Dynamic Monitoring of Platelet Deposition on Severely Damaged Vessel Wall in Flowing Blood
Effects of Different Stenoses on Thrombus Growth

Rititta Lassila, Juan Jose Badimon, Shankar Vallabhajosula, and Una Badimon

The formation of an arterial thrombus is a dynamic process that depends upon the characteristics of blood flow, the triggering substrate, and the blood components. We have developed and characterized a sensitive and specific computer-assisted nuclear scintigraphic method to study the dynamics of platelet deposition on severely damaged vessels both in vitro and in vivo in nonstenotic and stenotic flow conditions. Heparinized pig blood with Indium-111-labeled platelets was perfused for 50 minutes. Method variability in both static and flowing conditions was evaluated by Indium-111-labeled transferrin and Indium-111-labeled platelets. Positive scintigrams were obtained mainly in the presence of severe high grade stenoses on a thrombogenic substrate. Since the method is highly sensitive, computer-assisted axial dependence analysis was performed on the scintigraphic images to locate the thrombotic accumulation with respect to the area of the stenosis and to monitor the dynamic changes in platelet accumulation over time. Both in vitro and in vivo the highest level of platelet deposition occurred at the apex of the 80% stenosis, where embolization could be usually detected after 30 minutes of perfusion. This study is the first to assess the dynamics of thrombus growth in nonparallel flow streamlines such as are encountered in stenotic vessels. This method provides a new experimental tool with which to study factors affecting thrombus formation and stability.


A utopsy studies have demonstrated that acute myocardial infarction and sudden death are often associated with acute occlusive thrombosis.1,2 These studies have also commonly found platelet/fibrin microemboli distal to the thrombus in the microcirculation.3 Acute unstable angina is linked to underlying eccentric arterial lesions, which are suggestive of ruptured atherosclerotic plaque, thrombus, or both.4,5,6 These clinical findings suggest that thrombus formation in vivo involves a highly dynamic interaction between vessel wall and blood components. The flow characteristics, the severity of vessel wall damage, and the cellular and plasma environment regulate thrombus formation. Our group has previously observed that platelet accumulation on a severely damaged vessel wall is a dynamic process and that local flow conditions at the stenosis affect platelet deposition.7 However, the continuous detection of platelet accumulation over time is required to document the relation of local flow dynamics to cell wall interaction and kinetics of thrombus growth.

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This study was supported in part by NIH Grant HL-39840 and the Heart Research Foundation. Dr. Rititta Lassila was partly supported by fellowships from the Paulo Foundation and the Meilahti Foundation. Dr. Juan Jose Badimon is the Annenberg Scholar in Cardiology.

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Received March 6, 1989; revision accepted November 15, 1989.

The aim of the study was to develop a sensitive nuclear scintigraphic technique for continuous monitoring of platelet accumulation on severely damaged arterial walls. The effects of local nonstenotic laminar and stenotic flow conditions on thrombus formation have been assessed. The use of scintigraphic imaging of radiolabeled platelets has been an excellent tool for the detection of platelet deposition on different surfaces, biological or prosthetic.8-12 To improve these previously reported techniques, a more detailed spatial resolution was required to study narrowed vessels with small internal diameters. We found that the present method detects dynamic changes in thrombus growth, which are strongly influenced by the presence of stenosis. This method will be applicable for further studies of the pathophysiological aspects of arterial thrombosis.

Methods

Experimental Model

Blood (43 cc in 7 cc of acid-citrate-dextrose [ACD] anticoagulant) was drawn from Yorkshire albino pigs a day before the experiments. The platelets were isolated and labeled with 111In-tropolone and were re-injected into the animals as we have previously described.13 The labeling procedure required approximately 2 hours. The average efficiency of the platelet labeling in plasma was 58.7%±4.9% (±SEM) for in vitro perfusions and 51.8%±3.0% for ex vivo perfusions. The activity injected was 1.61±0.10 and 1.34±0.08 mCi, respectively. The radiolabeled platelets (1666×10^3±228×10^3/μl and

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Figure 1. Diagram of the perfusion chamber. A. Bottom part. B. Mounted chamber. C. View from above. D. Longitudinal cross-section of the substrate mounted in the perfusion chamber. The substrate was divided into segments to determine the axial dependence of platelet deposition or the distribution of platelets along the flow axis. Segment 4 is the location of the stenosis.

1653 x 10^3 ± 132 x 10^3/μl, respectively) were injected in a volume of 4.5 to 5 ml of plasma. Twenty-four hours later, the pigs were sedated with an intramuscular injection of ketamine-HCl-xylazine (Ketalar-Rompun); they were then intubated and connected to a respirator (Harvard respirator; Harvard Apparatus Co., Dover, MA). Anesthesia was maintained by sodium pentobarbital (i.v.) at a minimal effective dose. A saline dextrose (5%) solution (Travenol, Deerfield, IL) was administered via the marginal ear vein. Through left and right incisions, the carotid artery and jugular vein were isolated and catheterized. Blood was collected for determination of baseline hematocrit, platelet number, and prothrombin time. The animals were intravenously heparinized (120 U/kg bolus plus continuous infusion of 100 U/kg/h) to plasma heparin levels of 1.5 ± 0.1 U/ml (Coatest HP, Kabivitrum, Sweden). All procedures performed in this study were approved by the appropriate institutional guidelines and followed the American Heart Association Guidelines for animal research.

Perfusion Chamber

We used a tubular well-characterized perfusion chamber (Badimon 84), where a portion of the blood channel is replaced with a vascular surface, exposing the perfusion substrate directly to blood flow (Figures 1A and 1B). 13 14 15 The chamber has been recently modified to simulate the eccentric stenoses found in the coronary arteries. 7 In the top part of the chamber, protruders of different dimensions can be introduced to produce stenotic narrowing in the flow channel. The extent and length of the induced stenosis was determined under a dissection microscope by measuring the residual lumen of the channel with the mounted substrate in its place. Two grades of stenosis, 55% and 80%, were compared with the nonobstructed laminar flow. As a model of a severely damaged vessel wall, we exposed tunica media to the flowing blood in the perfusion studies. The tunica media was prepared from aortas harvested from normal pigs. After removing the surrounding connective tissue, the aortas were immediately frozen in liquid N₂ and stored at −70°C. At the time of the experiment, the aortas were thawed in Vassar saline buffer and opened longitudinally. Starting from one corner, intima was stripped off the proximal tubing and distal tubing.
vessel, and the deeper layer was used as the substrate by preparing segments (30 mm × 10 mm) for placement in the chamber as previously described.7

**Nuclear Scintigraphic Studies**

A gamma camera (Picker ZC) with a medium energy parallel hole collimator set on the 173 and 247 keV photo-peaks of Indium-111 was used. A matrix of 256×256 pixels and zoom factor 4 were selected to achieve the best spatial resolution. With this matrix size, 10 pixels corresponded to a vessel, and the deeper layer was used as the substrate by preparing segments (30 mm × 10 mm) for placement in the chamber as previously described.7

**Baseline Nuclear Scintigraphy Studies**

The method variability was assessed by using a soluble marker, 111In-transferrin, and a cellular marker, 111In-platelets, in static and flowing conditions with 0% and 80% stenosis. These baseline nuclear scintigraphic experiments enabled standardization of the variability in our system at different levels of 111In-activity, in both static and different flowing conditions in the absence of cell deposition. Static and flow conditions were analyzed to assess whether flow with new labeled cells into the acquisition area affected detection.

**Transferrin**

Ten milliliters of pig blood was taken into heparinized test tubes. Plasma transferrin was labeled with 111In-tropolone and added to 60 ml of autologous heparinized pig blood. Images were acquired under flow conditions (10 ml/min) for 30 minutes and in static conditions for 10 minutes. Two levels of activity, 25 µCi (low) and 140 µCi (high), were tested to study the range of activity achieved in the perfusion studies. Images were taken under three conditions: 1) blood was circulated through a cannula similar in size to the blood channel in the perfusion chamber, 2) blood was circulated through the chamber with a substrate (Gore-Tex) of low thrombogenicity in laminar conditions,16-18 and 3) blood was circulated through chambers including 55% and 80% stenosis. Total refers to all segments from 2 to 6 together. (See the Methods section for a fuller explanation.)

**Platelets**

Similar experiments with a low or high dose of radioactivity (25 µCi and 140 µCi) were performed with recirculating 111In (tropolone)-labeled autologous platelets added to 60 ml of blood. Perfusions were run as before: 1) without the substrate, 2) with the chamber perfusing Gore-Tex without stenosis, and 3) with the 80% stenosis. Static acquisitions were also obtained as per 1) and 2).

**Perfusion Studies**

Two kinds of perfusion studies were used to monitor platelet deposition on a thrombogenic substrate (pig aortic tunica media) in flowing blood over time (Figure 2).

**In Vitro Studies**

Sixty millimeters of pig arterial blood was drawn into a polypropylene tube. (See Figure 2A.) Blood was recirculated at 37°C (a water-bath) through a chamber with laminar flow conditions and through chambers including 55% and 80% stenosis in the flow channel. The flow rate (10 ml/min) was regulated by a peristaltic pump (Masterflex Model 7013, Cole Palmer, Chicago, IL). At the beginning of the experiments, the substrate, pig aortic tunica media, was equilibrated with Vassar saline (37°C) for 60 seconds, and thereafter blood flow and the acquisition were begun immediately. The laminar flow chamber had a theoretically calculated local wall shear rate of 212 s⁻¹, typical of an unobstructed medium-sized artery.14 A proximal and a distal part of the connecting tubings and the chamber were fixed under the gamma camera to a position that was defined with radioactive markers. The orientation to the direction of flow was checked in each experiment with a radioactive marker. Platelet deposition was continuously monitored up to 50 minutes unless there was an earlier acute occlusion.

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**Table 1. Baseline Nuclear Scintigraphy Studies: Acquisition with 111Indium-Transferrin at Two Levels of Activity**

<table>
<thead>
<tr>
<th>Perfusion conditions</th>
<th>25 µCi/30 ml of blood</th>
<th>140 µCi/30 ml of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (cpm)</td>
<td>SD (cpm)</td>
</tr>
<tr>
<td>a</td>
<td>4.5</td>
<td>1.7</td>
</tr>
<tr>
<td>b</td>
<td>5.0</td>
<td>2.1</td>
</tr>
<tr>
<td>c</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>5.8</td>
</tr>
<tr>
<td>Static conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>3.2</td>
<td>0.9</td>
</tr>
<tr>
<td>b</td>
<td>3.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>5.7</td>
</tr>
</tbody>
</table>

The acquisition included 15 frames (30 minutes) in the experiments where blood was recirculated through the chamber and five frames (10 minutes) in static experiments. Counts (cpm) represent the region of interest of 5x5 pixels (area of 5×5 mm) after the activity of the scintigraphy background was subtracted (mean, 1.5±SD 1.5). SD=standard deviation, CV=coefficient of variation.

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**Figure 2**

Two kinds of perfusion studies were used to monitor platelet deposition on a thrombogenic substrate (pig aortic tunica media) in flowing blood over time (Figure 2).
Afterward the substrate was divided into seven segments (1D). Segments 2, 3, 5, and 6 were 4 mm wide, and segments 1 and 7, 1.5 mm in length (entrance and exit regions of the chamber), were discarded (Figures 1C and 1D). With the matrix size of $256 \times 256$, 10 pixels corresponded to 10 mm. To obtain the underlying deposition (blood pool activity) of platelets in the region of interest, the radioactivity of the arriving blood in an equal-sized pixel area was subtracted from each region of interest. The activity per ROI thus achieved was called corrected counts per ROI ($cROI$). At the same time, the background activity was corrected. In the baseline studies, ROI were chosen to be 5 mm in length, and $cROI$ were achieved by subtracting the camera background. Before and after perfusion platelet number, hematocrit, heparin concentration, prothrombin time, and plasma $^{111}$In activity were monitored. Blood before and after perfusion was collected and was analyzed to measure the radioactivity. The amount of platelet deposition could thus be normalized by the known platelet number.\textsuperscript{17}

### Analysis of Data

At the end of the perfusion time, as previously reported,\textsuperscript{9} the substrate was unmounted and counted by gamma-well counting (TPD=total platelet deposition). Afterward the substrate was divided into seven segments, and each was counted to evaluate the axial dependence of the platelet deposition along the flow axis in the chamber (ADPD=axial dependence platelet deposition). Sections 1 and 7, 1.5 mm in length (entrance and exit regions of the chamber), were discarded (Figures 1C and 1D). Segments 2, 3, 5, and 6 were 4 mm wide, and segment 4 was 6 mm wide. Segment 4 encompassed the whole stenotic area and was large enough to avoid breaking a possible apical thrombus.

As before, the continuous imaging data were analyzed in two ways with computer assistance per each 2-minute frame: 1) evaluating the whole exposed substrate (TPD) and 2) outlining the corresponding regions of interest (ROI) according to the division of the substrate ex vivo and thus including areas with different local shear and flow conditions (ADPD) (Figures 1C and 1D). With the matrix size of $256 \times 256$, 10 pixels corresponded to 10 mm. To obtain the underlying deposition (blood pool activity) of platelets in the region of interest, the radioactivity of the arriving blood in an equal-sized pixel area was subtracted from each region of interest. The activity per ROI thus achieved was called corrected counts per ROI ($cROI$). At the same time, the background activity was corrected. In the baseline studies, ROI were chosen to be 5 mm in length, and $cROI$ were achieved by subtracting the camera background. Before and after perfusion platelet number, hematocrit, heparin concentration, prothrombin time, and plasma $^{111}$In activity were monitored. Blood before and after perfusion was collected and was analyzed to measure the radioactivity. The amount of platelet deposition could thus be normalized by the known platelet number.\textsuperscript{17}

### Data Management and Statistics

The results are expressed as means±SEM, unless otherwise stated. A dynamic picture of the cumulative imaging

### Table 2. Acquisition with $^{111}$Indium-Platelets at Two Levels of Activity

<table>
<thead>
<tr>
<th>Perfusion conditions</th>
<th>25 $\mu$Ci/30 ml of blood</th>
<th>140 $\mu$Ci/30 ml of blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (cpm)</td>
<td>SD (cpm)</td>
</tr>
<tr>
<td>a</td>
<td>4.0</td>
<td>1.8</td>
</tr>
<tr>
<td>b</td>
<td>4.4</td>
<td>1.9</td>
</tr>
<tr>
<td>c</td>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>4.5</td>
</tr>
</tbody>
</table>

### Table 3. Mean Hematocrit, Platelet Numbers, Prothrombin Time, and Heparin Units in Laminar and Stenotic Conditions

<table>
<thead>
<tr>
<th>Stenosis</th>
<th>N</th>
<th>Hematocrit (%)</th>
<th>Platelet number ($\times 10^9$ pt/ml)</th>
<th>Prothrombin time (sec)</th>
<th>Prothrombin time ratio</th>
<th>Heparin units (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% vitro</td>
<td>7</td>
<td>27.7±2.5</td>
<td>291±41</td>
<td>26.2±2.6</td>
<td>1.5±0.1</td>
<td>1.26±0.17</td>
</tr>
<tr>
<td>55% vitro</td>
<td>3</td>
<td>28.3±0.9</td>
<td>307±78</td>
<td>28.0±6.0</td>
<td>1.6±0.3</td>
<td>–</td>
</tr>
<tr>
<td>80% vitro</td>
<td>8</td>
<td>25.2±0.9</td>
<td>320±55</td>
<td>23.8±1.8</td>
<td>1.4±0.1</td>
<td>1.28±0.10</td>
</tr>
<tr>
<td>0% vivo</td>
<td>8</td>
<td>27.7±1.0</td>
<td>399±53</td>
<td>23.8±1.6</td>
<td>1.5±0.1</td>
<td>1.67±0.22</td>
</tr>
<tr>
<td>80% vivo</td>
<td>10</td>
<td>27.8±1.5</td>
<td>414±58</td>
<td>24.4±2.2</td>
<td>1.5±0.1</td>
<td>1.78±0.14</td>
</tr>
</tbody>
</table>

All values are the means±1 SEM.
data (platelet accumulation) was drawn as a function of the perfusion time. The effects of various degrees of stenosis (0%, 55%, 80%) were analyzed in each experiment by comparing platelet accumulation per frame (2 min): 1) in the whole exposed substrate (TPD) and 2) in the five segments of the substrate (ADPD). The analysis was done in each case by data obtained both from gamma-well counting and nuclear scintigraphy. The statistics were analyzed by Student's t test for paired and unpaired observations. The group means were compared by one or multiple factor analysis of variance with repeated measurement analysis. The correlation coefficients for linear regressions were analyzed, and the slopes were compared by t test. In each experiment, analysis of platelet deposition in the different cROI with respect to segment 4 (location of the stenosis) was performed. The significance of a change in radioactivity between subsequent frames within 95% confidence limits could be determined based on the standard deviation of the same level of radioactivity in the background experiments.

**Results**

**Baseline Nuclear Scintigraphy Studies**

The acquisition values of $^{111}$In-transferrin and $^{111}$In-platelets did not significantly differ (Tables 1 and 2). Corresponding levels of radioactivity led to the same levels of covariation. As expected, the highest counts showed the least variability. The presence of stenosis did not affect the results. The results under static and flow conditions did not differ.

**Perfusion Studies**

**In Vitro Perfusions**

The average hematocrit, platelet numbers, prothrombin time, and units of heparin are presented in Table 3. They did not differ significantly in the various perfusion conditions except in the studies with 80% stenosis, where there was a significant reduction in the platelet number after perfusion ($p<0.001$), probably due to the consumption of the platelets on the activating substrate.

The average linear regression lines of platelets deposited on the severely damaged wall versus perfusion time illustrate the kinetics of thrombus growth on the perfused...
area at laminar and stenotic (55% and 80%) flow conditions (Figure 3A). The mean slope of the linear regression was significantly increased in 80% stenosis, 2.83±0.72, over that in 0% stenosis, 0.74±0.31 (p<0.01). In 55% stenosis, the slope was 0.92±0.1% and did not reach statistical significance when compared with 0% stenosis. The peak of the stenosis (segment 4) showed the greatest platelet recruitment (Figure 3B). The corresponding slopes were 1.98±0.32 in 80%, 0.31±0.13 in 0%, and 0.31±0.16 in 55% stenosis (p<0.005 80% versus 0% and versus 55%). Analysis by repeated measurement analysis of variance (ANOVA) indicated that platelet deposition at the peak of the stenosis significantly changed at different flow conditions (0% and 55% versus 80% stenosis; factor A=stenosis, factor B=perfusion time; p<0.05).

The axial dependence analysis of the imaging results is presented in Figure 4, where representative examples are given for 0% and 80% stenosis. The peak of the stenosis showed most of the activity, which increased significantly with time (see segment 4). After 40 minutes of perfusion, activity dropped significantly (p<0.05) in comparison with the other frames nearby, suggestive of embolization. The post-stenotic areas had a tendency to accumulate more activity than the pre-stenotic ones, mainly when there was total occlusion.

The comparison of nuclear scintigraphic results with gamma-well counter results gave a highly significant correlation coefficient (r=0.98, p<0.01) (Figure 6A). As we have previously reported, at shorter perfusion times and by end-point analysis,7 platelets accumulated on the severely damaged vessel wall as a function of the increasing stenosis. We have found that the 80% stenosis led to the highest level of platelet deposition (p<0.01) (Figures 5B and 5C).

After 50 minutes of perfusion, the difference in platelet deposition was not statistically significant between laminar perfusions and 55% stenosis. None of the laminar perfusions or the perfusions with 55% stenosis occluded during the 50 minutes of perfusion. However, three of eight of the perfusions with 80% stenosis occluded. Thus, the perfusions with 80% stenosis were separately analyzed when occlusion occurred (Figure 5).

The axial distributions of platelet deposition along the blood flow axis as measured by gamma-well counting are shown in Figure 6. In agreement with our previous report at perfusion times up to 30 minutes,7 both the percentage distribution and the actual values of platelet deposition decreased distally along the flow axis in nonstenotic flow conditions (Figures 6A and 6E, p<0.01, one-factor ANOVA). The peak of the stenosis (segment 4) accumulated a significantly higher number of platelets than the pre-stenotic and post-stenotic segments (p<0.0001) (Figures 4 and 6). In those experiments with 80% stenosis where occlusion occurred, segment 4 had the largest platelet deposition (p<0.01), but significant platelet deposition occurred in post-stenotic segments as opposed to pre-stenotic ones (p<0.05).
**In Vivo Perfusions**

The average hematocrit, platelet numbers, prothrombin time, and heparin units did not differ significantly between 0% and 80% stenosis (Table 3).

As in the in vitro studies, the axial dependence patterns analyzed by nuclear scintigraphy show that nonstenotic flow conditions distributed platelets evenly on the substrate (Figure 7A), while in 80% stenosis the apex of the stenosis attracted most of the activity (Figure 7B). The average slope of thrombus growth at the segment 4 was significantly greater in 80% than in 0% stenosis, 2.13±0.27 versus 0.62±0.18, respectively, (p<0.005).

Platelet deposition on the substrate in the presence of 80% stenosis was over fivefold higher in comparison with laminar flow (TPD) (p<0.01) (Figure 8A). One of the 10 perfusions with 80% stenosis occluded at 40 minutes, while none of the laminar ones did. The axial dependence analysis (ADPD) by gamma-well counting showed that, in non-
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Figure 7. Axial dependence of the in vivo imaging results. Representative examples of nonstenotic conditions (A) and 80% stenosis without occlusion (B). *Frame 14 versus 16, 16 versus 18, and 23 versus 25, p<0.05. Symbols: + = segment 2, = segment 3, ○ = segment 4, ★ = segment 5, = segment 6. cROI = corrected regions of interest.

Discussion

This is the first quantitative nuclear scintigraphic assessment of the dynamics of thrombus growth on severely damaged arterial wall in flowing blood. This method dynamically monitors the interaction between the vessel wall and platelets in thrombus formation, growth, and stabilization. The nonparallel streamline conditions studied in these experiments mimic the circulation in atherosclerotic vessels more closely than studies performed in laminar flow with parallel streamlines.10,11,12

The standardization of the method and the basis for the statistical analysis were established by comparing Indium-111-labeled soluble transferrin with Indium-111-labeled cellular particles (platelets). The high sensitivity of the method permits analysis of the axial dependence of platelet deposition, as we had previously described with end-point analysis by gammawell counting.7 We have been able to measure thrombus growth changes in areas as small as 2 mm by 4 mm (2 pixels by 4 pixels, 256x256 matrix), which is a significant improvement in spatial resolution when compared with earlier techniques.6-12 This is especially pertinent in studies mimicking narrowed atherosclerotic arteries with small internal diameters. The triggering factors of thrombus growth leading to a significant level of detection are a thrombogenic wall and a high grade stenosis, which induce high shear conditions.20 The specificity of this method is confirmed by the comparable results achieved by gammawell counting (see Figure 5).

The Indium-111-activity of the arriving blood pool is subtracted per each frame; thus, the technique detects the actual cumulative platelet deposition. The variability of the acquisition itself becomes critical when the significance of the changes in thrombus growth is assessed. Our baseline nuclear scintigraphy studies show that with an Indium-111-activity higher than 50 cpm/ROI, the covariation is less than 20%. In the experiments with 80% stenosis, the initial thrombus growth always exceeded the level of 50 cpm in segment 4. Thus, the significance of the individual changes in platelet accumulation at different perfusion times can be validly analyzed by comparing frames. As a result, the technique enables analysis of the rate of thrombus growth as well as embolization.

The maximum platelet deposition concentrates on the peak of the stenosis. This area with high local shear forces enhances the conditions favoring platelet activation.7 The same results were achieved in vitro and in vivo. However, the level of platelet deposition under in vivo conditions exceeded that of in vitro in stenotic vessels. The reduced platelet reactivity in vitro could be due to platelet handling and recirculation as well as to absence of the influence by short-lived platelet-activating metabolites.21 As to axial dependence patterns under in vitro conditions (Figures 6A and 6E), the present results are in accordance with earlier in vitro and in vivo findings with perfusion times up to 30 minutes7 and with the in vitro results by Sakariassen and Baumgartner22 on collagen-coated surfaces and a short perfusion time (5 minutes). However, in in vivo perfusions there was no

stenotic flow conditions, the distribution was relatively even between the segments. However, the perfusions with 80% stenosis acquired the greatest number of platelets on the site of maximal stenosis. The area of flow recirculation, post-stenotic segments 5 and 6, also accumulated a significant number of platelets (Figures 8B to 8E).

Comparative Results of In Vitro and In Vivo Perfusion Conditions

The kinetics of thrombus growth and embolization, depending on the stenotic flow conditions, were similar in the in vitro and in vivo results. Embolization on the apex of 80% stenosis was seen seven of eight times (88%) in vitro and eight of 10 (80%) times in vivo (NS); however, the final platelet deposition was almost twice as high in vivo as in vitro (p<0.001). Both in vitro and in vivo, thrombus growth concentrated at the apex of the stenosis (Figures 4 and 7).
significant axial dependence of platelet deposition (Figures 8B and 8D). It may be that long perfusion times (>30 minutes) on very thrombogenic surfaces may induce large platelet deposits that could easily embolize from proximal areas to distal positions.

The slopes of the regression lines of the average time course of platelet deposition on the whole vessel, and similarly in the area of maximal stenosis where the platelets mainly accumulate, indicate that the kinetics of thrombus growth are related to the degree of stenosis (Figure 3). However, these average regression lines fail to give a true picture of the significant changes during perfusion (Figures 4 and 7) and thus conceal embolization.

This study was performed in anticoagulated blood. We selected heparin as the anticoagulant because it is often administered in clinical thrombotic events. High doses of heparin affect platelet deposition. The dose of heparin used in this study (1.26 to 1.78 U/ml) is in the low range with less effect on platelet deposition.

Unstable angina, acute myocardial infarction, and sudden death have been associated with thrombosis, often on arterial wall severely damaged due to plaque rupture, and microemboli are often found distal to the thrombus. Spontaneous intermittent coronary recanalization and reocclusion are frequent during the early phase of acute myocardial infarction, suggesting the dynamic nature of the event. Continuous monitoring of the dynamics of platelet deposition may help reveal the effects of cell-cell and cell-wall interactions that modulate acute thrombosis, embolization, and rethrombosis.

Acknowledgments
The authors thank Valentin Fuster for his expert comments, continuous support, and collaboration throughout the study. The assistance of Efim Mogilevsky is also appreciated.
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References

Index Terms: thrombosis • stenosis • nuclear scintigraphy
Dynamic monitoring of platelet deposition on severely damaged vessel wall in flowing blood. Effects of different stenoses on thrombus growth.
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doi: 10.1161/01.ATV.10.2.306
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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