Shear-Induced Platelet Aggregation Can Be Mediated by vWF Released From Platelets, as Well as by Exogenous Large or Unusually Large vWF Multimers, Requires Adenosine Diphosphate, and Is Resistant to Aspirin

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Fluid shear stress in arteries and arterioles partially obstructed by atherosclerosis or spasm may exceed the normal time-average level of 20 dyne/cm². In vitro, at fluid shear stresses of 30 to 60 dyne/cm² applied for 30 seconds, platelet aggregation occurs. At these shear stresses, either large or unusually large von Willebrand factor (vWF) multimers in the suspending fluid exogenous to the platelets mediate aggregation. Adenosine diphosphate (ADP) is also required and, in these experiments, was released from the platelets subjected to shear stress. At 120 dyne/cm², the release of endogenous platelet vWF multimers can substitute for exogenous large or unusually large vWF forms in mediating aggregation. Endogenous released platelet vWF forms, as well as exogenous large or unusually large vWF multimers, must bind to both glycoproteins Ib and the IIb/IIIa complex to produce aggregation. Shear-induced aggregation is the result of shear stress alteration of platelet surfaces, rather than of shear effects on vWF multimers. It is mediated by either large plasma-type vWF multimers, endogenous released platelet vWF forms, or unusually large vWF multimers derived from endothelial cells, requires ADP, and is not inhibited significantly by aspirin. This type of aggregation may be important in platelet thrombus formation within narrowed arterial vessels, and may explain the limited therapeutic utility of aspirin in arterial thrombosis.

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MATERIALS AND METHODS

Human blood was obtained on several different occasions from 11 normal individuals and one patient with severe von Willebrand’s disease (vWD). The blood was drawn into acid-citrate-dextrose, and platelet-rich plasma (PRP) was obtained by centrifugation. The pH was adjusted to 6.5 and platelets were pelleted. The platelets were resuspended and washed in a buffer solution (10 mmol/L HEPES, 145 mmol/L NaCl, 5 mmol/L KCl, 0.5 mmol/L NaHPO₄, 1 mmol/L MgSO₄, 0.1 mmol/L CaCl₂, 5.5 mmol/L glucose, and 3.5 g/L bovine serum albumin [BSA]; Calbiochem-Behring, San Diego), pH 7.0, containing sodium heparin (50 U/mL final concentration, from porcine intestine; Elkins-Sinn, Inc, Cherry Hill, NJ) and apyrase (2.5 U/mL final concentration; Grade V, Sigma Chemical Co, St Louis), and finally suspended for study in HEPES buffer (HEPES-NaCl-KCl-NaH₂PO₄-MgSO₄-glucose-BSA), pH 7.4, containing an increased concentration of CaCl₂ (1 mmol/L).
These platelets aggregated normally in response to collagen (2 μg/mL; Hormon-Chemie, Munich) and to 10 μmol/L ADP/fibrinogen (1 mg/mL; Calbiochem-Behring). We could not detect release of vWF antigen or vWF multimers into the buffer as a result of centrifugation, even when the monoclonal antibodies to both GPIb and GPIb/IIa were present in order to retard binding of any released vWF to platelet surface receptors. Furthermore, the lysates of platelets after centrifugation contained an apparently full complement of large and unusually large vWF multimeric forms.

The largest vWF multimeric forms in normal human cryoprecipitate were purified as described previously. Human umbilical vein endothelial cell supernatant containing unusually large vWF multimers were obtained from cells grown in serum-free, defined medium.

vWF antigen levels in normal and severe vWD plasma were quantified by solid-phase immunoradiometric assay (IRMA). vWF multimers in plasma, purified vWF preparations, endothelial cell supernatants, and platelets were separated by sodium dodecyl sulfate (SDS)-agarose gel electrophoresis, overlayed with rabbit IgG, and analyzed by autoradiography using 1% agarose and a continuous buffer system. The severe vWD patient studied has no detectable plasma or platelet vWF by IRMA, and no plasma or platelet vWF multimers by gel electrophoresis and autoradiography.

For the preparation and analysis of platelet lysates, normal blood was collected into purple-top tubes containing 3.7 mmol/L EDTA and the PRP was obtained by centrifugation. The platelets were sedimented, the plasma was removed, and the platelet pellet was washed three times in 0.01 mol/L Tris-HCl, 0.15 mol/L NaCl, 0.005 mol/L EDTA, pH 7.2. The pellet was then resuspended in the washing buffer, and the platelet count was adjusted to 50,000 to 150,000/μL. The platelet suspension (one part) was mixed with the denaturing solution (1.5 parts) consisting of 2% SDS, 8 mmol/L urea, 0.02 mol/L Tris-HCl, 2 mmol/L EDTA, pH 8.0, heated to 56°C for one hour, centrifuged, and the denatured platelet extract analyzed by SDS 1% agarose gel electrophoresis and autoradiography.

Dr Barry S. Coller of the State University of New York at Stony Brook generously provided purified monoclonal antibodies 6D1 and 10E5 directed against GPIb and either GPIIb or IIa, respectively. In experiments using the antibodies, platelets were incubated for five minutes before shearing with either 6 μg/mL of 6D1 or 10 μg/mL of 10E5, or with both antibodies in these concentrations.

In the experiments described below, shear stresses were applied to platelets suspended in the HEPES-albumin buffer, with or without added exogenous vWF or fibrinogen. Fluid shear stress promotes platelet-platelet collisions, and probably platelet surface alterations, in the platelet suspensions.

In some experiments, large vWF multimers purified from normal human cryoprecipitate were added (Fig 1A). These are identical to the larger vWF forms in normal platelet-poor plasma, and will be referred to as "large plasma-type vWF multimers." In other experiments, platelets were studied in the presence of supernatants obtained from human endothelial cells growing in serum-free, defined medium. These endothelial cell supernatants contain unusually large vWF multimers.

In other experiments, shear stress was applied to platelets suspended in buffer alone. In these experiments, available proteins were those released from the platelets themselves, including vWF multimers of large and unusually large forms (Fig 1B).

In some studies, creatine phosphate (Sigma; 5 mmol/L final concentration in HEPES buffer, pH 7.4) and creatine phosphokinase (Sigma; 200 units final concentration) were added to the platelet suspensions in order to metabolize ADP as it was released from sheared platelets. Either creatine phosphate alone or creatine phosphokinase alone was added to other platelet suspensions as controls.

A range of fluid shear stresses was applied to the platelet suspensions in a stainless steel viscometer with rotating cone and stationary plate. This instrument has been described in detail. The narrow gap between the cone and plate was filled with 0.6 mL of the platelet suspensions, which were sheared at ambient temperature for 30 seconds by rotating the cone at different speeds. This produced uniform fluid shear stresses ranging from 15 dyne/cm², a shear stress level within the range encountered in the normal arterial circulation, to 120 dyne/cm², a level of shear stress found distal to partially obstructed areas in small arteries and arterioles.

No vWF multimeric forms attached to the viscometer, either in the presence or absence of shear, and shear did not alter the apparent extent of multimerization of vWF in purified preparations or endothelial cell supernatants. Furthermore, shear-induced platelet aggregation was found to be independent of any minimal platelet-surface interactions that may occur.

Immediately after shearing, 10 μL of the test samples were dispensed in 20 mL Isoton II (Coulter Electronics, Inc, Hialeah, FL) containing 0.5% glutaraldehyde to fix platelet aggregates. Particle count and size distribution in each sample were then determined with an electronic particle counter (Model ZBI with Channelizer; Coulter Electronics, Inc) using a sample volume of 100 μL and a 50 μm diameter aperture. Particles with sizes ±20% of the mean...
platelet distribution in unsheared samples were considered as single platelets. The disappearance of single platelets was accounted for by the formation of platelet aggregates. Thus, the percent decrease in initial platelet counts was directly related to the percent increase in aggregated platelets.

In order to determine accurately the extent of release of platelet granular contents in the shear field, an assay system is required that can measure a released product directly without subjecting the platelets to centrifugal forces subsequent to the shear stress. For this reason, we quantified the release of ATP and ADP from normal and severe vWD platelets directly during shearing by a sensitive luminescence assay. The stainless steel lower platen of the viscometer was replaced by a glass platen of uniform thickness (3 mm). Firefly luciferin-luciferase (Sigma) was added to some platelet suspensions before shearing. ATP, released from sheared platelets, produced a luminescent reaction with luciferin-luciferase. The emitted light was transmitted through a glass fiber cable into a photomultiplier tube. The analog voltage was recorded in an IBM CS/9000 computer. The quantity of ADP released was estimated at 1.5 times the measured ATP release, based on the 3:2 concentration ratio of ADP:ATP in platelet dense granules.

Aspirinized platelets were prepared by either of two methods: the administration to normal donors of 640 mg of aspirin on the night before, and then again in the morning preceding the shear study; or the incubation of normal PRP for 30 minutes at ambient temperature with 55 mmol/L (final concentration) of acetylsalicylic acid (ASA; Sigma). To prepare the ASA solution, 10 mg of ASA were dissolved in 1 mL of absolute ethanol, and 9 mL of 0.154 mol/L NaCl were added. One hundred microliters of this solution were incubated with 10 mL of PRP before separation and washing of platelets. Control PRP was treated identically except that ASA was omitted from the incubation solution. Platelets in PRP after exposure to ASA in vivo or in vitro aggregated normally to ristocetin; however, response to arachidonic acid was absent, and there was no secondary wave of aggregation in response to ADP. Control PRP exposed to 100 μL of a 1:9 ethanol:NaCl solution aggregated normally to ristocetin, arachidonic acid, and ADP. A one-tailed student’s t distribution for paired data was used to analyze the shear-induced aggregation responses of aspirinized v non-aspirinized platelets.

RESULTS

There was little aggregation of normal platelets suspended in HEPES-albumin buffer alone (containing Ca2+ and Mg2+) when they were exposed to fluid shear stresses of 50 or 60 dyne/cm² for 30 seconds. These are levels of shear about 2.5- to threefold greater than the time-average level in the normal arterial circulation (Fig 2). At 50 dyne/cm², the platelets released 141 ± 45 nM/L of ADP (Fig 2), which is approximately 2% of the total releasable ADP.

Extensive shear-induced platelet aggregation occurred at 50 or 60 dyne/cm² applied for 30 seconds in the presence of 100, 50, or 25 U/dL of vWF antigen containing the largest plasma-type vWF multimeric forms purified from normal cryoprecipitate (Figs 2 and 3). Shear-induced platelet aggregation also occurred in the presence of only 2.5 U/dL of vWF antigen when this contained unusually large vWF multimeric forms from the supernatant of human endothelial cells grown in a serum-free, defined medium (Fig 3). Unusually large vWF multimers are considerably more potent, on the basis of relative antigen levels, in inducing platelet aggregation in the shear field than are the largest vWF multimers found in normal cryoprecipitate (Fig 3). It has previously been shown that unusually large vWF multimers are the active aggregating molecules in human endothelial cell supernatant.

In the presence of 100 U/dL of purified large plasma-type vWF forms, the application of 50 dyne/cm² shear stress for 30 seconds induced platelet aggregation that was accompanied by the release of 4% to 5% of the total releasable ADP, compared with 1% to 2% ADP release at the same shear stress in the absence of external vWF and aggregation (Fig 2).

Figure 3 demonstrates the effects of 60 dyne/cm² shear stress for 30 seconds on platelet suspensions in the presence of creatine phosphate and creatine phosphokinase. Removal of ADP as it was released from sheared platelets prevented shear-induced platelet aggregation mediated by the largest vWF multimers purified from normal cryoprecipitate, or by unusually large vWF forms in the supernatant of cultured human endothelial cells. (Unusually large vWF multimeric forms are five to 15 times more effective, based on comparative antigen levels, in initiating shear-induced platelet aggregation.) The inhibitory effect of creatine phosphate/creatine phosphokinase was not seen when either creatine phosphate/creatine phosphokinase...
was modestly suppressed. This was probably because of

Severe vWD platelets did, however, aggregate extensively in platelets, the aggregation of severe vWD multimers purified from cryoprecipitate. As seen with normal platelets, the aggregation of severe vWD platelets induced by 50 dyne/cm² in the presence of purified large plasma-type vWF multimers was accompanied by the release of 4% to 5% of total releasable ADP (Fig 2). Aggregation of severe vWD platelets in the presence of large plasma-type vWF forms was considerable, even at a shear stress of 30 dyne/cm². There was only a small amount of shear-induced platelet aggregation when fibrinogen, at 2 mg/mL, was present in the suspending buffer. When fibrinogen was present along with purified large vWF multimers in the buffer, shear-induced platelet aggregation mediated by vWF was modestly suppressed. This was probably because of

competition between fibrinogen and large vWF forms for GPIIb/IIIa binding sites on the platelet surface.

Normal platelets, in contrast to severe vWD platelets, have vWF multimers in their α-granules. These include vWF forms larger than those in normal human plasma, and similar to those found in cultured human endothelial cells (Fig 1B). In contrast to severe vWD platelets, normal platelets suspended in HEPES-albumin buffer alone aggregated extensively at the shear stress level of 120 dyne/cm² applied for 30 seconds in the absence of exogenous vWF (Fig 4B). It can be concluded from Figs 4A and B that endogenous platelet vWF, which is not present in severe vWD platelets but is released from normal platelets at the relatively high shear stress of 120 dyne/cm², is capable of supporting aggregation in the buffer suspension in the absence of added exogenous large vWF forms.

The quantity of vWF released from normal platelets (380,000/µL) in the shear field in the presence of monoclonal antibodies both to GPIb and GPIIb/IIIa (to retard binding to the platelets of released platelet vWF) is <1.5 U/dL, which is the limit of sensitivity of our immunoradiometric and autoradiographic procedures.
Additional shear-induced aggregation occurred if exogenous purified large vWF forms were provided to supplement the vWF released from normal platelet α-granules in the shear field. This augmentation was not quite so great if exogenous fibrinogen was present in the platelet suspensions to compete with the exogenous purified large plasma-type vWF multimers for platelet GPIIb/IIIa receptor sites.

It is also demonstrated in Fig 4B that shear-induced platelet aggregation in the presence of purified large plasma-type vWF multimeric forms is minimal at 15 dyne/cm² for 30 seconds, a shear stress within the range for normal time-average shear forces in the arterial circulation.

ADP, which is released during shear from platelet dense granules (along with the contents of α-granules) is a necessary cofactor for aggregation mediated by endogenous platelet vWF released from the platelets at relatively high shear stress. This conclusion is based on observations that the shear-induced aggregation of normal platelets in buffer alone was inhibited almost completely at 120 dyne/cm² when creatine phosphate and creatine phosphokinase were added to the suspending buffer (Fig 5). Thus, ADP is an essential co-factor for platelet aggregation at high shear stresses mediated by endogenous vWF released from platelets, as well as for platelet aggregation mediated by exogenous large and unusually large vWF forms at lower shear stresses (Fig 3).

At 120 dyne/cm², under the influence of ADP, endogenous vWF released from platelets apparently binds to both platelet GPIb and the GPIIb/IIIa complex. This follows from observations that monoclonal antibodies to both GPIb and GPIIb/IIIa inhibit aggregation mediated by ADP and vWF released from platelets at high shear stress (Fig 6). It has previously been found that exogenous large and unusually large vWF multimeric forms also bind to platelets in the shear field via both GPIb and the GPIIb/IIIa complex.\(^8\,^2\,0\)

When severe vWD PRP was exposed to a shear stress of 180 dyne/cm² and then removed from the viscometer, the subsequent addition of large plasma-type vWF multimers caused platelet aggregation (Fig 7). In contrast, when either normal platelet-poor plasma, purified large plasma-type vWF multimers, or endothelial supernatant containing unusually large vWF multimeric forms was subjected to shear stresses of 120 to 180 dyne/cm² for 30 seconds, these sheared vWF multimeric forms did not cause the aggregation of unsheared normal platelets, even in the presence of 500 nmol/L ADP (a concentration of ADP greater than that released from normal or severe vWD platelets during exposure to shear stress [Fig 2]).

Treatment of platelets with aspirin in vivo or in vitro had no statistically significant inhibitory effect on aggregation mediated by ADP and either endogenous vWF multimers released from platelets (Fig 8A), exogenous purified large plasma-type vWF multimeric forms (Fig 8B), or exogenous unusually large vWF multimeric forms from endothelial
cells (Fig 8C) when the platelet suspensions were exposed to shear stress of 30, 60, or 120 dyne/cm².

DISCUSSION

Little aggregation of normal platelets occurs at a shear stress of 15 dyne/cm² applied for 30 seconds in the presence or absence of exogenous large plasma-type vWF multimers. This shear stress level is within the time-average range encountered in normal arterial vessels. At 30 dyne/cm², and in most experiments at 60 dyne/cm², aggregation occurs only in the presence of exogenous large vWF multimeric forms. At higher shear stresses, 120 dyne/cm², endogenous vWF released from platelets can substitute for exogenous large vWF forms. This conclusion follows from the observation that a high level of shear stress induces the aggregation of normal platelets, but not the aggregation of severe vWD platelets, in a buffer suspension that does not contain exogenous large or unusually large vWF forms.

Endogenous human platelet vWF multimers are contained within α-granules (analogous to endothelial cell Weibel-Palade bodies) and include unusually large vWF multimeric forms similar to those found in endothelial cells and larger than the largest vWF multimers in normal human plasma. Unusually large vWF multimers derived from endothelial

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**Fig 7.** Effect of shear stress on platelets and on vWF multimers. Severe vWD PRP (600 µL; platelets 447,200 to 613,000/µL) were exposed to a shear stress of 180 dyne/cm² for 30 seconds. The PRP was removed from the viscometer immediately after shearing. Either 30 µL of HEPES-albumin buffer (as control) or 30 µL of purified large plasma-type vWF multimers (final concentration, 100 U/dL) was added to 400 µL of the sheared severe vWD PRP, and the extent of platelet aggregation determined by electronic particle counting. The five bars on the right each represent four to five experiments in which 400 µL samples of the following were exposed to shear stresses of 120 or 180 dyne/cm² for 30 seconds: HEPES-albumin buffer alone (as control); normal platelet-poor plasma; purified large plasma-type vWF multimers; or endothelial cell supernatant containing unusually large vWF multimeric forms. Following shear stress, the samples were removed from the viscometer, 200 µL were added immediately to 200 µL of unsheared washed normal platelets (600,000 to 1,200,000/µL), and the extent of platelet aggregation determined by particle counting in the presence or absence of 500 nmol/L ADP.

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**Fig 8.** Effect of aspirin on the aggregation of platelets in buffer alone (A), in the presence of purified large plasma-type vWF multimers (B), or in the presence of endothelial cell supernatant (C). Initial platelet counts before shear stress were 226,000 to 460,000/µL. In this as in previous figures, within this range of platelet concentrations there is no relationship between platelet numbers and the extent of shear-induced aggregation. (In individual experiments, initial aspirinized [□] and non-aspirinized [●] platelet counts differed by 10,000 to 88,000/µL.) The vWF antigen levels in B were 71 to 124 U/dL, and in C were 5.5 to 6.5 U/dL.
cells are exceptionally effective compared with vWF in
human plasma and cryoprecipitate and to the largest vWF
forms purified from cryoprecipitate in mediating platelet
aggregation in shear fields, as well as in supporting ristocetin-induced platelet aggregation (J.D. Olson and J.L. Moake, unpublished observations, 1987). (Unusually large vWF multimers do not cause the direct aggregation of unsheared platelets.) It is probable that the large and unusually large vWF forms released from sheared platelets are responsible, along with ADP as a co-factor, for mediating platelet aggregation in the absence of exogenous vWF multimers. Our experiments indicate that the release of only 2% to 3% of the total releasable ADP from platelets (300,000 to 353,000/µL) exposed to shear stresses above 30 dyne/cm² is sufficient to promote aggregation in the presence of 2.5 U/dL of exogenous unusually large vWF multimers derived from endothelial cells. The quantity of unusually large vWF forms released from this number of sheared platelets must be less than this amount until shear stresses reach levels of 120 dyne/cm². At or above this level, platelets aggregate in the shear field in the absence of exogenously added unusually large vWF multimers.

The potential role of endogenous vWF was not appreciated in our initial studies of the role of vWF multimeric forms in shear-induced aggregation, probably because in the earlier experiments with normal platelets suspended in severe vWD plasma there was some competition between vWF released from the platelets and plasma fibrinogen or fibronectin for binding to platelet IIb/IIIa receptors. A modest inhibitory effect of exogenous fibrinogen on the shear-induced aggregation mediated by exogenous large vWF multimers was shown in the present studies (Fig 4).

Platelet aggregation in the shear field mediated by endogenous vWF forms released from platelets, as well as aggregation mediated by exogenous large and unusually large vWF multimers, is inhibited by monoclonal antibodies to either GPIb or GPIIb/IIIa. These results indicate that both platelet receptor sites are involved in the interaction of large vWF forms with platelets in the shear field, regardless of whether the vWF is exogenous or released from platelet α-granules. This may be somewhat different than the situation that follows stimulation of platelet vWF release by thrombin. In the latter circumstance, released platelet vWF forms have been reported to bind predominantly to the GPIIb/IIIa complex. More platelet vWF may be released in response to thrombin compared to shear stress.

In addition to large or unusually large vWF multimers, shear-induced platelet aggregation over a wide range of shear stresses (30 to 120 dyne/cm²) requires the presence of 100 to 200 nmol/L of ADP. This conclusion is based on the quantity of ADP released during 50 dyne/cm² shear stress from normal or severe vWF platelets; and on the nearly complete inhibition of shear-induced platelet aggregation mediated by either exogenous large or unusually large vWF multimers, or by endogenous vWF forms released from platelets, when ADP is converted to ATP by creatine phosphate and creatine phosphokinase. ADP is released from platelets, and probably from erythrocytes and other blood cells when they are present, during the application of fluid shear stress.

We cannot determine the relative effects of fluid shear stress and ADP on the exposure or conformation of the platelet surface vWF receptors, GPIb and the GPIIb/IIIa complex, because both large or unusually large vWF multimers must bind to both GPIb and the GPIIb/IIIa complex in order to induce aggregation in the shear field. This latter conclusion is reinforced by our previous observations that neither Bernard-Soulier platelets (deficient in GPIb) or thrombasthenic platelets (deficient in GPIIb/IIIa) are capable of undergoing shear-induced, vWF-mediated aggregation.

We can detect no shear-modification of vWF multimeric forms in our agarose gel electrophoretic system. Furthermore, shear stresses of 120 to 180 dyne/cm² do not functionally alter the vWF multimers in normal PPP, in vWF purified from normal cryoprecipitate, or in endothelial cell supernatant. That is, the sheared vWF forms do not become capable of aggregating unsheared platelets. This is true even when sheared, purified large plasma-type vWF multimers are added together with ADP in a concentration in excess of that released from platelets in the shear fields. Shear forces do, however, modify the platelet surface so that aggregation occurs on the subsequent addition of large vWF multimeric forms. One hypothesis that is consistent with our data and different types of experiments by others is as follows. Shear forces modify the extent of exposure or structure of GPIb. This leads to the binding by GPIb of large or unusually large vWF multimers. Subsequently, the large or unusually large vWF multimers attach to the GPIIb/IIIa complex that has been modified by the combination of ADP, attachment of large or unusually large vWF forms to neighboring GPIb molecules and, possibly, directly by shear stress.

At moderately abnormal shear stresses in the 30 to 60 dyne/cm² range for 30 seconds, these large or unusually large vWF multimers must come from the suspending fluid exogenous to the platelets because insufficient quantities of unusually large vWF multimers are released from the sheared platelets. At a higher shear stress, 120 dyne/cm², either exogenous large or unusually large vWF forms, or the more substantial quantities of vWF multimers released from platelet α-granules at this higher level of shear, can bind to GPIb and the GPIIb/IIIa complex and induce aggregation in the presence of ADP.

Platelet aggregation mediated by nanomolar quantities of ADP, and by large or unusually large vWF multimers from plasma, platelets, or endothelial cells may be involved in the formation of platelet thrombi in narrowed arterial vessels in which the level of fluid shear stress is abnormally elevated. These diseased vessels may be narrowed permanently, as by atherosclerosis, or transiently, as by spasm.

Lysis of a small percentage of the platelets exposed to the shear stresses in our experimental system may be the source of the ADP required for vWF-mediated aggregation. We have previously determined that 1% to 2% of platelets lyse at shear stresses below 150 dyne/cm², regardless of whether
or not the platelets have been aspirinized. The quantity of platelet vWF (including a relatively small amount of unusually large vWF multimers) from these lysed platelets is apparently not usually sufficient to act as a cofactor with the ADP present in inducing platelet aggregation at shear stresses below 120 dyne/cm². Additional exogenous large or unusually large vWF multimeric forms are required. However, at levels of 120 dyne/cm² or above, the quantity of platelet vWF multimers (including unusually large forms) is sufficient to support, along with the small amount of ADP present, shear-induced aggregation in the absence of exogenous large or unusually large vWF multimers.

The time required for a platelet to traverse the flow area of an abnormal cardiac valve, or to pass through a partially obstructed region of the microcirculation, may be of the order of seconds (or fractions of seconds). O'Brien and Salmon² have recently found that shear-induced platelet aggregation occurs within three seconds in a capillary-type filter and that this platelet clumping is vWF-dependent. In their system, GPIIb/IIIa was apparently the major site of binding of vWF to the platelet surface, and they obtained no evidence that ADP was involved in the vWF-platelet binding.

Platelet aggregation of the type described in this report is not inhibited to a statistically significant extent by aspirin. This negative result would predict that aspirin therapy might be of limited utility in interfering with arterial thrombosis if the pathophysiology of this event involves shear-induced intravascular platelet aggregation mediated by: ADP from stimulated platelets, or from a few lysed platelets or red cells; and either large or unusually large vWF multimers from plasma or platelets, or unusually large vWF multimers from endothelial cells damaged or intensely stimulated in the region of a stenotic lesion. Our in vitro observations may help to explain why aspirin has not proved to be more effective in the prophylaxis and treatment of arterial thrombotic disease, and may provide direction for the development of new types of anti-thrombotic agents.

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Shear-induced platelet aggregation can be mediated by vWF released from platelets, as well as by exogenous large or unusually large vWF multimers, requires adenosine diphosphate, and is resistant to aspirin.

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