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## Effect of Wall Shear Rate on Thrombogenesis in Microvessels of the Rat Mesentery

Masaaki Sato and Norio Ohshima

**The role of hemodynamics on platelet thrombus formation was studied in venules and arterioles of the rat mesentery. Thrombus formation was induced by the fluorescent dye/light method for examination of the following factors: 1) the effect of wall shear rate on thrombus initiation, 2) the effect of wall shear rate on the growth of thrombi, and 3) the relation between platelet thrombus initiation and intraluminal velocity profile. The range of wall shear rate was up to approximately 1,000 1/sec in venules and from 640 to 2,900 1/sec in arterioles. Platelet thrombus initiation occurred more rapidly at higher wall shear rate in venules and at lower wall shear rate in arterioles. Thrombus initiation time was shortest around a wall shear rate of 900 1/sec in venules and around 700 1/sec in arterioles. Thrombus growth rate in venules was greatest at a wall shear rate of 1,500–2,000 1/sec. Thrombus initiation and its relation to blood flow was also examined in branched and curved microvessels. In these vessels platelet thrombi were also first initiated at the sites of higher wall shear rate in venules and of lower wall shear rate in arterioles. (*Circulation Research* 1990;66:941–949)**

**B**lood flow conditions have been shown to affect adhesion and aggregation of platelets to endothelium, both of which have been implicated in thrombus formation.<sup>1–5</sup> Baumgartner<sup>2</sup> has reported that platelet adhesion to subendothelium paralleled changes in blood flow velocity. Some other studies have indicated that growth rate of platelet thrombi and platelet adhesion were inhibited in the higher velocity range.<sup>1,3,4</sup> Although this phenomenon was also estimated theoretically by use of a physical model,<sup>6</sup> the details of interaction between blood flow and thrombi dynamics are still poorly understood. Furthermore, the effects of blood flow conditions on the thrombus formation seem to depend on many factors, such as the thrombus model used, the microvessel type (arteriole or venule), and the experimental conditions (in vivo or in vitro). Various techniques have been used for induction of endothelial cell injury and generation of platelet thrombi, including the application to microvessels of electrical,<sup>7</sup> mechanical,<sup>8</sup> biochemical,<sup>1</sup> or biolaser<sup>9,10</sup> stimuli.

We have reported that platelet thrombi are inducible in the microvasculature by the irradiation of filtered light in combination with the intravascular

administration of sodium fluorescein.<sup>11–13</sup> This phenomenon was first reported in cerebral microcirculation by Rosenblum and El-Sabban<sup>14</sup> in 1977. Their ultrastructural observations showed only minor endothelial vacuolation and no endothelial denudation by the light/dye injury.<sup>15</sup> We also confirmed the platelet aggregation without the endothelial denudation. However, in our cases (unpublished data), some high-density amorphous materials were present between the endothelial cells and platelets, as described by Herrmann and Voigt.<sup>16</sup> This thrombus formation model seems useful for elucidation of the mechanism of platelet thrombogenesis and its relation to microvessel blood flow. In a previous study, we used this thrombus model to examine the hemodynamics during growing of platelet thrombi and found that blood flow velocity at the stenosed portion by the thrombi dropped precipitously after reaching an almost constant level due to the progressive growth of the thrombus into the vessel lumen.<sup>13</sup> In this paper, the relation between thrombus formation and local hemodynamics are further examined. Rosenblum and El-Sabban<sup>17</sup> induced platelet aggregates in pial arterioles of mice using their methods and found the linear relation between platelet aggregation latency and wall shear rate. Similarly, we examined the effect of wall shear rate on the platelet thrombus initiation and growth at the different microvascular bed, including venules and other microvessels with complicated configuration such as arteriolar bifurcations and venular confluences. Because the platelet adhesion to the endothelium

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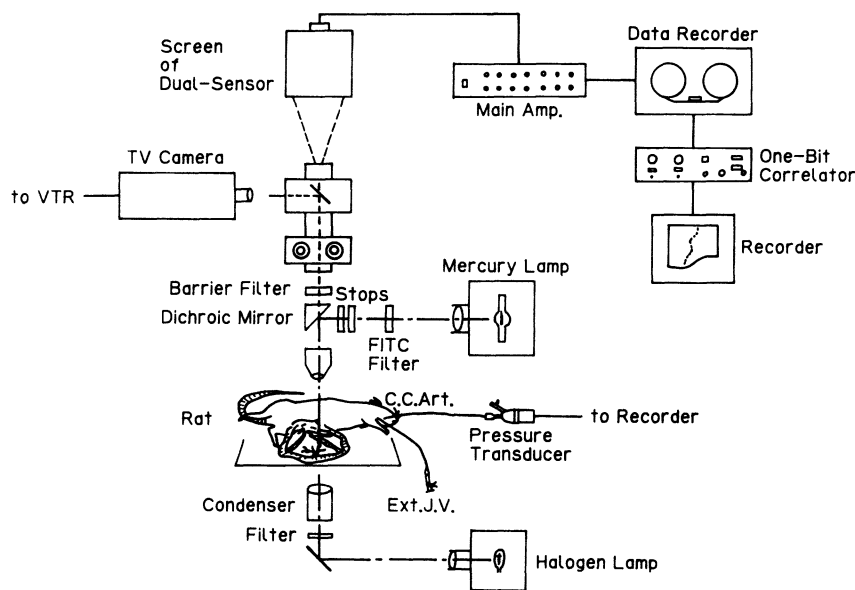


FIGURE 1. Schematic diagram of experimental apparatus. Screen of 10-channel dual-sensor is set above intravital microscope.

and/or the thrombi is directly related to the local platelet behavior around the vicinity of vessel wall, we will discuss the effects of wall shear rate of blood flow on the thrombus initiation and growth rather than the effects of the blood flow velocity.

### Materials and Methods

#### *Animal and Experimental Apparatus*

Ninety-nine Wistar rats weighing 200–300 g were anesthetized with pentobarbital in the femoral muscle (100 mg/kg). The intravital microscope television system used throughout the present experiments as well as techniques of animal experiments have been described in detail elsewhere.<sup>12,13,18</sup> A schematic diagram of the apparatus is shown in Figure 1. In short, a catheter for monitoring aortic pressure was placed in the common carotid artery, and the mesentery was placed into a saline bath maintained at a controlled temperature of 37° C. The mesentery around the ileum was spread out with the utmost care on a glass stage. The other parts of the intestines were covered with gauze. Microvessels in the mesentery were observed under transillumination from a halogen lamp. Arterioles of 20–50  $\mu\text{m}$  or venules of 30–90  $\mu\text{m}$  diameter were selected in which to produce microthrombi.

Platelet aggregation was induced in microvessels according to the method reported previously.<sup>12</sup> The filtered light, 400–500 nm in wavelength, was passed through an epi-illumination system from a mercury lamp and irradiated a selected microvessel through an objective lens (water immersion lens,  $\times 25$ , E. Leitz, Rockleigh, New Jersey). The area of irradiation was adjusted to about 100  $\mu\text{m}$  in diameter for straight microvessels on the focal plane by a field stop. Transillumination from a halogen lamp was subsequently discontinued for 1 minute and a solution of sodium fluorescein (2.5 wt/vol%) was injected through the external jugular vein (2 ml/kg body wt).

The irradiation by filtered light was continued throughout the observation. Simultaneously with the dye injection, the timer was started. The dynamic course of thrombus formation was continuously monitored by a TV camera and recorded on a videotape with the elapsed time. For measurement of the blood flow velocity and the radial velocity profile, a new 10-channel dual-sensor method was used.<sup>19</sup> The image of the observation field was projected onto a screen (20 cm in diameter) that was installed on the upper part of the microscope, as shown in Figure 1. Magnification of the projected image was in the range of  $\times 200$ – $\times 300$ . The tips of 10 pairs of glass fibers were embedded in the center of the screen in two lines 1.5-mm apart (5–7.5  $\mu\text{m}$  in microvessels). The other end of each fiber was connected to a silicon photosensor. The centerline axis of the microvessel image to be studied was adjusted to coincide with the direction of paired dual sensors. The changes in brightness of the sampling points detected by the sensors were recorded on a data recorder. After each experiment, the time-averaged (approximately 10-second) blood flow velocity was calculated by means of a cross-correlation technique previously described.<sup>20</sup> Using these methods, we performed the three protocols described below.

#### *Effect of Wall Shear Rate on Thrombus Initiation*

The concentration of applied sodium fluorescein was 50 mg/kg, and the irradiated light intensity was 20.7 mW/mm<sup>2</sup> for induction of platelet thrombus in a straight microvessel. As a measure of the thrombogenesis process, the time when the thrombus began to form (the initiation time,  $t_i$ ) was recorded. Separate experiments were performed for each microvessel because the injection of sodium fluorescein into the animal is essential in our method. Before induction of the platelet thrombus, the centerline blood flow velocity,  $v_c$ , was measured by use of the dual-

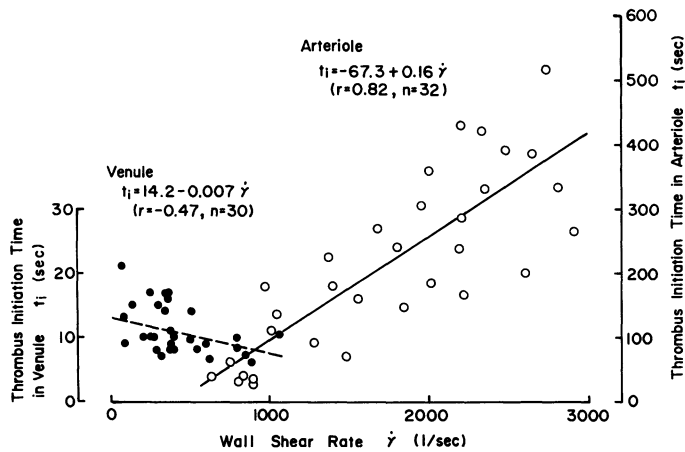


FIGURE 2. Effect of wall shear rate on thrombus initiation time in venules and arterioles.  $r$ , Correlation coefficient;  $n$ , number of data points (or rats).

sensor method in arterioles ( $n=32$ ) and venules ( $n=30$ ). Thus, the effect of wall shear rate,  $\dot{\gamma}$ , on the thrombus initiation time was examined. According to sophisticated experiments by Tangelder et al,<sup>21</sup> velocity profiles in vivo in arterioles both in systolic and diastolic phases are flattened as compared with a parabola. On the other hand, Baker and Wayland<sup>22</sup> have shown in vitro that the centerline velocity measured by the double-slit technique shows an 80% value of the actual centerline velocity. In this study, on the assumption of a parabolic profile of blood flow velocity as a first approximation, the time-averaged wall shear rate was estimated from the following equation using the centerline velocity,  $v_c$ , and the Baker-Wayland correction<sup>22</sup> as

$$\dot{\gamma} = 8v_c / 1.6D_i$$

where  $D_i$  is the internal vessel diameter.

#### Effect of Wall Shear Rate on Thrombus Growth Rate

In separate experiments, the effect of wall shear rate on the thrombus growth rate was examined in venules ( $n=22$ ).

One separate data point was also obtained from one separate animal as mentioned before. In these experiments, the light irradiation was temporarily stopped after the thrombus formation. During the light-off period the centerline blood flow velocity was measured at the narrowest portion (throat) of the lumen. The wall shear rate at the throat,  $\dot{\gamma}_t$ , was calculated using the narrowest internal diameter according to the correction written before. The shape of the lumen had the previously reported hourglass shape.<sup>12,13</sup> It was confirmed from preliminary experiments that the configuration and the volume of the forming thrombus in venules were unchanged during the first 20–30 minutes of the light-off period. The light-off period in this experiment depended on the measuring period of velocity, usually about 2 minutes. The light irradiation was then restarted, and the thrombus formation process was recorded on videotape. Two different levels of light intensity, 20.7 and 9.2 mW/mm<sup>2</sup>, were applied to the microvessels to change the growth rate of the thrombi. For analysis

of the detailed course of thrombus growth, the cross-sectional area of the thrombus at the throat and its ratio to that of the original lumen (percent area stenosis) were measured.

#### Platelet Thrombus Initiation and Velocity Profile

To examine the effect of velocity profile on the thrombus initiation, we looked at platelet thrombi occurring at arteriolar bifurcations ( $n=5$ ), venular confluences ( $n=7$ ), and arteriolar ( $n=2$ ) or venular ( $n=1$ ) curved vessels under the consideration of the vessel diameters, and branching and curved angles in each separate experiment. The time-averaged velocity profiles were measured first by use of the 10-channel dual-sensor method. Subsequently, the excitation light irradiated constantly the selected area 100–200  $\mu\text{m}$  in diameter. This diameter was decided under the consideration of the configuration and the vessel diameters. The region where the thrombus first formed was observed under the microscope.

### Results

#### Effect of Wall Shear Rate on Thrombus Initiation

In Figure 2 thrombus initiation time,  $t_i$ , in venules and arterioles is plotted against the wall shear rate. In venules the initiation time decreases as wall shear rate,  $\dot{\gamma}$ , over the range of 70 to 1,040 1/sec, as  $t_i = 14.2 - 0.007\dot{\gamma}$  ( $r = -0.47$ ,  $n = 30$ ,  $p < 0.01$ ). These wall shear rate ranges almost correspond with the range of 1.3 and 11.1 mm/sec of centerline peak velocity,  $v_c$ . The relation between  $t_i$  and  $v_c$  can be expressed by the equation  $t_i = 13.2 - 0.356 v_c$  ( $r = -0.24$ ,  $n = 30$ ,  $p < 0.2$ ). These results suggest that the initiation of platelet aggregation in venules in our model depends significantly on the wall shear rate rather than the blood flow velocity. In arterioles the initiation time becomes much longer in the higher wall shear rate range (640 to 2,900 1/sec),  $t_i = -67.3 + 0.16\dot{\gamma}$  ( $r = 0.82$ ,  $n = 32$ ,  $p < 0.001$ ). The relation between  $t_i$  and  $v_c$  can be expressed by the equation  $t_i = 21.2 + 13.0 v_c$  ( $r = 0.73$ ,  $n = 32$ ,  $p < 0.001$ ). The correlation coefficient to the wall shear rate is higher for arterioles, too. At comparable shear rate,



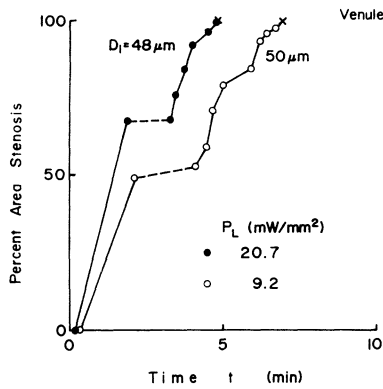


FIGURE 3. Typical examples of time course of change in percent area stenosis of platelet thrombus in growing process for two different levels of light intensity ( $P_L$ ). Broken lines mean excitation light-off period.  $D_i$ , internal diameter of venule before irradiation.

the initiation time is much longer in the arterioles than in the venules.

#### Effect of Wall Shear Rate on Thrombus Growth Rate

Two typical examples of the time course of change in percent area stenosis of platelet thrombus in venules are shown in Figure 3 for two different light intensities ( $P_L$ ). When the light irradiation was stopped, the growth of the thrombus also stopped (broken lines in Figure 3). During this period, the measurement of centerline blood flow velocity at the throat was performed. The thrombus started to grow again with reirradiation of the same spot. The growth rate of the thrombus in the radial direction was calculated from the slope of the growth curve of the percent area stenosis just after the reirradiation of excitation light shown in Figure 3. The growth rates of percent area stenosis of thrombus were calculated for 22 venules in all. The results were examined in terms of the wall shear rate at the throat in venules as shown in Figure 4. The data obtained in two different levels of light intensities show almost the same trend.

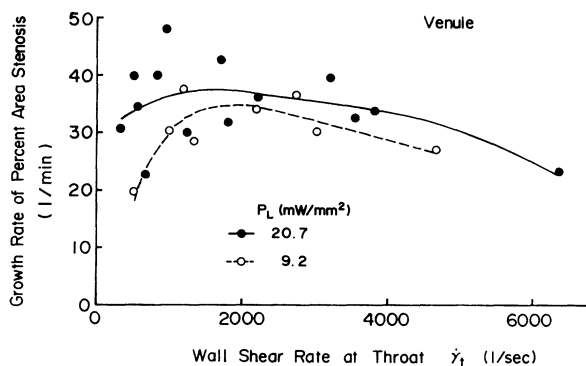


FIGURE 4. Effect of wall shear rate at throat on growth rate of percent area stenosis in venules under two different levels of light intensity. Solid and broken lines were obtained by polynomial approximation of degree 4 with least squares method.

The solid and broken lines in the figure were obtained by polynomial approximation of degree 4. Although these two curves have no statistically significant correlation coefficient ( $r=0.13$  [ $n=8$ ] for  $P_L=9.2$  mW/mm<sup>2</sup> and  $r=-0.33$  [ $n=12$ ] for  $P_L=20.7$  mW/mm<sup>2</sup>), the growth rate of platelet thrombus seems to have a maximum value around a wall shear rate of 2,000 1/sec for  $P_L=9.2$  mW/mm<sup>2</sup> and 1,500 1/sec for  $P_L=20.7$  mW/mm<sup>2</sup>.

#### Platelet Thrombus Initiation and Velocity Profile

The velocity profiles were measured at an arteriolar bifurcation, a venular confluence, or a site where arterioles or venules curve. The platelet thrombus was then induced by constant irradiation of the microvessel under study. The results are shown in Figures 5 and 6. The velocity profiles measured at the sections identified by chain lines in Figures 5 and 6 seem to support the mass conservation of flow within approximately 15% error. At present we cannot understand whether this error came from the intrinsic problem of the measuring system or the complicated configuration of microvessels. Since in this paper we will discuss qualitatively the velocity profile, the above question will be addressed in a future study.

At the bifurcation of arterioles the blood flow velocity in both daughter vessels becomes much higher along the outer walls. Adhesion and aggregation of platelets were first found in the sites of the inner wall, where shear rates are low. The formed platelet thrombus is shown as a shadowed area in Figure 5a and is also clearly seen in the photograph in Figure 5b. A large circle drawn with a broken line indicates the irradiated area of excitation light. At the confluence of venules shown in Figures 6a and 6b, the velocity profile just after the confluence is slightly distorted and shows higher velocity along the outer wall, and the initiation of thrombus formation is localized in this area. This kind of experiment was performed for 15 different microvessels of arterioles and venules. In these microvessels, induced platelet thrombus was judged to occur first either at the site of higher wall shear rate or lower wall shear rate. The data obtained are summarized in Table 1, which also shows mean value of peak velocity. In arterioles, two in seven cases showed the thrombus formation at the site of higher wall shear rate. Blood flow velocities in these cases have much lower values than those in other cases. In venules, the first thrombus formation was found at the site of lower wall shear rate in two cases.

#### Discussion

Using the light/dye method for induction of the platelet thrombus in microvessels in vivo, we found that thrombus initiation occurred mainly at higher wall shear rates (but below 1,000 1/sec) in venules. In arterioles thrombus initiation occurred at lower wall shear rates (above 640 1/sec). Especially at the same wall shear rate conditions, the initiation of platelet aggregation in arterioles was much prolonged com-

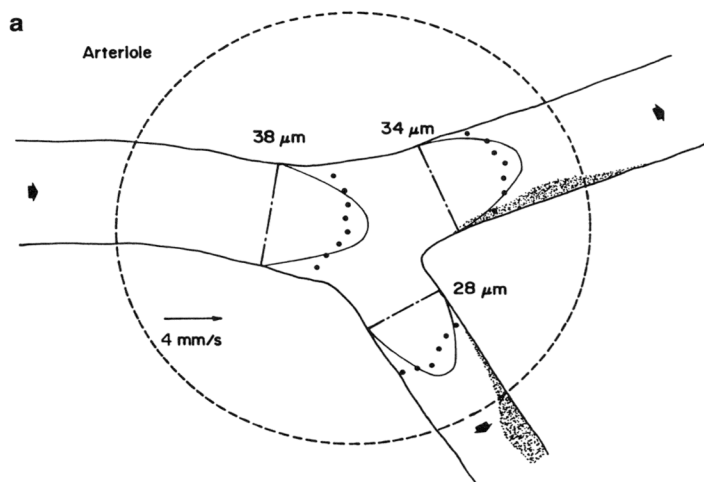
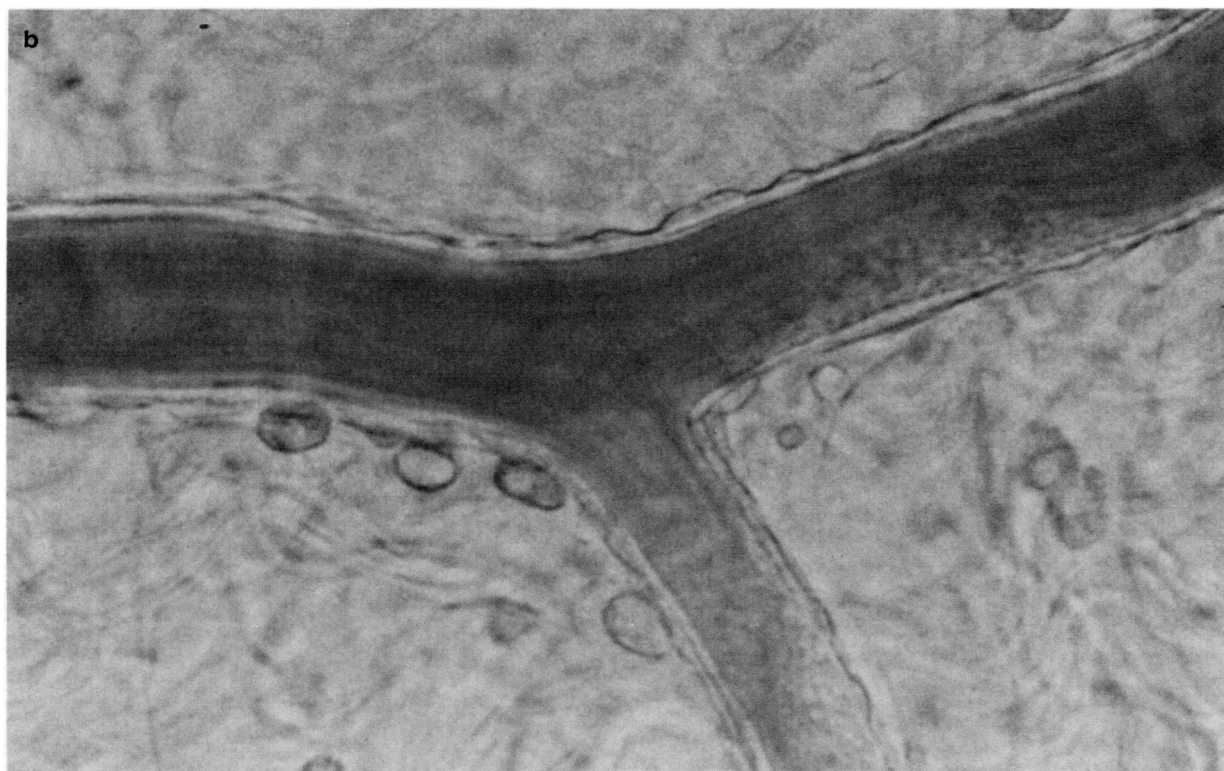


FIGURE 5. Blood flow velocity profiles and thrombus initiation sites at an arteriolar bifurcation. Panel a: Dots represent velocities measured by dual-sensor method. Parabolic solid lines are velocity profiles corrected by method of Baker and Wayland<sup>22</sup> on assumption of an ideal Poiseuille flow in a straight tube. These parabolic solid lines give reference information on distortion of measured velocity profiles. Short arrows in microvessels indicate blood flow direction. Length of long arrow expresses magnitude of velocity written below; distance from chain line to dots and parabolic solid lines shows magnitude of velocity in microvessels. Broken circular line is area irradiated by excitation light. Numbers beside chain lines are internal diameters of microvessels. Same spot is shown in photograph (panel b).



pared with that in venules. We speculated from these results that functional characteristics of the arteriolar endothelial cells may be different from those of the venular cells. Similar wall shear rate dependence of the initiation of platelet aggregation was observed in

the microvessels with complicated configuration, such as venular confluences and arteriolar bifurcations. Furthermore, the growth rate of the platelet thrombus in venules showed a maximum around a wall shear rate of 1,500–2,000 1/sec.

TABLE 1. Sites of First Occurrence of Induced Platelet Thrombi in Branched and Curved Microvessels

Site of thrombus initiation	Arteriole (n)		Venule (n)	
	Bifurcation	Curved	Confluence	Curved
Higher wall shear rate	1 (1.9 mm/sec)	1 (3.5 mm/sec)	5 (4.3 mm/sec)	1 (5.0 mm/sec)
Lower wall shear rate	4 (10.9 mm/sec)	1 (20.0 mm/sec)	2 (4.9 mm/sec)	

Mean value of peak flow velocity in vicinity of thrombi is given in parentheses. Velocity is corrected according to results of Baker and Wayland.<sup>22</sup> n, Number of sites.



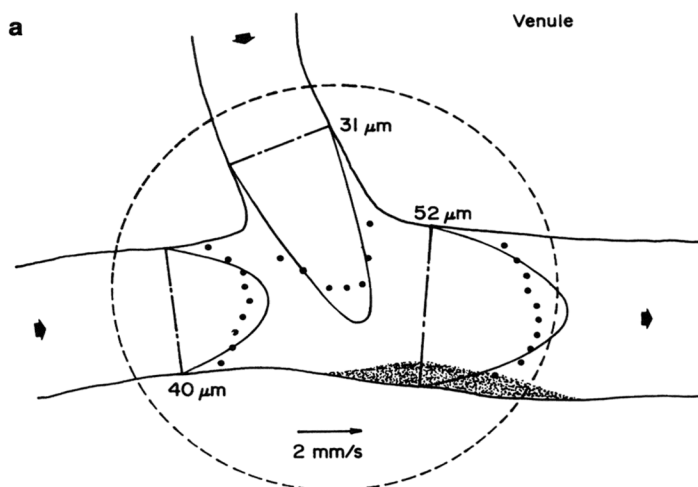
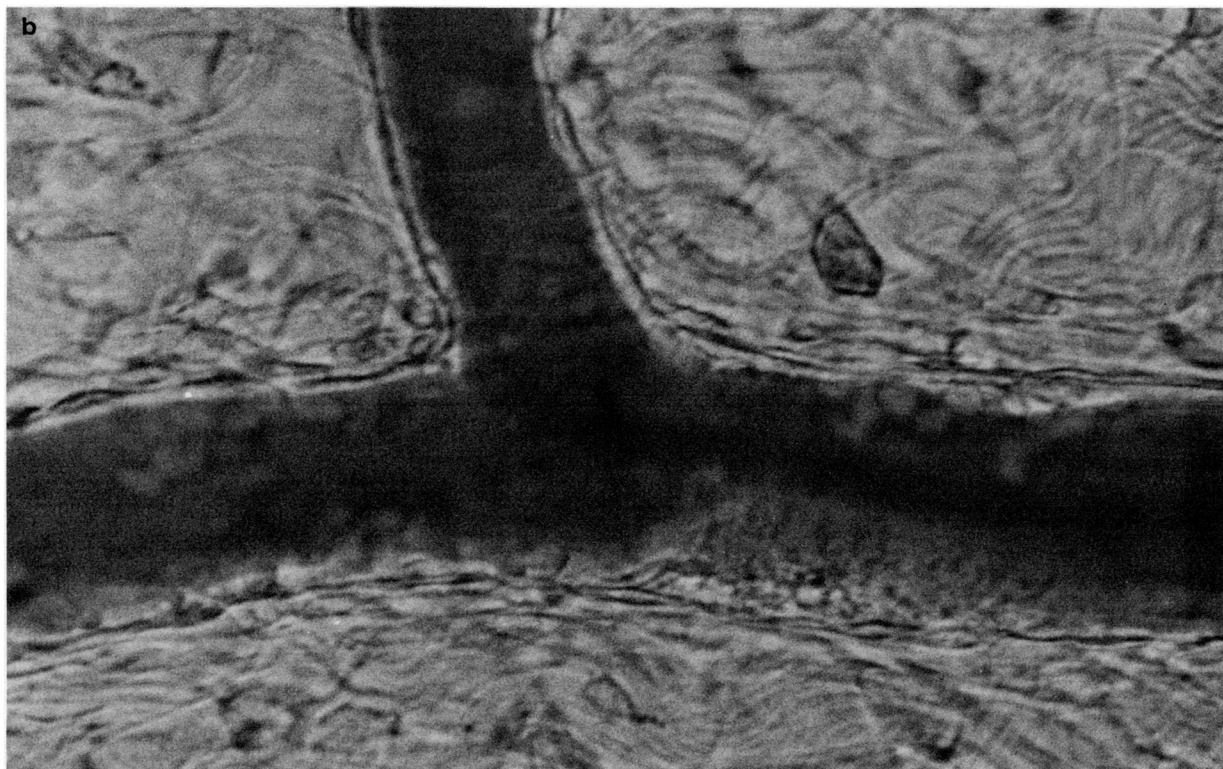


FIGURE 6. Blood flow velocity profiles and thrombus initiation site at a venular confluence. Panel a: Dots represent velocities measured by dual-sensor method. Parabolic solid lines are velocity profiles corrected by method of Baker and Wayland<sup>22</sup> on assumption of an ideal Poiseuille flow in a straight tube. These parabolic solid lines give reference information on distortion of measured velocity profiles. Short arrows in microvessels indicate blood flow direction. Length of long arrow expresses magnitude of velocity written below; distance from chain line to dots and parabolic solid lines shows magnitude of velocity in microvessels. Broken circular line is area irradiated by excitation light. Numbers beside chain lines are internal diameters of microvessels. Same spot is shown in photograph (panel b).



This light/dye model of platelet aggregation was first reported by Rosenblum and El-Sabaan<sup>14</sup> in 1977. Then we also reported the same phenomena in 1981.<sup>11</sup> This light/dye model has the following advantages: 1) The experimental procedure is very simple, and we can produce the platelet aggregates in arbitrary parts of any microvessels; 2) this method quantifies thrombus formation in a reproducible manner, a result that conventional *in vivo* measurements have not been able to achieve; and 3) the present model seems applicable to several artificial situations as well as to physiological conditions. The damage to endothelial cells seems to be relatively small, and the platelet aggregation on the cells is induced without

denudation of the endothelium. On the other hand, a major drawback of the present model is that the observation is unrepeatable in a single animal because the fluorescent dye must be injected intravenously. Rosenblum<sup>23</sup> and Povlishock et al<sup>15</sup> have pointed out in ultrastructural studies that platelet aggregation in either venules or arterioles begins when only minor endothelial vacuolation or lucency is noted. Endothelial cell dissolution and/or sloughing with exposed basement membrane was also shown to be a much later event.<sup>15</sup> These researchers have demonstrated that platelet aggregation in injured pial arterioles is inhibited by pretreatment of mice with cyclooxygenase inhibitors, with hydroxyl



radical scavengers, and with a stimulator or releaser of prostaglandin.<sup>23</sup> They have especially stressed that radicals seem responsible for endothelial injury in the light/dye model. Herrmann pointed out that platelet aggregation initiated by excitement of fluorescein isothiocyanate-dextran (the light/dye reaction) may involve local production of singlet oxygen.<sup>24</sup> We also examined the effects of active oxygens using their scavengers for the platelet aggregation produced by the light/dye method in microvessels of the rat mesentery.<sup>25</sup> In our results superoxide dismutase was the most effective in inhibition of the initiation and growth of platelet aggregation. Although there is still a difference in the effective scavengers of active oxygen, it should be stressed that some active oxygens are playing important roles in the platelet aggregation by this light/dye model.

In venules the inhibition time of platelet aggregation steadily decreased with increasing wall shear rate in the range of 70 and 1,040 1/sec and significantly depended on the wall shear rate rather than the blood velocity. This result suggests that adhesion of platelets to the endothelial cells and aggregation in the vicinity of the wall are influenced by the local flow conditions expressed as the wall shear rate. Some reports have examined influences of blood flow velocity or shear rate on platelet aggregation in venules. Begent and Born<sup>1</sup> applied ADP iontophoretically to the microcirculation for induction of "white bodies" (platelet thrombi) in microvessels in the hamster cheek pouch. They measured both the mean blood flow velocity and the growth rate constant of the white body produced in venules. The growth rate of the platelet thrombus increased with increasing mean blood flow velocity up to a sharp maximum at velocities of 0.3–0.4 mm/sec. With higher velocities the growth rate decreased and remained approximately constant, at about half the maximum, as the mean flow velocities increased from about 0.6 to about 2.5 mm/sec. With velocities greater than 3.0 mm/sec, no platelet thrombi formed. In their results, mean blood flow velocity of 0.3–0.4 mm/sec in venules with diameters of 40–70  $\mu\text{m}$  corresponded approximately to the wall shear rate of 40–80 1/sec. At these wall shear rates, the growth rate of white bodies became maximum. Although we have no data below the wall shear rate of 70 1/sec, our data (Figure 2) indicate relatively high values of the time,  $t_i$ , around lower wall shear rate of about 100 1/sec and a decrease in  $t_i$  with increasing wall shear rate. At blood flow velocities above 3 mm/sec (approximately 500 1/sec in wall shear rate) in the study by Begent and Born,<sup>1</sup> no platelet thrombi formed. But this was not the case for our results, as seen in Figure 2.

In arterioles the initiation time of thrombus formation was prolonged almost linearly with increases in wall shear rate from 640 to 2,900 1/sec. Rosenblum and El-Sabban<sup>17</sup> reported similar results in wall shear rate range of 200 to 1,400 1/sec in cerebral arterioles with the same light/dye method. Their results also showed that the time required for initiation of plate-

let aggregation was more significantly related with wall shear rate than with blood flow velocity. Arfors et al<sup>3</sup> measured the growth rate of platelet microthrombi induced by laser injury in the ear chamber arterioles of conscious rabbits. Blood flow velocity had a variable effect on the number of emboli from sites of laser injury. At velocities above 2.5 mm/sec the number of emboli remained relatively constant; between 1.0 and 2.5 mm/sec the number of emboli tended to increase, and there was a greater variation at this level; and below 1.0 mm/sec the number of emboli decreased. The relation between the number of emboli and blood flow velocity in arterioles obtained by Arfors et al<sup>3</sup> was similar to that in venules reported by Begent and Born.<sup>1</sup>

Few reports have examined the effect of blood flow conditions on platelet thrombus initiation in both arterioles and venules. Results for rat mesenteric arterioles and venules obtained by Seiffge and Kremer<sup>5</sup> by use of the laser-induced thrombus model are similar to ours. In their results on venules, more platelet aggregations tended to be induced in higher blood flow conditions, but the correlation was not significant. From our results, it should be stressed that the initiation of platelet thrombus started much more rapidly in venules than in arterioles even at the same wall shear rate or blood flow velocity. The difference on the platelet thrombus formation between arterioles and venules has been pointed out by Rosenblum,<sup>23</sup> who proposed three possible factors: 1) difference in degree of endothelial damage by the light/dye model, 2) blood flow velocity, and 3) produced substances that influence aggregation. From our results, the second reason will be denied; however, blood flow conditions still affect the platelet thrombus initiation in the same kind of microvessels. From the microcirculatory point of view, we should attribute the difference to the function of endothelial cells. It is often observed, for example, that flowing leukocytes in the center of arterioles start to roll on the endothelium in venules at almost the same wall shear rate or blood flow velocity in both microvessels.

In large vessels, Baumgartner<sup>2</sup> examined in vivo and in vitro the interaction of platelets with the surface of rabbit aorta that had been selectively denuded of endothelium by a balloon catheter. He found that at reduced blood flow fewer platelets adhered to the subendothelial surface in vivo. Similarly, fewer platelets adhered to the subendothelial surface at low blood flow velocities in vitro, and as a result no platelet thrombi formed. In a similar experiment carried out by Turitto et al,<sup>4</sup> human blood was exposed to subendothelium in an annular perfusion chamber for wall shear rates ranging from 50 to 10,000 1/sec. They reported that rates of adhesion increased with wall shear rate up to 650 1/sec; at higher values of shear no significant increase was observed. Phenomena of platelet adhesion in the region of higher wall shear rate is inconsistent with our data, probably due to differences in experimental

models. In their model, endothelium was denuded and subendothelial structures were exposed.

The relation between the thrombus initiation time and the wall shear rate was almost linear in venules and arterioles in the rat mesentery. The relation between the site of thrombus initiation and the profile of blood flow velocity observed in Figures 5 and 6 is consistent with the results shown in Figure 2: thrombi were easily inducible at higher wall shear rate in venules but at lower wall shear rate in arterioles. The platelet thrombogenesis can be considered in three phases: 1) a platelet transport to the vicinity of the damaged endothelium, 2) an initial adhesion of platelet to the surface, and 3) an aggregation of platelets on adhered platelets. This process of thrombus formation on the endothelium is substantially related to blood flow and shear rate conditions. From our experimental results on venules, the initiation of thrombus formation was promoted in the range of higher wall shear rate (Figure 2). In this wall shear rate range platelet transport is likely to govern the process of thrombus formation. On the other hand, adhesion to endothelium and aggregation of platelets are likely inhibited at higher shear rates occurring in arterioles because platelets are removed from damaged endothelium. The shear rate also plays a similar role in the growing process of platelet thrombus (Figure 4). These results explain why thrombi were initiated at the site of higher wall shear rates in arterioles and lower wall shear rates in venules in the exceptional cases shown in Table 1. In the two cases in which the thrombus initiated at the site of higher wall shear rate in arterioles, the blood flow velocity was much lower than in other cases.

Generally, optimal shear flow conditions may exist for initiation of platelet adhesion to a wall and growing of thrombus, as shown by Begent and Born<sup>1</sup> and Arfors et al.<sup>3</sup> Richardson<sup>6,26</sup> analyzed this problem in terms of the theory of aggregation in shear flow. He interpreted the low flow rate results as indicating that the thrombus was enlarged by capture of all platelets that approached it closely enough as they were swept along in the blood flow. This means that the first process of platelet transport mentioned at the beginning of this section might be dominant. Tangelder et al<sup>27</sup> have reported that the platelet concentration near the wall was approximately twice the concentration in the center of arterioles in the rabbit mesentery. This phenomenon is explained by an interaction between the platelets and the red blood cells; thus, it is strongly suggested that the hemodynamics of blood cells are playing very important roles in platelet adhesion to the endothelium and thrombogenesis. Furthermore, we considered that shear stress might inhibit platelet adhesion and/or remove the aggregates at higher flow conditions. On the other hand, fluid-mechanical forces (shear stresses) are known to activate directly the platelets and to initiate chemical changes resulting in the hemostatic aggregation of platelets. Richardson<sup>6,26</sup> proposed that platelets have a finite time delay

between activation and development of a sufficient adhesive. Using this idea, he explained the results obtained by Begent and Born<sup>1</sup>: The growth rate factor of the thrombi decreased at higher blood flow rates, and at extreme it went to zero. He deduced the activation delay time at 0.1–0.2 second from the hamster cheek-pouch experiments.<sup>26</sup> On the other hand, Born<sup>28</sup> speculated that a platelet flowing within a distance no greater than its own diameter would pass an injury site 100  $\mu\text{m}$  long in about 2 msec. The discrepancy is so large that other possible reasons should be investigated in future studies. Finally, hemodynamic factors, especially wall shear rate, are considered to be strongly related to each phase in the process of thrombogenesis mentioned above, as either promoting or inhibitory factors.

### Conclusion

The platelet thrombus model used in this experiment proved useful in the study of the effect of hemodynamics on thrombus formation and growth. In venules higher wall shear rates (up to about 1,000 1/sec) promoted the initiation of thrombus formation. In arterioles higher wall shear rates (up to 2,900 1/sec) inhibited the initiation. These results were also found in branched or curved microvessels. At the bifurcation of arterioles, the initial aggregation of platelets was found at the site of lower wall shear rate. At the confluence of venules, the initiation started at the site of higher wall shear rate. The growth rate of thrombi in venules was affected by the blood flow and showed a maximum value around a wall shear rate of 1,500–2,000 1/sec. These local phenomena on platelet adhesion and aggregation are considered to depend on wall shear rate rather than blood flow velocity.

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KEY WORDS • microcirculation • platelet • thrombogenesis • wall shear rate