The Platelet Function Defect of Cardiopulmonary Bypass


The use of cardiopulmonary bypass (CPB) during cardiac surgery is associated with a hemostatic defect, the hallmark of which is a markedly prolonged bleeding time. However, the nature of the putative platelet function defect is controversial. In this study, blood was analyzed at 10 time points before, during, and after CPB. We used a whole-blood flow cytometric assay to study platelet surface glycoproteins in (1) peripheral blood, (2) peripheral blood activated in vitro by either phospholipid monolayer, the thromboxane (TXA)2 analog U46619, or a combination of adenosine diphosphate and epinephrine, and (3) the blood emerging from a bleeding-time wound (shed blood). Activation-dependent changes were detected by monoclonal antibodies directed against the glycoprotein (GPIIb-IX) and GPIIb-IIIa complexes and P-selectin. In addition, we measured plasma glycocalcin (a proteolytic fragment of GPIb) and shed-blood TXB2 (a stable breakdown product of TXA2). In shed blood emerging from a bleeding-time wound, the usual time-dependent increase in platelet surface P-selectin was absent during CPB, but returned to normal within 2 hours. This abnormality paralleled both the CPB-induced prolongation of the bleeding time and a CPB-induced marked reduction in shed-blood TXB2 generation. In contrast, there was no loss of platelet reactivity to in vitro agonists during or after CPB. In peripheral blood, platelet surface P-selectin was negligible at every time point, demonstrating that CPB resulted in a minimal number of circulating degranulated platelets. CPB did not change the platelet surface expression of GPIIb-IIIa complex in peripheral blood, as determined by the platelet binding of fibrinogen and a panel of monoclonal antibodies. In summary, CPB resulted in (1) a markedly deficient platelet reactivity in response to an in vivo wound, (2) normal platelet reactivity in vitro, (3) no loss of the platelet surface GPIIb-IX and GPIIb-IIIa complexes, and (4) a minimal number of circulating degranulated platelets. These data suggest that the "platelet function defect" of CPB is not a defect intrinsic to the platelet, but is an extrinsic defect such as an in vivo lack of availability of platelet agonists. The near universal use of heparin during CPB is likely to contribute substantially to this defect via its inhibition of thrombin, the preeminent platelet activator.

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patients were undergoing their first open heart procedure. All patients required coronary artery bypass grafting, while one required concomitant valvular replacement. No patient had a history suggestive of an underlying hemostatic disorder. The age of the subjects in the study was 62 ± 2.3 years (mean ± SEM; n = 20). Nineteen of the 20 subjects were male. All patients underwent a standard CPB procedure using a membrane oxygenator. The mean aortic cross-clamp time was 50.4 ± 4.4 minutes and the mean duration of CPB was 109.9 ± 7.2 minutes. Patients received intravenous heparin at an initial dose of 4 mg/kg, followed by additional doses as necessary to maintain the activated clotting time (ACT) greater than or equal to 999 seconds. At the completion of CPB, heparin was reversed with protamine sulfate. Heparin reversal was verified by confirming that the ACT had returned to the preoperative value. Maximal hypothermia during CPB was a core temperature of 26.8 ± 1.0°C, as determined by a bladder thermometer (Bard, Boston, MA). Seven patients required coronary artery bypass grafting, while one required concomitant valve replacement.

Maximal hypothermia on CPB was a core temperature of 26.8 ± 1.0°C, as determined by a bladder thermometer (Bard, Boston, MA). Seven patients required coronary artery bypass grafting, while one required concomitant valve replacement.

### Table 1. Blood Sampling Time Points Before, During, and After CPB

<table>
<thead>
<tr>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE OP</td>
<td>Preoperative</td>
</tr>
<tr>
<td>PRE HEP</td>
<td>After the start of anesthesia and surgery, but before heparin and CPB</td>
</tr>
<tr>
<td>HEP</td>
<td>5 minutes after heparin administration, but before the start of CPB</td>
</tr>
<tr>
<td>CPB NT</td>
<td>After the start of CPB (normothermic conditions)</td>
</tr>
<tr>
<td>CPB HT</td>
<td>Beginning of maximal hypothermia on CPB</td>
</tr>
<tr>
<td>CPB 45</td>
<td>45 minutes after the start of CPB (hypothermic conditions)</td>
</tr>
<tr>
<td>CPB END</td>
<td>Completion of CPB, immediately following administration of protamine</td>
</tr>
<tr>
<td>POST 2</td>
<td>2 hours after completion of CPB</td>
</tr>
<tr>
<td>POST 24</td>
<td>24 hours after completion of CPB</td>
</tr>
<tr>
<td>POST 48</td>
<td>48 hours after completion of CPB</td>
</tr>
</tbody>
</table>

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Flow cytometric analysis of platelet surface glycoproteins in peripheral blood. A whole-blood flow cytometric method was used. The method has previously been described in detail and includes no centrifugation, gel filtration, vortexing, or stirring steps that could artifactually activate platelets. Briefly, the method was as follows: the first 2 mL of blood drawn was discarded and then blood was drawn into a sodium citrate Vacutainer (Becton Dickinson, Rutherford, NJ) and, within 15 minutes, diluted in modified Tyrode’s buffer (137 mmol/L NaCl, 2.8 mmol/L KCl, 1 mmol/L MgCl₂, 12 mmol/L NaHCO₃, 0.4 mmol/L Na₂HPO₄, 0.35% bovine serum albumin, 10 mmol/L HEPES, 5.5 mmol/L glucose, pH 7.4). This method of dilution of blood into buffer before the addition of agonist was based on the method of Shattil et al. Then the blood is diluted into buffer to decrease the platelet concentration, thereby preventing platelet-to-platelet aggregation after the addition of agonist. This dilution is critical because quantitation of the amount of surface antigen by flow cytometry requires that platelets by analyzed individually. After dilution, the sample was incubated at 22°C with buffer only or an agonist: either phorbol myristate acetate (PMA; Sigma, St Louis, MO), the stable thromboxane (TX)A₂ analog U46619 (Cayman Chemical, Ann Arbor, MI), purified human α-thrombin (provided by Dr John Fenton II, New York Department of Health, Albany) together with 2.5 mmol/L glycolyl-1-propyl-l-arginyl-l-proline (Calbiochem, San Diego, CA) (an inhibitor of fibrin polymerization) or a combination of adenosine diphosphate (ADP; Bio/Data, Hatboro, PA) and epinephrine (Sigma). In kinetic studies, the samples were then fixed with formaldehyde (1% final concentration) at various time points after the addition of the agonist. In all other studies, fixation with formaldehyde (1% final concentration) was performed 15 minutes after the addition of the agonist, as previously described. After fixation, all samples were diluted and incubated at 22°C for 15 minutes with (1) a saturating concentration of a biotinylated monoclonal antibody (directed against either P-selectin, the GPIb-IIIa complex, or the GPIb-IX complex) and (2) a saturating concentration of either fluorescein isothiocyanate (FITC)-conjugated GPIV-specific monoclonal antibody OKM5 or FITC-conjugated GPIVa-specific monoclonal antibody Y2/51. The samples were then incubated at 22°C for 15 minutes with phycoerythrin-streptavidin (Jackson Immunoresearch, West Grove, PA). When monoclonal antibody M148 was used, the incubation step with phycoerythrin-streptavidin was unnecessary because the antibody was purchased directly conjugated with R-phycoerythrin.) Within 24 hours of antibody tagging, the samples were analyzed in an EPICS Profile flow cytometer (Coulter Cytometry, Hialeah, FL). After identification of platelets by gating on both FITC positivity and their characteristic light scatter, bind-

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ing of the biotinylated monoclonal antibody was determined by analyzing 5,000 individual platelets for phycoerythrin fluorescence. In addition to platelets, OKM5 binds to monocytes, but these were completely gated out by size (forward-light scatter).

It has previously been shown that this method (fixation before antibody incubation) results in no significant differences in fluorescence intensity between samples analyzed immediately and samples analyzed within 24 hours of antibody tagging. We confirmed this finding, and also demonstrated that collection of blood into a Vacutainer did not result in platelet activation (Fig 1).

In addition to our standard method of determination of binding by fluorescence intensity relative to a maximally degranulated platelet, some samples were analyzed, as indicated in the Results, by the percentage of P-selectin-positive platelets. The percentage of P-selectin-positive platelets at different time points during CPB was defined as the percentage of platelets that had an SI2 fluorescence greater than 98% of the platelets in samples from the preoperative time point (without an added exogenous platelet agonist).

In a subgroup of patients, the response of washed platelets to thrombin was studied. Peripheral blood was collected as described above and the platelets separated and washed in modified Tyrode's buffer as previously described. The platelets (75,000/μL) were incubated with 0.05, 0.1, or 1 U/mL of purified human α-thrombin or buffer only, and then analyzed by flow cytometry for the binding of a biotinylated monoclonal antibody (either S12, PAC1, 7E3, or 6D1) as previously described. PAC1 does not bind well to fixed platelets. For this reason, as distinct from all other antibodies used in this study, PAC1 was incubated with platelets before fixation, as previously described.

Flow cytometric analysis of platelet surface P-selectin in shed blood. The platelet surface expression of P-selectin in blood emerging from a standardized bleeding time wound was analyzed by the whole-blood flow cytometric method. The method has been previously described in detail. Duplicate standardized bleeding times were performed on the forearm of patients with the Simplate II device. The blood emerging from the bleeding-time wound (shed blood) was collected with a micropipet at 2-minute intervals until the bleeding stopped. After each pipetting, any residual blood at the bleeding-time wound site was removed with filter paper. Immediately after collection at each time point, the pipetted blood was added to a microfuge tube containing sodium citrate, fixed for 30 minutes at 22°C with formaldehyde (1% final concentration), and diluted 1:10 by volume in modified Tyrode's buffer. As described above, the fixed diluted whole-blood samples were then labeled with the FITC-conjugated GPⅣ-specific monoclonal antibody OKM5 and the biotinylated P-selectin-specific monoclonal antibody S12, and the individual platelets analyzed in an EPICS Profile flow cytometer to assess S12 binding.

Radioimmunoassay of shed-blood TXB2. TXB2 is a stable metabolite of TXA2 and an important marker of platelet activation. The shed-blood assay method has been previously described. A standardized bleeding-time wound was performed, as described above. The shed blood emerging from the wound was aspirated through a blunt needle into a tuberculin syringe coated with heparin (1,000 U/mL) and containing 20 μL of ibuprofen for each 1 mL of blood (1.9 mg/mL). Samples were collected every 30 seconds until a 600-μL aliquot of blood was obtained. The TXB2 concentration of the plasma was determined with an RIA kit (New England Nuclear, Boston, MA).

Plasma glycoprotein C (GPIb) assay. Plasma glycoprotein C was determined by a modified version of a previously described competitive inhibition assay. A subsaturating concentration of FITC-conjugated monoclonal antibody 6D1 (1.2 μg/mL) was incubated at 22°C for 20 minutes with either (1) test plasma that had been filtered through a 0.22-μm Acrodisc (Gelman, Ann Arbor, MI) and the pH buffered to 7.4; or (2) various concentrations of purified glycoprotein C (prepared as previously described). Samples were then incubated at 22°C for 20 minutes with fixed, washed platelets (final concentration, 100,000/μL) and diluted 20-fold in modified Tyrode's buffer, pH 7.4, before the platelet binding of 6D1 was analyzed by flow cytometry. Linear regression analysis was used to generate a stan-
standard curve from 0 to 70 nmol/L from the purified glycocalicin samples. The plasma glycocalicin concentration of unknown plasma samples was derived from this standard curve.

Ristocetin-induced binding of von Willebrand factor to platelets. The ristocetin-induced binding of normal von Willebrand factor to washed platelets from the patient was determined by the following method: the patient’s platelets were washed as previously described, fixed in 1% formaldehyde, and resuspended at a concentration of 75,000/µL in modified Tyrode’s buffer, pH 7.4. Twenty microliters of the platelet suspension was incubated at 22°C for 15 minutes with 20 µL of pooled platelet-poor plasma from normal donors (as a source of von Willebrand factor) and 5 µL of ristocetin (BioData, Horsham, PA; final concentration, 1.4 mg/mL). The mixture was then incubated at 22°C for 15 minutes with 0.028 mg/mL of either polyclonal FITC-conjugated anti-von Willebrand factor goat IgG antibody (Atlantic Antibodies, Stillwater, MN) or FITC-conjugated nonspecific goat IgG (Atlantic Antibodies). The sample was then diluted 16-fold in modified Tyrode’s buffer, pH 7.4, and analyzed by flow cytometry. The fluorescence of the sample incubated with the nonspecific goat IgG was subtracted from the sample incubated with the anti-von Willebrand factor antibody.

Fibrinogen binding to platelets. Fibrinogen binding to ADP-stimulated washed platelets was determined by a direct binding assay using radioiodinated fibrinogen, as previously described.

Hematocrit and platelet counts. Hematocrit and platelet counts were measured using a J.T. Electronic Particle Counter (Coulter).

RESULTS

Effect of CPB on platelet reactivity in vivo. To determine the effect of CPB on platelet reactivity in vivo, the time-dependent up-regulation of platelet surface P-selectin was analyzed by whole-blood flow cytometry in the blood emerging from a standardized bleeding-time wound (Fig 2). The previously reported time-dependent up-regulation of platelet surface P-selectin was observed in samples obtained from bleeding time incisions performed preoperatively (PRE OP time point in Fig 2) and after the start of anesthesia and surgery but before heparin and CPB (PRE HEP time point in Fig 2). However, this in vivo up-regulation of P-selectin was almost abolished 5 minutes after heparin administration but before the start of CPB (HEP time point in Fig 2) and 45 minutes after the start of CPB (CPB 45 time point in Fig 2). The in vivo up-regulation of P-selectin returned to near normal within 2 hours after the completion of CPB (POST 2 time point in Fig 2) and was completely normal within 24 hours after the completion of CPB (POST 24 time point in Fig 2). The maximal up-regulation of P-selectin in shed blood collected during bypass and postbypass was 19.3% ± 3.2% (mean ± SEM; n = 14) and 111.9% ± 16.8%, respectively, of the prebypass value. Thus, the maximum up-regulation of P-selectin in shed blood collected during bypass was significantly decreased as compared with the mean values in shed blood collected before and after bypass (F statistic = 33.97, df = 2.28, P < .001, analysis of variance for repeated measures).

To provide further evidence that platelet reactivity in vivo is deficient during CPB, TXB₂ (a stable metabolite of TXA₂) was assayed in the shed blood emerging from the standardized bleeding-time wound. TXB₂ generation in shed blood was reduced 5 minutes after heparin administration but before the start of CPB (HEP time point in Fig 3) and decreased further 45 minutes after the start of CPB (CPB 45 time point in Fig 3). Generation of TXB₂ in shed blood returned toward normal after the completion of CPB (CPB END, POST 2, and POST 24 time points in Fig 3).

The CPB-induced inhibition of shed-blood P-selectin up-regulation and TXB₂ generation paralleled the effect of CPB on the bleeding time. The bleeding time (minutes ± SEM; n = 16) at the indicated time points was 6.2 ± 0.3 (PRE OP), 6.7 ± 0.3 (PRE HEP), 10.4 ± 0.5 (HEP), 32.8 ± 3.2 (CPB 45), 14.3 ± 1.0 (POST 2), and 11.5 ± 1.8 (POST 24). This CPB-induced marked prolongation of the bleeding time was...
To assess the reactivity of platelets to thrombin, the patients' platelets were washed free of heparin. CPB did not inhibit the thrombin-induced up-regulation of platelet surface P-selectin (Fig 5A), up-regulation of the GPIIb-IIIa complex (Fig 5B), or down-regulation of the GPIb-IX complex (data not shown). However, as expected, when the thrombin reactivity of platelets in whole blood containing the therapeutically administered heparin was measured, platelets were completely unreactive to thrombin at the following time points: 5 minutes after heparin administration, but before the start of CPB; after the start of CPB (normo-

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**Fig 3.** The effect of CPB on TXB₂ generation in response to a standardized vascular injury in vivo. The abbreviations listed on the horizontal axis refer to the perioperative time points, as defined in Table 1. At each of these perioperative time points, a standardized bleeding-time wound was performed and the shed blood was collected every 30 seconds into a heparinized syringe containing ibuprofen. The TXB₂ concentration of the plasma was determined by radioimmunoassay. Data are the mean ± SEM; n = 16.

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**Fig 4.** A) Effect of CPB on platelet reactivity to PMA, as determined by whole-blood flow cytometry. Abbreviations listed on the horizontal axis refer to the perioperative blood sampling time points, as defined in Table 1. Peripheral blood samples were incubated with PMA 0 µmol/L ( ), 0.25 µmol/L ( ), or 10 µmol/L ( ). (A) Platelet surface expression of P-selectin, as determined by monoclonal antibody S12. The fluorescence intensity of platelets incubated with PMA 0 µmol/L at the preoperative (PRE OP) time point was assigned a value of 100 U. (B) Platelet surface GPIIb, as determined by monoclonal antibody 6D1. The fluorescence intensity of platelets incubated without PMA at the preoperative time point was assigned a value of 100 U. Data are the mean ± SEM; n = 16.
plex.

Whole-blood flow cytometric method that did not require washing. To avoid these potential artifacts, we used a single peripheral blood sampling time points, as defined in Table 1.

The platelets were washed, incubated with thrombin (U/mL) 0.05 (e), 0.1 (f), 1.0 (g), and analyzed by flow cytometry. (A) Platelet surface expression of P-selectin, as determined by monoclonal antibody S12. (B) Platelet surface expression of the GPIIb-IIIa complex, as determined by monoclonal antibody PAC1. The fluorescence intensity of platelets incubated with thrombin 1 U/mL at the preoperative (PRE OP) time point was assigned a value of 100 U.

Figure 5. The effect of CPB on thrombin reactivity of washed platelets. Abbreviations listed on the horizontal axis refer to the periperaoperative peripheral blood sampling time points, as defined in Table 1. The platelets were washed, incubated with thrombin (U/mL) 0 (c), 0.05 (e), 0.1 (f), 1.0 (g), and analyzed by flow cytometry. (A) Platelet surface expression of P-selectin, as determined by monoclonal antibody S12. (B) Platelet surface expression of the GPIIb-IIIa complex, as determined by monoclonal antibody PAC1. The fluorescence intensity of platelets incubated with thrombin 1 U/mL at the preoperative (PRE OP) time point was assigned a value of 100 U. Data are mean ± SEM; n = 4.

Effect of CPB on platelet surface GPIIb-IIIa complex. The platelet surface GPIIb-IIIa complex was analyzed by whole-blood flow cytometry. CPB did not result in any significant change in the platelet surface expression of the GPIIb-IIIa complex, as determined by monoclonal antibodies (7E3, 10E5, and M148) directed against different epitopes near the fibrinogen-binding site on the GPIIb-IIIa complex and a monoclonal antibody (Y2/51) directed against GPIIIa (Fig 6F, G, H, and I).

Direct measurement of the binding of exogenous fibrinogen to platelets activated in vitro with ADP (reflecting exposure of the fibrinogen-binding site on the GPIIb-IIIa complex) demonstrated an increase in the $K_D$, from 0.43 to 0.54 μmol/L and no loss of binding sites during CPB (Fig 6J).

Effect of CPB on the presence of circulating activated platelets. The presence of activated platelets circulating in peripheral blood was investigated by the platelet binding of PAC1 (Fig 5B, open circles) demonstrated an increase in the $K_D$, from 0.43 to 0.54 μmol/L and no loss of binding sites during CPB (Fig 6J).

Effect of CPB on thrombin reactivity of washed platelets. The platelet surface expression of the GPIIb-IX complex can be markedly reduced by platelet activation and washing. To avoid these potential artifacts, we used a whole-blood flow cytometric method that did not require any centrifugation, gel filtration, vortexing, or stirring steps. As determined by this method, CPB did not result in significant change in the platelet surface expression of the GPIIb-IX complex, irrespective of whether the monoclonal antibody used was directed against the von Willebrand factor binding site on GPIb (Fig 4B, closed circles, and Fig 6A), the thrombin binding site on GPIb (Fig 6B), GPIX (Fig 6C), or the GPIIb-IX complex (Fig 6D).

In addition, we examined the ristocetin-induced binding of von Willebrand factor to platelets. This binding, which reflects the availability of GPIb as a platelet receptor for von Willebrand factor, was unchanged during and after CPB (Fig 6E).

Furthermore, we assayed the patients' plasma for glycosaminoglycan, a proteolytic product of GPIb. Plasma glycosaminoglycan did not rise above the preoperative concentration at any time point during or after CPB (Fig 7). The observed reduction in plasma glycosaminoglycan during CPB was probably dilutional, because the reduction paralleled the reductions in hematocrit (Fig 7) and serum albumin (data not shown).

Effect of CPB on the presence of circulating activated platelets. The presence of activated platelets circulating in peripheral blood was investigated by the platelet binding of PAC1 (open circles) demonstrated an increase in the $K_D$, from 0.43 to 0.54 μmol/L and no loss of binding sites during CPB (Fig 6J).

Discussion

The most important factor contributing to the hemostatic defect associated with CPB is generally considered to be a platelet function defect. However, the precise nature of the putative CPB-induced platelet function defect is controversial.

In this study, we demonstrate that CPB results in
Platelet Function in Cardiopulmonary Bypass

In this study, we have demonstrated that CPB inhibits platelet reactivity in vivo, as determined by two independent markers of platelet activation in shed blood: up-regulation of platelet surface P-selectin and TXB2 generation. These defects paralleled the CPB-induced prolongation of the bleeding time, which is generally accepted to be the hallmark of the hemostatic defect of CPB.

Effect of CPB on platelet reactivity in vivo. The shed blood emerging from a standardized bleeding-time wound has been used in a number of previous studies as a reflection of in vivo activation of platelets and other components of the hemostatic system. In this study, we have demonstrated that CPB inhibits platelet reactivity in vivo, as determined by two independent markers of platelet activation in shed blood: up-regulation of platelet surface P-selectin and TXB2 generation. These defects paralleled the CPB-induced prolongation of the bleeding time, which is generally accepted to be the hallmark of the hemostatic defect of CPB.

Effect of CPB on platelet reactivity in vitro. The results of studies of the effect of CPB on platelet aggregation, as determined by standard nephelometric methods, are inconsistent. These inconsistencies probably result in part from the fact that platelet aggregation, especially in a complex clinical setting, is semiquantitative and subject to standardization problems. Furthermore, most of the reported platelet aggregometry studies during CPB were performed in platelet-rich plasma without normalizing the platelet count. The CPB-induced “platelet aggregation defect” may therefore simply reflect the CPB-induced thrombocytopenia.

In this study, platelet reactivity in vitro was analyzed not by aggregometry, but by whole-blood flow cytometric assay of peripheral blood samples obtained before, during, and after CPB. CPB did not result in a defect in platelet reactivity in vitro, as determined by agonist-induced up-regulation of platelet surface P-selectin and the GPIIb-IIIa complex, and down-regulation of the GPIb-IX complex. The results were the same irrespective of whether the in vitro platelet agonist was PMA, the stable TXA2 agonist U46619, a combination of ADP and epinephrine, or thrombin (in a washed platelet system), thereby excluding the possibility of a CPB-induced signal transduction defect.

Effect of CPB on the platelet surface GPIb-IX complex. Some, but not all, previous studies have concluded that a CPB-induced modest decrease in platelet surface GPIb, a receptor for von Willebrand factor, may play a role in the pathogenesis of the CPB-induced platelet dysfunction. The study by George et al has been widely interpreted as evidence for a CPB-induced reduction in platelet surface GPIb. However, in George’s study all values for platelet surface GPIb during CPB were within or close to the normal range. van Oeveren et al reported a 25% reduction in GPIb during CPB, but, unlike the study by George et al and the present study, these investigators centrifuged and
gel-filtered the platelets before assay, thereby introducing the possibility of an artefactual in vitro decrease in platelet surface GPIb as a result of proteolysis or activation. Rinder et al reported a maximal reduction of platelet surface GPIb of 28%, but the only significant reduction in GPIb was at the completion of and after CPB. Thus, Rinder et al did not observe a significant reduction in platelet surface GPIb during CPB, when the bleeding time is most prolonged.

In this study, we used a flow cytometric method that allowed us to study the platelet GPIb-IX complex in whole blood, thereby avoiding potential artefactual reductions in platelet surface GPIb. As demonstrated by this method, CPB did not result in a decrease in the platelet surface expression of the GPIb-IX complex. The possibility of a CPB-induced conformational change in the GPIb-IX complex, or CPB-induced proteolysis of a fragment of the GPIb-IX complex, was excluded by the lack of change in the platelet binding of a panel of monoclonal antibodies (6D1, TM60, AK1, and FMC25) known to be directed against different epitopes on the GPIb-IX complex. Further, by a combination of methods, the present study demonstrates that CPB is not associated with a loss of platelet surface GPIb.

Effect of CPB on the platelet surface GPIIb-IIIa complex. Some, but not other, previous studies have concluded that a CPB-induced modest decrease in the platelet surface GPIIb-IIIa complex, the fibrinogen receptor, plays a role in the pathogenesis of the CPB-induced platelet dysfunction. Using washed platelet methods, Wenger et al reported a decrease of 25% in the whole-platelet GPIIIa content during CPB and a decrease of 41% in the platelet binding of exogenous fibrinogen. Dechavanne et al also used a washed platelet method and reported a 32% decrease in the platelet surface GPIIb-IIIa complex during CPB. Using a whole-blood method, George et al reported only a slight decrease in platelet surface GPIIb during CPB. Rinder et al, using a whole-blood method, reported a 21% (but statistically insignificant) decrease in the platelet surface GPIIb-IIIa complex after CPB. However, van Oeveren et al detected a modest increase in both platelet surface GPIIb-IIIa and ADP-induced fibrinogen-binding during CPB. Finally, using a whole-blood flow cytometric method, Abrams et al reported that fibrinogen binding to the platelet GPIIb-IIIa complex was slightly increased during CPB, as determined by the binding of a monoclonal antibody (PAC1) directed against the fibrinogen binding site on the GPIIb-IIIa complex and by the binding of a monoclonal antibody (9F9) directed against platelet-bound fibrinogen.

In the present study, as demonstrated by a whole-blood flow cytometric assay, CPB did not result in a decrease in the platelet surface expression of the GPIIb-IIIa complex. The possibility of a CPB-induced conformational change in the GPIIb-IIIa complex, or CPB-induced proteolysis of a fragment of the GPIIb-IIIa complex, was excluded by the lack of change in the platelet binding of a panel of monoclonal antibodies (7E3, 10E5, M148, and Y2/51) known to be directed against different epitopes on the GPIIb-IIIa complex. In addition, we directly studied the binding of exogenous fibrinogen to the GPIIIb-IIIa complex and found no CPB-induced reduction. Finally, we demonstrated that irrespective of whether peripheral blood samples were drawn before, during, or after CPB, maximal in vitro stimulation
of washed platelets by thrombin resulted in the same exposure of the fibrinogen-binding site on the GPIIb-IIIa complex, as determined by monoclonal antibody PAC1. Thus, by a combination of methods, the present study demonstrates that CPB is not associated with a loss of the platelet surface GPIIb-IIIa complex.

Effect of CPB on platelet degranulation. Electron microscopic methods, fraught with the possibility of artifactual in vitro platelet degranulation, have resulted in reports of circulating partially degranulated platelets during CPB in some, but not other, studies. More recently, a number of investigators have studied the effect of CPB on the binding of monoclonal antibodies directed against granule antigens that are only present on the platelet surface after degranulation. In washed platelet systems, Nieuwenhuis et al found a modest increase during CPB of the platelet binding of a monoclonal antibody directed against a 53-Kd lysosomal antigen, whereas Dechavanne et al found that CPB did not result in an increase in the platelet surface expression of P-selectin. Using whole-blood methods that are much less likely than washed platelet methods to result in artifactual in vitro platelet degranulation, both George et al and Abrams et al demonstrated that CPB resulted in no significant increase in the platelet surface expression of P-selectin. By comparable methods, Corash reported a modest, transient increase in P-selectin–positive platelets during CPB. Rinder et al used a whole-blood flow cytometric assay and found a 29% increase in P-selectin–positive platelets at the end of CPB, but the amount of increase in P-selectin on the P-selectin–positive platelets was modest.

Consistent with these previous studies, the present whole-blood flow cytometric study of peripheral blood samples during CPB showed that although there was a 55% increase (2.0% to 3.1%) in the number of circulating degranulated platelets during CPB, the overall P-selectin expression on the surface of the platelets was minimal (Fig 4A, closed circles). These data suggest that the reported CPB-induced increases in plasma β-thromboglobulin and platelet factor 4 originate from either (1) degranulated platelets that are rapidly cleared from the circulation (possibly by circulating monocytes and neutrophils), (2) noncirculating degranulated platelets adherent to synthetic surfaces or vessel walls, (3) platelet lysis in vivo or in vitro, and/or (4) artifactual in vitro degranulation and secretion as a result of separation of plasma from platelets before the performance of the assays.

The role of heparin in the platelet function defect of CPB. Thrombin, the most important platelet agonist in vivo, is functionally deficient during CPB because of the presence of high circulating concentrations of heparin. Although thrombin bound to fibrin clots is relatively protected from inhibition by heparin-antithrombin III, the high concentrations of heparin used during CPB (4.5 U/mL with an ACT ≥999 seconds in this study) are sufficient to completely overcome this protection. The importance of heparin inhibition of thrombin in the platelet function defect of CPB is suggested by the finding that 5 minutes after heparin administration, but before the start of CPB, (1) the in vivo up-regulation of platelet surface P-selectin was almost completely abolished (HEP time point in Fig 2), (2) the in vivo generation of TXA2 was almost maximally inhibited (HEP time point in Fig 3), (3) the bleeding time was prolonged, and (4) platelets in whole blood were completely unreactive to thrombin in vitro.

The present study provides evidence for two distinct effects of heparin on platelet function during CPB. First, heparin augments platelet activation in whole blood exposed to an exogenous platelet agonist in vitro (Fig 4). Second, as discussed above, heparin suppresses platelet activation in vivo via inhibition of endogenous thrombin. Thus, although heparin augments the actiavability of platelets, the platelets are not in fact activatable in vivo because thrombin, the preeminent agonist, is unavailable.

In addition to heparin, other extrinsic factors such as hypothermia and fibrinolytic activity may contribute to the platelet function defect associated with CPB. These factors are currently under investigation in our laboratory.

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