Axial Dependence of Platelet-Collagen Interactions in Flowing Blood
Upstream Thrombus Growth Impairs Downstream Platelet Adhesion

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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 (<650 s\(^{-1}\)). After 5 minutes of perfusion at a shear rate of 650 s\(^{-1}\), 4.6 \times 10^6 platelets were deposited on the most upstream 20 mm\(^2\) of the collagen surface, in contrast to 2.2 \times 10^5 platelets/20 mm\(^2\) 14 mm farther downstream. Depletion 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.


Platelet deposition on artery subendothelium in vivo and in vitro is a dynamic event.\(^{1,2}\) 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.\(^{3,4}\) 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.\(^5,6\) 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 fibrils\(^7\) and subendothelial surfaces,\(^3,8,9\) 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 considerations\(^5,6\) 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 noncollagenous 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 (\approx 5 \mu g/ml and \approx 20 ng/ml, respectively), and they bind to subendothelial components.\(^10,11\) VWF bound to artery subendothelium and to various collagens mediates adhesion of platelets.\(^10,12\) In contrast, TSP appears not to be required for normal platelet adhesion, at least not to the pericellular matrix of

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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.

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, alpha-chymotrypsin-treated rabbit artery subendothelium consisting of islands of collagen fibrils embedded in elastin, and plastic coverslips coated with collagen fibrils 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, 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. Girma (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. Clemenson, (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 Bomstein (University of Washington, Seattle, Washington). The antiserum did not cross-react with collagen types I, III, IV, and V or with VWF, laminin, or fibrinogen. Characterization of this antiserum was previously reported.

Lyophilized normal rabbit IgG was purchased from Sigma Chemical Company (St. Louis, MO). Plastic coverslips (22×60 mm, Thermanox) were purchased from Miles Laboratories (Elkhart, IN) and fibrillar equine collagen (Collagen Reagent Horm, 1 mg/ml) from Hormon Chemie (Munich, West Germany). Aspirin and ⁵¹Cr (4 μCi/ml 0.9% NaCl) were obtained from Bayer (Leverkusen, 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. 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.

Aspirin 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 few 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 Sakariassen et al., with some minor modifications. Briefly, platelets in platelet-rich plasma prepared from centrifuged blood 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 7.4), 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 ⁵¹CrCl₂/ml. After labeling at 22°C for 20 minutes, the platelets were washed free of exogenous ⁵¹CrCl₂ 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 centrifugations (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. 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. 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. 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 Na₂HPO₄, 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) x 10 (width) x 0.6 (height) mm and 226 (length) x 10 (width) x 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 tubings 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 76.0 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) x 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, Thörigen, 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)²⁴ and as thrombus height/min (μm/min).²⁵

**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 area 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.** 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 sliced proximal (prox.) and distal (dist.) collagen-coated coverslips. Axial number of deposited platelets in citrated blood was calculated from the average thrombus area per micron section length at axial positions of 1, 8, and 14 mm on the proximal and distal collagen coats, as previously reported.²¹ 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.** 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, 48% 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-
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).

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.² 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-surface interactions on surfaces known to trigger rapid thrombus growth (collagenous),¹⁸ and on surfaces known to be mild inducers of thrombus growth (subendothelial),⁶,¹⁸ 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

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*The text continues with similar content, discussing various experimental results and conclusions.*
AXIAL DEPENDENCE OF PLATELET DEPOSITION

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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 aggre-
gation in plasma induced by a variety of agonists. 15, 16
Platelet-collagen interactions were quantified with stan-
dard 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

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 21 and alpha-chymotrypsin-treated rab-
tor aorta (a surface consisting of native-type collagen
fibrils and elastin), 18 showed pronounced axial decreases
in surface coverage with platelets and in thrombus dimen-
sions. Larger mural thrombi prevailed on these surfaces,
and only a few single adherent platelets were occasionally

Figure 8. 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 assess-
ment of thrombus density (A), thrombus area (B), and thrombus
height (C) at downstream positions of 2 mm on the proximal
collagen coat (•) and of 14 mm on the distal collagen coat (O).
Values are mean±SEM, n=4 to 9. Significance was determined
relative to the respective determinations at 2 mm (*p<0.05,
**p<0.01, ***p<0.001).

Figure 9. Effect of aspirin on the axial percent surface coverage
with platelets (A), thrombus density (B), thrombus area (C), and
thrombus height (D) at downstream positions of 2, 8, and 14 mm
on the proximal collagen coat (•). Control blood samples (O). A
shear rate of 650 s⁻¹ was maintained for 5 minutes. Values are
mean±SEM, n=5. Significance was determined relative to the
respective control determinations (*p<0.05, **p<0.01).

(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
to control blood samples had no effect on the axial platelet-
collagen interactions (results not shown).
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 ⁵¹Cr-radiolabeled platelets and citrated blood, respectively. The lower value observed with ⁵¹Cr-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, remained constant for at least 10 minutes, and exceeded by manyfold the growth rate previously reported on subendothelium.² This enhanced thrombogenicity contrasts with the reversible thrombus growth on subendothelium, which appears maximal at 5 minutes.¹

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.³ 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 VWF and TSP did not affect the axial decrement of platelet-collagen interactions. Collagen-bound VWF enhanced the surface cover-
age with platelets (results not shown), as previously reported. The enhancement paralleled the amount of VWF used for preincubation, similar to that reported for subendothelium. 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. 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 aggregation could also play a role at this high shear rate. Translocation of platelets and/or platelet aggregates from the upstream thrombi 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.  

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 and/or platelet aggregates from the upstream thrombi 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.

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