Axial Dependence of Platelet-Collagen Interactions in Flowing Blood

Upstream Thrombus Growth Impairs Downstream Platelet Adhesion

Kjell S. Sakariassen and Hans R. Baumgartner

Vascular subendothelium and collagenous surfaces were exposed to flowing citrated blood. Platelet interactions with these surfaces were investigated at various axial distances from the upstream end of the exposed surfaces. A pronounced axial decrease in surface coverage with platelets and in thrombus dimensions was encountered on collagenous surfaces. This phenomenon was observed at shear rates of 200 to 2000 s⁻¹, but was most pronounced at low shear rates (<550 s⁻¹). After 5 minutes of perfusion at a shear rate of 650 s⁻¹, 4.5 x 10⁶ platelets were deposited on the most upstream 20 mm² of the collagen surface, in contrast to 2.2 x 10⁶ platelets/20 mm² 14 mm farther downstream. Deposition of von Willebrand factor and/or thrombospondin from the boundary layer of the blood flow was not responsible for this. Collagen-bound von Willebrand factor enhanced the surface coverage with platelets without affecting the axial decrement, while pretreatment of the collagen surface with thrombospondin had no effect at all. However, partial inhibition of thrombus growth by aspirin reduced the axial decrements, and less thrombogenic surfaces as human and rabbit subendothelium, which induced only a few small thrombi, produced virtually no axial differences in platelet adhesion. Raising the shear rate to 2600 s⁻¹ also gave no axial differences in platelet-collagen adhesion; it did, however, give an axial increase in thrombus dimensions. This increase was neutralized after the addition of antibody against human platelet thrombospondin to the blood. Our data are consistent with the view that platelet-surface interactions are limited by the arrival of platelets to the surface at shear rates below 650 s⁻¹. Surfaces that induce rapid-growing upstream thrombi may deplete the boundary layer for platelets, resulting in decreased platelet adhesion and thrombus growth farther downstream. At higher shear rates, when the platelet supply to the surface is not a limiting factor, thrombospondin released from upstream thrombi appears to enhance downstream thrombus growth and/or thrombus stability. (Arteriosclerosis 9:33–42, January/February 1989)

Platelet deposition on artery subendothelium in vivo and in vitro is a dynamic event.¹² Platelets adhere rapidly to the subendothelium and subsequently produce mural thrombi. The growth of these thrombi is reversible, and most of the aggregated platelets have disappeared within 20 minutes, apparently without affecting platelets adherent to the deendothelialized area.

Axial platelet-surface interactions have not been studied in detail, although previous reports occasionally reported differences in axial platelet deposition on various surfaces.³⁴ A variety of mechanisms, physical as well as chemical, may be responsible for this phenomenon. Theoretical considerations have predicted that upstream deposition of platelets depletes the boundary layer (the layer of the blood flow streaming adjacent to the surface) of platelets, resulting in less deposition on downstream areas.⁵⁶ Indeed, decreased axial platelet deposition on collagen fibrils was reported recently.⁴ Partial inhibition of thrombus growth by aspirin was reported to enhance platelet adhesion to collagen fibrils⁷ and subendothelial surfaces,³⁵ indicating that consumption of platelets from the boundary layer by growing thrombi decreases the rate of platelet-surface adhesion. These observations lend support to the theoretical considerations⁵⁶ predicting that depletion of platelets in the boundary layer results in decreased platelet-surface adhesion. However, local shear stresses may also play a role, as was indicated by the observation of translocation of platelets and/or platelet masses from upstream thrombi on collagen fibrils to downstream noncoagulogenous areas.³

Depletion of von Willebrand factor (VWF) and thrombospondin (TSP) from the boundary layer could also influence the axial platelet deposition. Both proteins are present in relatively low amounts in plasma (~5 μg/ml and ~20 ng/ml, respectively), and they bind to subendothelial components.¹⁰¹¹ VWF bound to artery subendothelium and to various collagens mediates adhesion of platelets.¹₀¹² In contrast, TSP appears not to be required for normal platelet adhesion, at least not to the pericellular matrix of

From the Department of Pharmaceutical Research, F. Hoffmann-La Roche & Company, Basel, Switzerland.

Address for reprints: Kjell S. Sakariassen, Ph.D., Department of Pharmaceutical Research, F. Hoffmann-La Roche & Company, CH-4002 Basel, Switzerland.

Received February 18, 1988; revision accepted July 26, 1988.
endothelial cells. However, both proteins play a role in platelet-platelet cohesion, as was demonstrated in ex vivo perfusion experiments with blood from patients with von Willebrand’s disease and in studies with the aggregometer using antibodies against TSP, 13, 19.

We report on characterization and quantitation of platelet-surface interactions in flowing citrated blood at well-defined axial positions on a variety of surfaces. Nonmodified rabbit and human artery subendothelium, 6, 17 alphachymotrypsin-treated rabbit artery subendothelium consisting of islands of collagen fibrils embedded in elastin, 18 and plastic coverslips coated with collagen fibrils 19 were used. We observed a pronounced axial decrease in platelet adhesion and thrombus dimensions on the collagenous surfaces that was not caused by depletion of VWF or TSP. Our data indicate that thrombi growing rapidly upstream deplete the boundary layer of platelets, resulting in decreased platelet adhesion and thrombus dimension on downstream positions of the exposed surface. We show that this axial phenomenon is more pronounced at low shear rates (<650 s⁻¹), and disappears at high shear rates (2600 s⁻¹), observations that are consistent with the theoretical considerations, 5, 6 assuming reduced downstream platelet adhesion caused by depletion of platelets from the boundary layer by upstream growing thrombi.

Methods

VWF purified from human plasma was provided by J. P. Gimma (Hôpital de Bicêtre, Paris). The preparation possessed intact multimeric structure, and contaminating fibrinogen and fibronectin were not detected. TSP purified in the presence of CaCl₂ from human platelets was provided by K. J. Clementson, (Theodor Kocher Institute, Berne, Switzerland). Gradient polyacrylamide gel (6% to 12%) electrophoresis and silver staining revealed a homogeneous preparation. Lyophilized rabbit antiserum directed against human platelet TSP was provided by Paul Bornstein (University of Washington, Seattle, Washington). The antiserum did not cross-react with collagen types I, III, IV, and V or with VWF, laminin, or fibronectin. Characterization of this antiserum was previously reported. 11, 20 Lyophilized normal rabbit IgG was purchased from Sigma Chemical Company (St. Louis, MO). Plastic coverslips (22×60 mm, Thermomax) were purchased from Miles Laboratories (Elkhart, IN) and fibrilar equine collagen (Collagen Reagent Hom, 1 mg/ml) from Hormon Chentie (Munich, West Germany). Aspin and 51Cr (4 μCi/ml 0.9% NaCl) were obtained from Bayer (Laverkusen, West Germany) and from Fleurus (Belgium), respectively.

Blood Samples

Blood from healthy individuals was collected in 1/10 volume 108 mM trisodium citrate, and the plasma citrate concentration was adjusted to 20 mM, as previously described. 21 All individuals denied having been subjected to any medication during the 10 days prior to the donations. Their hematocrits (40% to 48%) and platelet counts (1.2 to 2.5×10¹¹/L) were within normal ranges.

Aspin dissolved in 130 mM NaCl, 2 mM KCl, 12 mM NaHCO₃, 2.5 mM CaCl₂, 0.9 mM MgCl₂, and 20 mM trisodium citrate, pH 7.4, was added to a low blood samples at a concentration of 1 mM. The aspirinated blood samples were subsequently incubated at 37°C for 30 minutes and then used for perfusion experiments. Control blood samples from the same individuals were similarly treated, but without the presence of aspirin in the buffer.

Lyophilized rabbit anti-human TSP and a control rabbit IgG fraction were dissolved in distilled water and added to blood samples at a concentration of 66 μg/ml. Blood samples with added antibodies were incubated at 37°C for 10 minutes and successively used in perfusion experiments.

Blood Reconstituted with 51Cr-radiolabeled Platelets

Reconstituted blood perfusates with radiolabeled 51Cr-platelets were made up from citrated blood according to Sankariassen et al. 10 with some minor modifications. 21 Briefly, platelets in platelet-rich plasma prepared from citrated blood by centrifugation (at 200 g, 22°C, 10 minutes) and diluted with one volume of 130 mM NaCl, 2 mM KCl, 12 mM NaHCO₃, and 20 mM trisodium citrate (pH 5.0), giving a final pH of 6.2, were pelleted (at 500 g, 22°C, 10 minutes) and subsequently resuspended in the same buffer (pH 6.0) and in the presence of 2 μCi 51CrO₄⁻/ml. After labeling at 22°C for 20 minutes, the platelets were washed free of exogenous 51CrO₄⁻ by three successive centrifugations (at 500 g, 22°C, 10 minutes) with the same buffer (pH 6.0) at pH 6.2 in the various platelet suspensions. Platelet-free plasma and three-times-washed erythrocytes were prepared by centrifugation (at 3000 g, 4°C, 30 minutes; and at 3000 g, 22°C, 2×5 minutes and 1×20 minutes, respectively), as recently described in detail. 21 The blood perfusates were reconstituted with 51Cr-platelets and washed erythrocytes in autologous 20 mM trisodium citrate plasma at platelet counts and hematocrits similar to those of the corresponding citrated blood samples, 1.2 to 2.4×10¹¹/L and 44% to 46%, respectively.

Preparation of Collagen and Collagen/Protein-coated Coverslips

Plastic coverslips (22×18 mm) were spray-coated with 30 μg equine collagen/cm², as previously reported. 22, 23 The collagen-coated coverslips were stored at 22°C for about 16 hours before they were used in perfusion experiments. Some collagen-coated coverslips were incubated with 0.4 ml of purified VWF or TSP suspended in the buffer used to dissolve aspirin, but without trisodium citrate, at 22°C for 20 minutes. 12, 19 Control surfaces were exposed to the buffer only. TSP concentrations of 1.5, 15, 150, and 435 μg/ml and VWF concentrations of 0.15, 1.5, and 15 μg/ml were used. Incubations were followed by a perfusion of 50 ml phosphate-buffered saline (PBS: 59 mM NaH₂PO₄, 1 mM NaH₂PO₄, and 75 mM NaCl, pH 7.4) at a shear rate of 650 s⁻¹ at 37°C in order to remove nonspecifically attached VWF and TSP. The surfaces were not exposed to air, but used immediately in perfusion experiments.
Preparation of Artery Segments

Artery segments from rabbit aorta and human umbilical arteries were used.

Deendothelialization of rabbit aorta was performed in situ in anesthetized rabbits by means of the balloon catheter technique. Alpha-chymotrypsin treatment of rabbit aortic subendothelium was carried out according to Baumgartner. This treatment produces a surface consisting of islands of collagen fibrils embedded in elastin.

Deendothelialization of human umbilical arteries was carried out in vitro with a brief exposure to air. All segments were used within 3 days.

Parallel-Plate and Annular Perfusion Chambers

Parallel-plate perfusion chambers with two coverslip holders, one proximal and one distal, were used (Figure 1). The chambers were otherwise similar to the original parallel-plate chamber, except for the length and the height of the rectangular blood flow slits. The flow slits were 83 (length) × 10 (width) × 0.6 (height) mm and 226 (length) × 10 (width) × 0.4 (height) mm, respectively. The average heights at the collagen surface were 0.624 and 0.424 mm due to the depth of the recess of the coverslip holder and the thickness of the collagen coat. The original annular perfusion chamber with annular width of 1.3 mm was used for the artery segments.

Perfusions

Perfusions were carried out at 37°C with pulsatile blood flow. Twenty ml of perfusate was recirculated from a container with Silastic tubing for periods of 2, 5, and 10 minutes after preperfusion of the various surfaces with 40 ml PBS at 37°C. The perfusions were terminated by removing the Silastic tubing from the perfusate container, resulting in successive removal of blood from the chamber by the pump. The coverslip was immediately removed from the chamber and briefly rinsed in PBS and then immersed in freshly prepared fixative at 4°C.

Average flow rates in the parallel-plate perfusion chambers were 7.6, 24.6, 49.2, and 75.0 ml/min (chamber with 0.624-mm slit height) and 56.4 ml/min (chamber with 0.424-mm slit height), which correspond approximately to shear rates at the collagen surface of 200, 650, 1300, 2000, and 2600 s⁻¹, respectively. Average flow rates in the annular perfusion chamber were 130.0 and 118.2 ml/min, corresponding to a shear rate of 650 s⁻¹ at the rabbit aortic subendothelium (vessel wall thickness ≈ 0.10 mm) and at the human artery subendothelium (vessel wall thickness ≈ 0.15 mm), respectively.

Axial Assessment of Platelet Deposition with ⁵¹Cr-Platelets

Coverslips perfused with ⁵¹Cr-platelets were sliced in nine strips of 10 (width) × 2 (length) mm and successively transferred to a gamma counter (Model 1185, Searle, Chicago, Illinois) for registration of ⁵¹Cr-count. Platelet deposition on each of the nine strips was expressed as number of ⁵¹Cr-platelets/20 mm².

En Face Preparations

En face preparations on coverslips for light microscopy were fixed and stained according to Muggli et al.

Fixation, Embedding, and Axial Sections of Arteries and Collagen Coats

Fixation and Epon embedding of the collagen coats with successive removal of coverslips and postembedding in Epon were carried out according to Sakariassen et al. The vessel segments were fixed and embedded in Epon according to Baumgartner.
Semithin sections perpendicular to the direction of the blood flow were produced at axial positions of approximately 2, 8, 14, and 17 mm on the vessel segments and at 1, 2, 3, 8, 9, 14, and 15 mm on the collagen coats.

**Standard and Computer-assisted Morphometry of Semithin Sections**

Percent surface coverage with platelets and thrombi more than 5 μm in height were scored with standard morphometry. Thrombus parameters were measured with computer-assisted morphometry (unpublished observations.) The microscope image of the sections was displayed on a color video monitor (Sony, PVM-2060ME) by a video camera (JVC KY-1900E) fitted on the tube of the microscope (Zeiss) with a final magnification on the screen of ×2700. All platelet deposits identified were registered by contouring the objects manually with an electromagnetic pen on a graphic tablet. Contours and image were superimposed on the monitor, and both were contrasted by a color effect generator (RGB mixer, EL-Elektronik, Basel, Switzerland). Management of data and data processing were performed with a BIVAS program (Heinz Meyer, DataLab, Thötgen, Switzerland) and an Apple Ile computer. Data were printed on an Epson printer (Epson, model FX-80). Thrombi were defined as platelet masses higher than 2.5 μm. The total number of thrombi was registered and expressed as average number of thrombi per 100-μm sectional length (thrombus density). Sectional thrombus areas (μm²/μm) were calculated by the program, and sectional thrombus heights (in microns), the base to peak distance of the thrombi, were registered and expressed as average area and height. Thrombus growth was expressed as thrombus area/mm section length min⁻¹ (μm²/μm min⁻¹)² and as thrombus height/min (μm/min).²

**Statistical Analysis**

The significance of difference for grouped data was calculated with Student's t test. p values <0.05, <0.01, and <0.001 were considered significant. Linear regression analyses were calculated with the Apple Ile computer by using the BIVAS TAT program.

**Results**

**Assessment of Axial Platelet Deposition on Collagen by Using ⁵¹Cr-radiolabeled Platelets**

Axial deposition of platelets on collagen was investigated by using ⁵¹Cr-radiolabeled platelets (Figure 2) after perfusions for 5 minutes at a shear rate of 650 s⁻¹.

A significant axial decrease in platelet deposition was observed (Figure 2). The decrease was significant on both collagen coats (p<0.05), with r values (linear regression analysis) of 0.90 (proximal) and 0.82 (distal). A steeper axial decrease, however, was observed in citrated blood (nonreconstituted blood) when the number of deposited platelets was calculated from the average thrombus areas according to Sakariassen et al.²¹ The average axial decrease yielded 52% on the proximal collagen coat and increased further to 85% on the distal collagen coat, while the corresponding figures in reconstituted blood with ⁵¹Cr-platelets were 28% and 60%, respectively.

![Figure 2](image_url)

**Figure 2.** Axial assessment of number of deposited platelets in reconstituted citrated blood with ⁵¹Cr-platelets (●) and in citrated blood (○). Axial number of deposited platelets in reconstituted blood was measured by ⁵¹Cr-counting of 2 by 10-mm strips (20 mm²) of silicil proximal (prox.) and distal (dist.) collagen-coated coverslips. Axial number of deposited platelets in citrated blood was calculated from the average thrombus area per micrometer section length at axial positions of 1, 8, and 14 mm on the proximal and distal collagen coats, as previously reported. Blood from the same individuals was used in both sets of experiments, and platelet counts and hematocrits in reconstituted blood were adjusted to the corresponding values in citrated blood. Five minute perfusions were at shear rates of 650 s⁻¹. Values are mean±SEM, n=3. *p<0.05, **p<0.01, ***p<0.001.

![Figure 3](image_url)

**Figure 3.** Effect of time, 2 minutes (●), 5 minutes (●), and 10 minutes (○) at a shear rate of 650 s⁻¹, on the axial percent surface coverage with platelets at downstream positions of 2, 8, and 14 mm on the proximal (prox.) and distal (dist.) collagen coats. Values are mean±SEM, n=4. Significance was determined relative to the respective determinations at 2 mm downstream on the proximal collagen coat ( ●p<0.05, ●●p<0.01, ●●●p<0.001).

**Effect of Time**

The effects of time on axial surface coverage with platelets (Figure 3) and on thrombus density and dimensions (Figure 4) were quantified with standard morphometry and computer-assisted morphometry. Perfusions at a shear rate of 650 s⁻¹ were maintained for 2, 5, and 10 minutes.

A significant axial decrease was seen in surface coverage with platelets of about 63% at 2 minutes, 45% at 5 minutes, and 35% at 10 minutes from 2 mm downstream on the proximal collagen coat to 14 mm downstream on the distal collagen coat (Figure 3).
Thrombus density and thrombus dimensions were measured at two axial positions. Axial decrease in thrombus density was detected at 2 minutes, but not at 5 and 10 minutes (Figure 4A). Conversely, axial decrease in thrombus area and height was documented at all time points measured (Figures 4B and 4C). The average decrements were 82% (2 minutes), 65% (5 minutes), and 71% (10 minutes) in area, and 35% (2 minutes), 33% (5 minutes), and 44% (10 minutes) in height. The average growth rate in area remained constant, but was about three- to 5.5-fold higher at the upstream position than at the downstream position (Figure 5A); however, the concomitant average growth rate in height, which was highest at the upstream position, decreased with time (Figure 5B).

Light micrographs of semithin sections (Figure 6) show the pronounced axial decrements in surface coverage with platelets and in thrombus area and height. The thrombi appeared firmly attached to the collagen coat with platelets that had migrated into the collagen meshwork.

**Effects of Shear Rate**

The effects of shear rate on axial surface coverage with platelets (Figure 7) and on thrombus density and dimensions (Figure 8) were quantified with standard morphometry and computer-assisted morphometry, respectively. Shear rates of 200, 650, 1300, 2000, and 2600 s⁻¹ were maintained for 5 minutes.

The axial decrease in surface coverage with platelets was most pronounced at the lowest shear rate (200 s⁻¹) (Figure 7). Increasing shear rates yielded progressively less axial decrease. Average decrements were 79% at a shear rate of 200 s⁻¹, 48% at 650 s⁻¹, 33% at 1300 s⁻¹, 23% at 2000 s⁻¹, and 0% at 2600 s⁻¹ from 2 mm downstream on the proximal collagen coat to 14 mm downstream on the distal collagen coat.

Thrombus density, area, and height were measured at the same axial positions. Axial decrease in thrombus density was observed only at shear rates of 200 s⁻¹ and 650 s⁻¹, which averaged 71% and 35%, respectively (Figure 8A). The axial drop in thrombus area appeared more pronounced and yielded average figures of 86% at shear rates of 200 s⁻¹, 72% at 650 s⁻¹, 47% at 1300 s⁻¹, and 38% at 2000 s⁻¹, respectively. However, an axial increase of 32% was observed at a shear rate of 2600 s⁻¹ (Figure 8B). The corresponding axial decreases in aver-
arteriosclerosis

Figure 6. Light micrographs (×820) of sections cut perpendicular to the direction of blood flow on the proximal collagen coat at axial positions of 3, 9, and 15 mm. Perfusion was at a shear rate of 650 s⁻¹ for 5 minutes. Arrow indicates the direction of the blood flow.

Figure 7. Effect of shear rate, 200 s⁻¹ (□), 650 s⁻¹ (■), 1300 s⁻¹ (●), 2000 s⁻¹ (▲), and 2600 s⁻¹ (△) maintained for 5 minutes on the axial percent surface coverage with platelets at downstream positions of 2, 8, and 14 mm on the proximal (prox.) and distal (dist.) collagen coats. Values are mean±SEM, n=4 to 9. Significance was determined relative to the respective determinations at 2 mm downstream on the proximal collagen coat (*p<0.05, **p<0.01, ***p<0.001).

Average thrombus heights were shear rate–dependent as well, and averaged 41% at a shear rate of 200 s⁻¹, 33% at 650 s⁻¹, 21% at 1300 s⁻¹, and 12% at 2000 s⁻¹, respectively. An axial increase of 19% was observed at a shear rate of 2600 s⁻¹ (Figure 8C).

However, shortening the perfusion time to 3 minutes at a shear rate of 2600 s⁻¹ resulted in axial decreases in platelet-collagen interactions. The axial decrease in surface coverage with platelets was 28% (64.9±3.7% proximal and 46.9±1.6% distal, n=4; it was 29% for thrombus area (8.0±0.6 μm²/μm proximal and 5.7±0.3 μm²/μm distal) and 14% for thrombus height (14.5±0.6 μm proximal and 12.4±0.7 μm distal). An axial increase of 11% was observed for thrombus density (2.8±0.1/100 μm proximal and 3.2±0.2/100 μm distal).

Effect of Aspirin

To test whether depletion of platelets from the boundary layer plays a role in the axially decreased platelet-collagen interactions, consumption of platelets by growing thrombi was partially inhibited by the addition of aspirin to the blood samples. Previous data have shown that aspirin in citrated blood inhibits the growth of thrombi. 8 The axial surface coverage with platelets, thrombus density, and thrombus dimensions were quantified with standard morphometry and computer-assisted morphometry following 5-minute perfusions at a shear rate of 650 s⁻¹ (Figure 9).

Addition of aspirin to citrated blood abolished the axial decrease in surface coverage with platelets (Figure 9A), but more thrombi were observed upstream (Figure 9B); these were smaller in area than those encountered in control blood samples (Figure 9C). No differences in area were noted at axial positions of 8 and 14 mm. The average thrombus height was significantly lower at all axial positions (Figure 9D).

Effect of Thrombogenicity

A second set of experiments was performed to check whether depletion of platelets from the boundary layer plays a role in the axial dependence phenomenon. Axial platelet-collagen interactions on surfaces known to trigger rapid thrombus growth (collagenous), 10 and on surfaces known to be mild inducers of thrombus growth (subendothelial), 11 were compared by means of standard morphometry after perfusion for 5 minutes at a shear rate of 650 s⁻¹.

Pronounced axial decrease in surface coverage with platelets was measured on alpha-chymotrypsin–treated
rabbit artery subendothelium and equine collagen (Figure 10A). Virtually no axial decrease was observed on artery subendothelium from humans and rabbits. An axial decrease in surface coverage with thrombi was measured on the collagenous surfaces, while only a few thrombi were occasionally encountered on the subendothelial surfaces (Figure 10B).

**Effect of Anti-TSP**

Rabbit antiserum raised against human platelet TSP was added to some blood samples to see whether the antiserum could affect the axially increased thrombus dimensions at a shear rate of 2600 s⁻¹. Recent findings have shown that antibody to TSP inhibits platelet aggregation in plasma induced by a variety of agonists. Platelet-collagen interactions were quantified with standard morphometry and computer-assisted morphometry after 5-minute perfusions at a shear rate of 2600 s⁻¹.

Anti-TSP had no effect on the axial surface coverage with platelets (Figure 11A) or on the thrombus density (Figure 11B). However, the antiserum neutralized the axial increase in thrombus area and height, resulting in no differences in axial thrombus dimensions (Figures 11C and 11D, respectively). Addition of normal rabbit IgG to control blood samples had no effect on the axial platelet-collagen interactions (results not shown).

**Discussion**

This is the first study to focus on platelet-surface interactions at well-defined axial positions in relation to the direction of the blood flow. Particular emphasis is placed on the characterization of these interactions and on the factors that affect them. We demonstrate that platelet thrombus growth occurs most rapidly on the upstream portion of the thrombogenic surface, and that this growth impairs platelet adhesion and thrombus growth further downstream. We have termed this axial behavior “axial dependence of platelet-surface interactions.”

Thrombus growth appears to be a prerequisite for the axial dependence. This was demonstrated by the fact that the two most thrombogenic surfaces used, collagen-coated coverslips and alpha-chymotrypsin-treated rabbit aorta (a surface consisting of native-type collagen fibrils and elastin), showed pronounced axial decreases in surface coverage with platelets and in thrombus dimensions. Larger mural thrombi prevailed on these surfaces, and only a few single adherent platelets were occasionally
encountered. In contrast to this, the two subendothelial surfaces, human umbilical artery and rabbit aorta, triggered only a few small thrombi, and axial decrements in platelet-surface interactions were virtually absent.

Detailed characterization of the axial dependence phenomenon was performed after 2-, 5-, and 10-minute perfusions at a shear rate of 650 s⁻¹. The number of deposited platelets at 5 minutes yielded average decreases of 60% and 85%, by using reconstituted blood with ⁶⁷Cr-radioabeled platelets and citrated blood, respectively. The lower value observed with ⁶⁷Cr-platelets may have been caused by the in vitro processing, which impairs platelet reactivity, resulting in decreased platelet adhesion and thrombus dimensions (unpublished observations). Morphometric assessment revealed that the axial decrement was caused by a pronounced drop in surface coverage with platelets (48% at 5 minutes) and in thrombus dimensions, particularly in area (65%). The relatively smaller drop in thrombus height (33%) and corresponding decrease in the time-dependent growth rate may have been caused by the local shear stresses, which bend mural thrombi toward the surface, paralleling the direction of the blood flow (unpublished observations). However, the growth rate in area, upstream and downstream, was constant for at least 10 minutes, and exceeded by manyfold the growth rate previously reported on subendothelium. This enhanced thrombogenicity contrasts the reversible thrombus growth on subendothelium, which appears maximal at 5 minutes.

The apparent physical nature of the axial dependence phenomenon was substantiated in perfusion experiments at various shear rates and with aspirin-containing blood. The axial decrease in platelet-collagen interactions appeared most pronounced at shear rates that prevail in the largest (200 s⁻¹) and middle-sized (650 s⁻¹) arteries. The axial decrements leveled off gradually in concert with increasing shear rates. However, axial decrease was still present at a shear rate of 2600 s⁻¹ after shortening the perfusion time to 3 minutes. Perfusion times longer than 3 minutes appear to mask the axial dependence. Nonetheless, the observations support the theory of platelet depletion from the boundary layer of the blood flow, an effect that is gradually overcome by increasing shear rates that enhance the radial transport of platelets toward the boundary layer and the surface, and that may increase the translocation of platelet masses from upstream to downstream positions. Thus, at low shear rates, the consumption of platelets by growing thrombi exceeds the radial platelet transport toward the boundary layer, while at higher shear rates the consumption is gradually compensated by the net increased flux of platelets to the boundary layer. Reduction of platelet consumption from the boundary layer by partial inhibition of thrombus growth with aspirin, which abolished the axial dependence, further substantiates the proposed physical explanation of axial-dependent platelet-surface interactions.

Exposure of the collagen coating to WVF and TSP did not affect the axial decrement of platelet-collagen interactions. Collagen-bound WVF enhanced the surface cover-
AXIAL DEPENDENCE OF PLATELET DEPOSITION  
Sakariassen and Baumgartner

age with platelets (results not shown), as previously reported.12,16 The enhancement paralleled the amount of VWF used for preincubation, similar to that reported for subendothelium.10 Apparently TSP is not involved in platelet attachment to collagen, since neither pure TSP (results not shown) nor anti-TSP affected the surface coverage with platelets. These findings are consistent with recent data showing that TSP in the pericellular matrix of endothelial cells is not required for normal platelet adhesion.13 Anti-TSP, however, did neutralize the axial increase in thrombus dimensions as observed at a shear rate of 2600 s⁻¹. This surprising observation indicates that platelets deposited upstream may influence passing platelets to interact more efficiently further downstream. Alternatively, shear stress–induced platelet aggregation26 could also play a role at this high shear rate. Translocation of platelets and/or platelet aggregates from the upstream thrombus to downstream positions by the high shear forces is less likely to explain the experimental findings, because the presence of anti-TSP in the perfusate affected only thrombi located at downstream positions. The effect of the anti-TSP on the downstream thrombi is puzzling. The data do not help determine whether TSP stabilizes the large downstream thrombi and/or promotes platelet–platelet interaction directly, but they are consistent with previous observations, which reported inhibition of platelet aggregation by anti-TSP in the aggregometer device.15,16

Our data highlight the need for careful evaluation of platelet-surface interactions at well-defined axial positions before any firm conclusions are drawn, particularly in comparative studies. The balance between the platelet supply to the boundary layer and the consumption of platelets by the surface becomes extremely important when thrombogenic surfaces are used. In our previous studies with artery segments,17 and with equine-collagen-coated coverslips,23 only the midpiece of the segments (0.7 to 1.0 cm long) and the collagen coats were routinely inspected. It is noted, however, that substantially longer vessel segments are needed to observe significant changes in axial dependence. Our previous experiences with collagen coating were always conducted in the presence of aspirin, a condition that virtually abolishes the axial dependence. Morphometric evaluation at the upstream position (1 mm proximal to the flow inlet) is recommended for routine purposes, irrespective of the surface used.

Acknowledgments

We thank J. P. Girma, Hôpital de Bicêtre, Paris, for purified VWF; K. J. Clemetson, Theodor Kocher Institute, Berne, Switzerland, for purified TSP; and Paul Bornstein, University of Washington, Seattle, for rabbit anti-human-TSP serum. C. Michael is thanked for technical assistance.

References

1. Baumgartner HR, Muggli R. Adhesion and aggregation: morphological demonstration and quantitation in vivo and in vitro. In: Gordon JL, ed. Platelets in biology and pathology, vol 1. Amsterdam: North Holland, 1976:23–60

2. Baumgartner HR, Sakariassen KS. Factors controlling thrombus stability on arterial lesions. Ann NY Acad Sci 1985; 454:162–177

3. Baumgartner HR, Muggli R, Tschopp TB, Turitto VT. Platelet adhesion, release and aggregation in flowing blood: effect of surface properties and platelet function. Thromb Haemost 1976;35:124–130

4. Adams GA, Feuerstein IS. Platelet accumulation on collagen: drugs which inhibit arachidonic acid metabolism and affect intracellular cyclic AMP levels. Thromb Haemost 1984; 52:45–49

5. Turitto VT, Baumgartner HR. Platelet deposition on subendothelium exposed to flowing blood. Mathematical analysis of physical parameters. Trans Am Soc Artif Intern Organ 1975;21:593–600

6. Turitto VT, Weiss HJ, Baumgartner HR. Rheological factors influencing platelet interaction with vessel surfaces. J Rhac 1979;23:735–749

7. Tschopp TB. Aspirin inhibits platelet aggregation on, but not adhesion to collagen fibrons: an assessment of platelet adhesion and deposited platelet mass by morphometry and 51Cr-labeling. Thromb Res 1977;11:619–632

8. Weiss HJ, Tschopp TB, Baumgartner HR. Impaired interaction (adhesion-induced aggregation) of platelets with the subendothelium in storage pool disease and after aspirin ingestion—a comparison with von Willebrand’s disease. N Engl J Med 1976;295:819–824

9. Sakariassen KS, Benga JD, de Groot PhG, Sixma JJ. Comparison of platelet interaction with subendothelium of human and renal arteries and the extracellular matrix produced by human venous endothelial cells. Thromb Haemost 1984;57:60–65

10. Sakariassen KS, Bolhuis PA, Sixma JJ. Human blood platelet adhesion to artery subendothelium is mediated by factor VIII–von Willebrand factor bound to the subendothelium. Nature 1979;279:636–638

11. Mumper SM, Rauji GJ, Bornstein P. Interaction of thrombospondin with extracellular matrix proteins: selective binding to type I collagen. J Cell Biol 1984;98:645–652

12. Houdijk WPM, Sakariassen KS, Nieuwlaat PFEM, Sixma JJ. Role of factor VIII–von Willebrand factor and fibronectin in the interaction of platelets in flowing blood with monomeric and fibrillar human collagens types I and III. J Clin Invest 1985;75:531–540

13. Houdijk WPM, de Groot PG, Nieuwlaat PFEM, Sakariassen KS, Sixma JJ. Subendothelial collagens and platelet adhesion. Van Willebrand factor and fibronectin, not thrombospondin, are involved in platelet adhesion to extracellular matrix of human vascular endothelial cells. Arteriosclerosis 1986;6:24–33

14. Turitto VT, Weiss HJ, Baumgartner HR. Platelet interaction with rabbit subendothelium in von Willebrand’s disease: altered thrombus formation distinct from defective platelet adhesion. J Clin Invest 1984;74:1730–1741

15. Leung LLK. Role of thrombospondin in platelet aggregation. J Clin Invest 1984;74:1764–1772

16. Dixit VM, Haverstick DM, O’Rourke KM, et al. A monoclonal antibody against human thrombospondin inhibits platelet aggregation. Proc Natl Acad Sci USA 1985;82:3844–3848

17. Baumgartner HR. The role of blood flow in platelet adhesion, fibrin deposition, and formation of mural thrombi. Microvasc Res 1973;5:167–179

18. Baumgartner HR. Platelet interaction with collagen in flowing blood. I. Reaction of human platelets with alphachymotrypsin-digested subendothelium. Thromb Haemost 1977;21:13–18

19. Sakariassen KS, Fressinaud E, Girma J-P, Baumgartner HR, Meyer D. Mediation of platelet adhesion to fibrillar collagen in flowing blood by a proteolytic fragment of human von Willebrand factor. Blood 1980;66:1715–1719

20. Rauji GJ, Mumper SM, Abbot-Brown D, Bornstein P. Thrombospondin: synthesis and secretion by cells in culture. J Cell Biol 1982;95:351–354
ARTERIOSCLEROSIS  VOL 9, NO 1, JANUARY/FEBRUARY 1989

21. Sakarlassen KS, Muggli R, Baumgartner HR. Measurements of platelet interaction with components of the vessel wall in flowing blood. In: Hawiger JJ, ed. Platelets. Methods in enzymology vol. 169. New York: Academic Press 1988:37–70

22. Muggli R, Baumgartner HR, Techopp TB, Keller H. Automated microdensitometry and protein assay as a measure for platelet adhesion and aggregation on collagen-coated slides under controlled flow conditions. J Lab Clin Med 1980; 95:195–207

23. Sakarlassen KS, Aart PAMM, de Groot PG, Houdijk WPM, Stam JJ. A perfusion chamber developed to investigate platelet interaction in flowing blood with human vessel wall cells, their extracellular matrix, and purified components. J Lab Clin Med 1983;102:522–535

24. Baumgartner HR, Tourtto VT, Welas HJ. Effects of shear rate on platelet interaction with subendothelium in citrated and native blood. II. Relationships among platelet adhesion, thrombus dimensions, and fibrin formation. J Lab Clin Med 1980;95:208–211

25. Aart PAMM, van den Broek SATM, Prins GW, Kullken GDC, Stam JJ, Heethaar RM. Nonhomogeneous distribution of blood cells in flow, studied in vitro with a laser Doppler technique [Dissertation]. Dordrecht, Netherlands: ICG Printing, 1985:51–67

26. Belval TK, Helluma JD, Sollis RT. The kinetics of platelet aggregation induced by fluid-shearing stress. Microvasc Res 1984;28:279–288

27. Sakarlassen KS. The role of factor VIII–von Willebrand factor in platelet adherence to human artery subendothelium [Dissertation]. Dordrecht, Netherlands: ICG Printing, 1984. 210 pp

Index Terms: axial dependence • shear rate • thrombus growth • platelet adhesion • collagen • perfusion chamber