subendothelium is decreased in citrated blood at a shear rate of 800 s⁻¹ and, more strikingly, at shear rates of 1,300 s⁻¹ and above.30 In nonanticoagulated blood, adhesion is decreased at shear rates of 2,600 s⁻¹ and above, but not at 1,300 s⁻¹ or below.28,30 Platelet thrombus formation is decreased, even under conditions in which adhesion is normal.28

TSA. Four previously described27 patients (M.C., C.G., L.W., and M.Mo) with Glanzmann’s TSA were studied. All four patients have markedly reduced or absent amounts of the GPIb-IIa complex and their platelets do not aggregate with 50 µmol/L adenosine diphosphate (ADP) or epinephrine, nor with 20 µg/mL collagen. Platelet adenosine nucleotide and serotonin values are normal. The average value for the platelet count was 212,000 ± 21,000/µL and for serum TxB2 was 1,176 ± 260 pmol/µL. Platelet thrombus formation on subendothelium is absent at all shear rates.27 Platelet adhesion to subendothelium in citrated blood is decreased at shear rates of 800 and 2,600 s⁻¹ caused by a defect in platelet spreading.27 In nonanticoagulated blood, adhesion is normal at these shear rates, but platelet spreading is defective.27 Fibrin formation and FPA production are increased.31

BSS. Two previously reported29,30 patients (A.J. and T.H.) whose findings are typical of the BSS were studied. Previous studies on these patients have shown absent platelet aggregation with ristocetin,31 decreased platelet GPIb content,33 impaired platelet adhesion to subendothelium in citrated blood at shear rates of 800 s⁻¹ and above,27,30,31 and decreased adhesion in nonanticoagulated blood at shear rates of 650 s⁻¹ and above.30 Platelet-thrombus formation is also markedly defective.31 The average value for the platelet count was 84,000/µL and for serum TxB2 was 462 pmol/µL.

SPD. Four patients (L.V., W.A., N.R., and J.V.) with the albinism variant of β-SPD (Hermansky-Pudlak syndrome) were studied. All four patients were of Puerto Rican ancestry and had the typical clinical features of this syndrome, including a history of excessive bruising and bleeding at surgical procedures, ocularcutaneous albinism, and nystagmus. Platelets in β-SPD are specifically deficient in dense granule substances.23,33,34 The average values (±SE) for these substances, assayed as described previously,23 in the platelets of these four patients (versus normal control subjects) were: adenosine triphosphate (ATP) = 3.53 ± 0.23 µmol/10¹¹ (v 4.86 ± 0.13); ADP = 0.32 ± 0.04 µmol/10¹¹ (v 2.56 ± 0.07); ATP/ADP = 11.44 ± 4.70 (v 1.91 ± 0.04); serotonin = 21 ± 8 µmol/10¹¹ (v 288 ± 8). The average value for the platelet count was 317,000 ± 22,000/µL, and for serum TxB2 was 1,130 ± 230 pmol/µL.

αβ-SPD. Five previously reported patients (4 members of family C and patient J.C.) have combined deficiencies of both α and β
dense granules (αδ-SPD). In family C, α-granule numbers (78% of normal) and α-granule substances (average values 50% of normal) are reduced. A more severe α-granule defect is present in patient J.C., whose platelets contain approximately 20% of the normal amount of α-granule number and substances. The average values for dense granule substances (assayed on multiple occasions) in these five patients are: ATP = 4.0 ± 0.25, ADP = 0.81 ± 0.11, ATP/ADP = 5.31 ± 0.96, serotonin = 138 ± 28. The average value for the platelet count was 164,000 ± 23,000/μL, and for serum TxB2 was 350 ± 174 pmol/mL.

In nonanticoagulated blood of both albino δ-SPD patients and αδ-SPD patients, platelet thrombi on subendothelium are decreased at shear rates of 650 s⁻¹ and above.

**Thrombocytopenia.** Four adult patients with the characteristic findings of chronic ITP were studied. The average value for the platelet count was 20,000 ± 4,000/μL, and for serum TxB2 was 317 ± 175 pmol/mL. No evidence for an underlying disease that might have accounted for the thrombocytopenia was found in any of these patients.

**Other Studies**

The hematocrit values in the various patient and normal groups varied from 38 ± 3 (ITP) to 43 ± 4 (vWD). Serum PF4, compared with normal values (8,804 ± 1,008 ng/mL), was significantly decreased in αδ-SPD (1,700 ± 345) and ITP (1,093 ± 268) and was normal in the other patient groups. Average plasma fibrinogen values varied from 207 to 281 mg per 100 mL in the various groups.

**Curve Fitting**

The data obtained from control subjects and patient groups were fitted to second-order (TxB2, PF4, and FPA) or third-order (volumes) polynomials using a SigmaPlot computer program, version 4.0 (Jandel Scientific, Corte Madera, CA). This program uses a Marquardt-Levenberg algorithm to provide best-fit values for the equation coefficients, their SD, and the 95% or 99% confidence limits of the curve. From these values the initial slope and the parameter values at any 30-second interval could be calculated. The initial slope for each parameter was taken as the first-order coefficient derived from curve fitting of the data obtained during the first 6 minutes of bleeding only. Although these curves provided excellent fits for the experimental data and enabled calculation of several parameters of interest, they are entirely empirical and do not necessarily imply any particular model of hemostasis. In particular, the calculation of the initial slope is intended to reflect the rate of bleeding during the early stages of hemostasis and not the instantaneous rate of bleeding at time zero after transection of the vessel.

**Numerical Analysis**

Average values for the parameters were determined by a method in which the area under a curve is evaluated numerically by means of an integration formula obtained by interpolating parabolas through a number of pivotal points of the integrand, using the area under the parabolas as an approximation to the area under the integrand (Simpson’s rule) and dividing by the time interval over which the area was determined.

**Statistical Analysis**

The significance of the difference between two means was evaluated by Student’s t-test. For multiple data points (formation of TxB2 by incremental concentrations of thrombin), differences between controls and patient groups were compared by analysis of variance (ANOV).

**RESULTS**

**Bleeding-Time Blood Volumes**

Figure 1 shows, for each subject group, the average bleeding-time blood volumes versus time. In normal subjects, blood volume increased to a maximum value at 2 minutes of bleeding.
and then declined until bleeding ceased at a maximum of 8.5 minutes. In all patient groups, bleeding continued throughout the entire 15-minute study period.

The fitted third-order curves for the first 6 minutes of bleeding are also shown in Fig 1. Although the prolonged bleeding in all disorders markedly increased the total volumes obtained at 15 minutes (data not shown), the parameters of early bleeding were not uniformly increased in the various patient groups. Thus, compared with the normal value of 12.23 ± 0.20, the initial slopes were significantly increased ($P < .05$) in patients with ITP (15.32 ± 0.29), BSS (16.50 ± 0.2), αδ-SPD (15.64 ± 0.18), and δ-SPD (17.46 ± 0.27), but not in patients with vWD (12.47 ± 0.19) or TSA (11.03 ± 0.13). The same general pattern was observed for the measured 2-minute volumes, which showed further the significantly ($P < .05$) increased bleeding in δ-SPD (57.1 ± 8.9 μL v 34.5 ± 3.8 μL in controls), but not in TSA (39.1 ± 11.1; $P = NS$) or vWD disease (42.7 ± 10; $P = NS$).

$TxB_2$

Figure 2 depicts the average group values for $TxB_2$ in the bleeding-time samples, as well as the fitted second-order curves. Except that the experimental values are somewhat higher than those in the previous report, the fitted second-order curve for the expanded group of normal subjects shows, as shown previously, the relatively slow increase in $TxB_2$ during the early time points and the more rapid increase that coincides with slowing, and then cessation, of bleeding.

$TxB_2$ in bleeding-time blood was markedly decreased in ITP patients, indicating its platelet origin. $TxB_2$ was also markedly decreased in patients with αδ-SPD, in whom decreased platelet malondialdehyde production from arachidonate was reported previously, and was undetectable in any of the samples obtained during the first 5 minutes in the two patients with BSS. In contrast, the initial slopes of the fitted curves in patients with vWD and δ-SPD were not decreased, compared with controls (2.84 ± 0.14 and 3.07 ± 0.14 v 1.96 ± 0.20), although decreased values were obtained at 5 minutes (7.0 ± 1.3 and 10.9 ± 1.5 v 40.1 ± 3.0 pmol/mL). In both disorders, the average $TxB_2$ concentration during the first 5 minutes was also less than that in controls (6.2 ± 1.8 and 5.7 ± 1.6 v 14.3 ± 4.6 pmol/mL), as reported in other studies, but the differences were not significant.

In contrast to the findings in every other platelet disorder, $TxB_2$ levels were increased ($P < .05$) in TSA compared with controls, as indicated by the increased values for the initial slope (16.42 ± 0.40 v 1.96 ± 0.26), and the average values (36.2 ± 6.6 v 14.3 ± 4.6 pmol/mL) during the first 5 minutes.

Enhanced $TxB_2$ production in thrombathenes was also observed in vitro (Fig 3A). $TxB_2$ production induced by a range of thrombin concentrations was significantly greater ($P < .05$, ANOV) in thrombasthenic GFP than in normal GFP. This increased capacity for $TxB_2$ formation is also evident in these patients' serum $TxB_2$ value when this value is corrected for their lower platelet count (see Materials and Methods). However, no increased $TxB_2$ production was observed in normal platelets in which the GPIIb-IIIa complex had been blocked by incubation in vitro with the complex-specific MoAb LJ-CP8 (Fig 3B).

$PF4$

Figure 4 depicts the PF4 values and fitted second-order curves in controls and patient groups. The results for PF4...
were, in general, similar to those for TxB₂. Decreased PF4 generation was observed in ITP, BSS, and in patients with αδ-SPD, whose platelets contain reduced amounts of PF4. As with TxB₂, the initial slopes of the fitted curves, compared with controls (201 ± 8), were not decreased in vWD (261 ± 10) and δ-SPD (285 ± 9), whereas the 5-minute values (750 ± 135 and 1,104 ± 126 v 1,518 ± 82 ng/mL) were significantly reduced (P < .05) in both cases.

Also, as with TxB₂, both the initial slope (476 ± 9 v 201 ± 8) and the average concentration of PF4 (1,145 ± 182 v 695 ± 112 ng/mL) during the first 5 minutes were significantly increased (P < .05) in patients with TSA.

**FPA**

The FPA values and fitted second-order curves for FPA are shown in Fig 5. In contrast to TxB₂ and PF4, the calculated initial slope for FPA was not decreased in any of the patient groups, including ITP and BSS (data not shown). As with TxB₂ and PF4, the initial slope was greatly increased in TSA (906 ± 16 v 245 ± 40). The FPA value at 5 minutes in thrombasthenics (3,055 ± 162 ng/mL) was not significantly different than control (3,400 ± 400), whereas these values were significantly decreased in all other patient groups (P < .05, data not shown). The greatest decrease in FPA concentrations throughout the bleeding period was found in patients with vWD (average concentration, 459 ± 42 v 1,263 ± 219 ng/mL in controls; P < .05).

**DISCUSSION**

The patterns of TxB₂, PF4, and FPA concentrations observed in the bleeding-time blood of patients with well-defined disorders of platelet function, together with the abnor-
malities of platelet adhesion, thrombus formation, and fibrin deposition previously described in these patients, may provide some insights into mechanisms of platelet activation and of thrombin generation that occur normally after transection of blood vessels. In addition, differences in the initial blood flow that were observed among these patients may reflect specific properties of platelets that modulate vascular tone.

Blood Flow

Blood volumes in normal subjects increased during the early stages of bleeding, reaching a maximum at approximately 2 minutes (Fig 1). Previous studies have shown that vessels contract rapidly after their transection, possibly caused by neuronal or direct smooth muscle stimulation, or endothelin derived from injured endothelial cells. After contraction, the vessels rapidly dilate, which could account for the increased blood volumes in sequential samples obtained during the first 2 minutes after the incision.

The mechanisms involved in vascular dilatation are not well understood, but the increased early bleeding observed in ITP and some of the other patient groups, as reflected by the calculated initial slopes of the fitted curves, suggests that substances released from platelets, which are known to influence vascular tone, may modulate this response. The increased early bleeding in δ-SPD suggests that platelet dense granule substances, such as ATP and serotonin, may play an important role in regulating vessel diameter after transection, as they have been shown previously to influence the time to the arrest of bleeding.

The role of ATP and serotonin in influencing vascular tone is complex. In large arteries, these substances directly cause vessel contraction if endothelial cells are injured or absent. However, in the presence of endothelium, they induce the cells to synthesize endothelium-derived relaxing factor (EDRF), which has been identified as either nitric oxide (NO) or a substance that yields NO under appropriate conditions, and which causes large vessel to dilate. The effects of ATP and serotonin on smaller vessels, in the presence or absence of endothelial cell injury, is less clear. However, the increased early blood flow observed in patients with δ-SPD suggests that the absence of these substances from the milieu of a transected vessel in a bleeding-time wound may result in a more rapid dilatation of the vessel.

The initial bleeding was also increased in patients with BSS, but not in those with vWD or TSA. These findings are consistent with the hypothesis suggested below that platelet adhesion (and hence the subsequent dense-granule secretion) may be normal initially on the various wound surfaces in TSA and vWD, but is more severely impaired in BSS.

Platelet Activation

For normal subjects, Figs 2 and 4 show the relatively slow increase in TXB₂ and PF4 during the early bleeding-time samples and the more rapid increases that coincide with slowing and then cessation of bleeding. The almost total absence of TXB₂ and PF4 in all bleeding-time samples (early and late) from patients with ITP is consistent with the platelet origin of these substances. Plausible sources for these substances are platelets that are adherent to collagen and/or those that are recruited to form a hemostatic plug at the ends of transected vessels or along the edges of the
V-shaped wound. In addition, nonadherent platelets in the flowing blood could also be activated by thrombin. However, previous findings that early formation of TxB₂ is normal in patients with severe deficiencies of factor V or X, in whom FPA levels were undetectable, suggest that thrombin may not be absolutely required for early platelet activation in bleeding-time wounds.

The normal values for TxB₂ and PF4 in early bleeding-time samples of patients with vWD, as reflected by the initial slopes of the fitted curves, is somewhat surprising in view of the defect in platelet adhesion that has been shown in this disorder. However, this defect is highly shear-rate dependent and was observed in nonanticoagulated blood only at shear rates greater than 1,300 s⁻¹, a value that might be anticipated at the proximal ends of the transected arterioles. Thus, the normal early values for TxB₂ and PF4 in vWD suggest that these substances are derived from platelets that have adhered initially to collagen (or other substrates) within the lower shear environment of the wound surface, at the ends of transected venules, or at the ends of the transected distal arterioles, where flow is reversed. This would also be consistent with the normal initial blood flow noted above in vWD.

The most pronounced reductions in TxB₂ and PF4 were observed in the two patients with BSS. Because BSS platelets can produce TxB₂ and secrete PF4 (See Materials and Methods), their total absence (TxB₂, Fig 2) or marked reduction (PF4, Fig 4) in bleeding-time wounds could be caused by an impairment of adhesion that is more severe, and that is observed at lower shear rates, than that in platelets from patients with vWD. Whether such an impairment may be related solely to the GPIb defect, or to additional defects in BSS platelets, is not clear at present.

In contrast to the variably decreased values for TxB₂ and PF4 in early bleeding-time samples, reduced amounts of these substances were found in later bleeding-time blood from all of the patient groups except thrombasthenics (see below). In normal subjects, the formation of a hemostatically effective plug consisting of closely packed platelets during the later phase of bleeding (2 to 5 minutes) probably accounts for the rapid increases in the concentrations of TxB₂ and PF4 (Figs 2 and 4), as well as the sharp decrease in blood volume. One possible explanation for the reduced amounts of TxB₂ and PF4 in the patient groups could be a simple dilution effect by the larger blood volumes obtained in these patients, compared with normal subjects. However, the observation that these substances were not reduced in thrombasthenics, in whom the blood volumes were similarly increased relative to normal subjects, argues against this possibility. A more likely explanation for the reductions seen at later time points in the various patient groups is an impairment of platelet thrombus formation at the ends of transected vessels, as has been previously shown in biopsy specimens of bleeding-time wounds in humans or pigs with vWD. This impairment in vWD may result directly from the absence of vWF, which has been shown to mediate thrombus formation on subendothelium and incorporation of platelets into polymerizing fibrin through its ability to bind both GPIb and GPIIb-IIIa. For reasons discussed earlier, a similar reduction in platelet activation products would also be anticipated in BSS, in which impaired platelet thrombus formation on subendothelium has also been described. In δ-SPD, impaired thrombus formation could result from the deficiency of dense-granule ADP, which has been shown to modulate thrombus formation on subendothelium and on transected vessels.

Thus, the reduced TxB₂/PF4 values in the late bleeding-time samples of the patient groups are consistent with previously described abnormalities of platelet function in these disorders and provide further support for the importance of platelet-granule substances, and the interaction of vWF with its platelet receptors, in activating platelets and promoting hemostatically effective platelet plugs in normal hemostasis.

FPA

In contrast to the platelet activation substances, FPA levels in the early bleeding-time samples, as shown by the calculated initial slopes, were not decreased versus controls in any of the disorders studied, including ITP, and were increased in TSA, strongly suggesting that platelets are not required for fibrin formation during the early stages of hemostasis. In biopsy specimens of bleeding-time wounds obtained 1 to 2 minutes after incision, fibrin was found initially along the sides of the wound, and subsequently at the periphery of the platelet plugs that protrude from the ends of transected vessels. In previous studies we have shown that platelets play a role in fibrin deposition on subendothelium at high shear rates (>650 s⁻¹ in the model tested), but are not required at lower shear rates, as might be found in some areas of the bleeding-time wounds. Thus, the normal or increased FPA values found in all of the patients groups in early bleeding-time specimens are consistent with these previous observations, and suggest that initial fibrin formation occurs independently of platelets in the low-shear environment of the wound edges.

However, the FPA values at 5 minutes and beyond were modestly decreased in all the patient groups except TSA. This corresponds to a relatively late stage in the arrest of bleeding in normal subjects, at which time the fibrin cap at the periphery of the hemostatic plug becomes greatly enlarged. Thus, in contrast to the initial fibrin formation that appears to occur independently of platelets under low-shear conditions in the wound, platelets may be required for later fibrin formation in the higher shear environment at the periphery of the hemostatic plugs. These plugs could provide the primary surface on which both thrombin generation and fibrin formation are localized, thereby consolidating the processes responsible for the final arrest of bleeding.

The greatest reduction in FPA levels, and hence presumably in thrombin generation in later bleeding-time samples, was found in vWD. In previous studies of FPA formation in bleeding-time blood from patients with coagulation de-
fits we found that thrombin generation was normal in hemophilic patients and appeared to occur primarily via a tissue factor/Factor VII-mediated pathway. Thus, while the marked reduction of FPA in vWD is not likely to be caused by the concomitant factor VIII deficiency, it might be explained by earlier findings that patients with vWD may have an abnormality in the expression of tissue factor. 

TSA

The increased values for both the platelet activation indicators TxB₂ and PF4 and the coagulation indicator FPA obtained in the early bleeding-time samples in TSA, which was in marked contrast to the results obtained in all other platelet disorders, require special attention, particularly in view of the current interest in preventing thrombosis and reocclusion of thrombosed vessels with agents that can block the receptor properties of GPIIb-IIIa. The increased FPA production is consistent with increased fibrin formation and FPA production on subendothelium observed in ex vivo studies using thrombasthenic blood. Because it is highly unlikely that platelet thrombus formation occurs to any significant extent in thrombasthenic patients (although masses of platelets entrapped within fibrin have been described in bleeding time wounds), the TxB₂ and PF4 in early bleeding-time samples from these patients most likely originate from platelets adherent to collagen (or other adhesive substrates) and/or from thrombin-induced activation of circulating platelets within the wound. In vitro dose-response studies (Fig 3A) showed that greater than normal amounts of TxB₂ were produced at equivalent thrombin concentrations by thrombasthenic platelets, which lack the GPIIb-IIIa receptor complex, but not by normal platelets in which the receptor was blocked by a complex-specific MoAb (Fig 3B). It is not clear whether the increased platelet activation evident in early bleeding-time samples is related to the actual absence of the GPIIb-IIIa complex on the platelet surface (rather than just the loss of receptor function), to the acquisition of an enhanced TxA₂ production in circulating thrombasthenic platelets, or to an increased production of thrombin, as may be reflected by the increased FPA values. In addition, these possibilities may not be independent of each other. However, whatever mechanisms are involved in TSA, it is entirely possible that increased activation of platelets and coagulation might also occur in vivo in normal subjects receiving agents that block the GPIIb-IIIa receptor, and this effect could counteract their intended antithrombotic properties.

Finally, as indicated earlier, although the curves that were fitted to the data in the present study enabled the calculation of several parameters of interest, they were entirely empirical and do not necessarily imply any particular model of hemostasis. Thus, it may be appropriate to develop models for the production of platelet-fibrin plugs that will predict the pattern of bleeding and the appearance of platelet and coagulation activation products in blood from bleeding time, and other types of wounds, and which may be tested experimentally by measurements similar to those used in the present study.

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