Correlations Between Morphological Changes in Platelet Aggregates and Underlying Endothelial Damage in Cerebral Microcirculation of Mice

S. Said, PhD; W.I. Rosenblum, MD; J.T. Povlishock, PhD; G.H. Nelson, MS

**Background and Purpose:** The aim of this study was to test the hypothesis that, once formed, platelet aggregates may injure underlying cerebrovascular endothelium. Such injury could make the same site selectively attractive to the next wave of passing emboli or activated platelets. This vicious circle could account for repetitive, stereotypic symptoms in transient ischemic attacks.

**Methods:** In pial arterioles, minor endothelial injury was produced by a laser/dye technique. After various periods of platelet aggregation at the damaged site, the vessels were fixed in situ for electron microscopic study. The degree of platelet activation (rounded and/or degranulated forms) was evaluated by counting these forms in the electron photomicrographs. These counts were related to the degree of endothelial damage ascertained in the micrographs. Other statistical relations were also examined.

**Results:** Endothelial damage progressed in parallel with the duration of platelet aggregation and the degree of platelet degranulation at the site. Correlations were number of activated platelets versus degree of damage, \( r = .43, P < .03 \); duration of aggregation versus damage, \( r = .52, P < .01 \); and number of degranulated platelets versus the degree of endothelial damage, \( r = .83, P < .001 \). If an aggregate embolized, endothelial damage did not appear to progress. No correlation existed between the duration of exposure to the laser and the degree of injury.

**Conclusions:** The parallel between changes in platelets and endothelial damage could represent either an effect of endothelium on platelets or an effect of platelets on endothelium. Although the former alternative cannot be totally ruled out, the observations seem to fit best the hypothesis that progressive endothelial damage can result from increasing activation and degranulation of overlying platelets. *(Stroke, 1993;24:1968-1976.)*

**KEY WORDS** • cerebral ischemia, transient • endothelium • platelet aggregation • mice

The long-held belief that endothelial denudation is a prerequisite for platelet aggregation was challenged by Povlishock et al. and Povlishock and Rosenblum, who demonstrated that subtle endothelial perturbation, without denudation, was sufficient to trigger platelet aggregation. Whereas it now appears that subtle endothelial injury is sufficient to attract platelets, it has also been suggested that once recruited, the platelets themselves are capable of contributing to further endothelial change. The damaging consequences of platelet recruitment were first alluded to in the vascular beds of the systemic circulation. However, this linkage was also recently suggested in the cerebral microvasculature. Fujimoto et al. and more recently Yamazaki et al., who used ADP and arachidonic acid to induce platelet aggregation, argued that the presence of the platelet aggregate caused the subsequent denudation of the underlying endothelium. However, they did not provide an analysis of the relation between the degree or duration of platelet aggregation and the degree of endothelial damage. Moreover, many of the preceding studies did not involve the microcirculation, and none took place in the cerebral circulation. Thus, the question remained as to whether platelet aggregates do exacerbate damage in the cerebral microvessels.

A better understanding of the contribution of platelets to continuing endothelial damage in the cerebral microcirculation is of more than merely academic interest, since it is conceivable that the damaging consequences of platelet aggregation could contribute to a host of microcirculatory abnormalities seen in both animals and humans. Such may be the case in transient ischemic attacks, in which initial subtle endothelial injury in an arteriolar segment could recruit platelet emboli from or platelets activated by events occurring at an upstream site (such as a carotid artery thrombus). The recruited mass of platelets would then enhance the degree of endothelial injury by releasing damaging mediators. The mass might then disintegrate as platelet aggregates are prone to do, leaving behind a site more prone to attract passing platelets from the next shower of emboli, which will cause further endothelial damage.
This repetitive process could result in transient ischemic attacks and account for the stereotypical nature of many of these attacks. Ultimately, permanent vascular occlusion might then occur as a result of the repeated, ever-worsening repeated, permanent platelet deposition of ever-worsening permanent platelet deposition at the site.

The following study tests the hypothesis that in cerebral arterioles, platelet aggregation contributes to endothelial damage. Through the ultrastructural assessment of various stages of platelet aggregation and the parallel endothelial changes, we were able to demonstrate a significant parallel between the duration of platelet aggregation and the degree of endothelial damage. Moreover, there was a correlation between the number of activated platelets and the degree of underlying endothelial damage and an even greater correlation between the number of degranulated platelets and the degree of damage in adjacent endothelium.

Materials and Methods

Surgical Procedure

The procedures used here have been described in detail elsewhere. Essentially, 44 adult male mice (ICR strain, Harlan-Sprague Dawley, Indianapolis, Ind) were used. After anesthesia was induced with urethan (2 mg/kg IP), a tracheostomy was performed. The pial vessels were exposed through a craniotomy (4 x 3 mm) over the right parietal cortex, and the dura was carefully excised to expose the delicate arachnoid and the underlying pial vessels. The exposed site was continuously irrigated with artificial cerebrospinal fluid at pH 7.35. During the procedure, body temperature was maintained at 37°C. The pial vessels under the arachnoid were observed through a microscope. The vessels were illuminated from the side by a halogen lamp and fiberoptic guide. The microscope was fitted with the objective lens turret of a Leitz metallurgic illuminator. The metallurgic illuminator was disconnected from the turret. This exposed a side port on the turret at right angles to the objective lens. To damage the endothelium at a preselected site, the beam of a 6-mW helium-neon laser (Spectra Physics, Mountainview, Calif) was directed through the side port and downward through the objective lens by the optics within the turret. Thus, the laser beam epi-illuminated its target and appeared as a red dot. An infinity-corrected x 20 objective was used to focus the laser beam over an area 18 μm in diameter.

The laser alone is innocuous. Damage is produced only when the bed is sensitized by intravascular blue dye such as Evans blue. In this case, we injected by tail vein 0.5% Evans blue in normal saline (25 mg/kg) 30 minutes before the start of the study. As previously described, short laser exposures (20 to 30 seconds) usually induced no platelet aggregation and no morphologically recognizable endothelial damage; longer exposures were required to induce an aggregate.

Experimental Design

In our experimental design, we used a previously described procedure. An arteriole 30 to 50 μm in diameter possessing a straight segment 200 μm long was randomly selected. A map was drawn of this vessel and adjacent vessels. One site on the vessel was exposed to the laser for 30 seconds to produce a minimal injury, and the site was noted on the prepared map. After the 30 seconds, the laser was discontinued, and the site was observed for up to 3 minutes. If an aggregate formed during that time, the vessel was topically fixed immediately and processed in accordance with the protocols detailed below. Conversely, if no aggregation occurred, a second site 140 to 200 μm upstream from the first site was exposed to the laser until an aggregate formed. This event was also recorded on the prepared map. Because the laser beam interfered with the observations, the following procedure was used to monitor the upstream site: the site was exposed to laser injury for 30 seconds, followed by a 5-second observation period under halogen illumination to determine whether platelet aggregation occurred. If no aggregate formed, another 15-second laser exposure was initiated, followed by another 5-second observation period. This pattern was repeated until an aggregate was identified. The duration of laser exposure required to induce an aggregate was variable. Although the reason underlying such variability is unknown, it is probable that variations in the thickness of the arachnoid and the vessel wall, the contour of the brain surface, and the binding of Evans blue to albumin and its clearance all contributed to this variable response. After aggregation was induced at the upstream site, the laser was discontinued, the induction time was recorded, and continuous observation of both sites was then initiated. The appearance of an adherent mass of platelets at the downstream site signified the “capture” of platelets embolizing from the upstream site or activated at the upstream site. When capture occurred at the downstream site, the vessel was immediately fixed. If no capture occurred, the vessel was fixed after a preselected period of time had elapsed after initiation of aggregation at the upstream site. Three time periods were used: 5, 10, and 15 minutes. In some instances, the aggregate at the upstream site embolized and re-formed during the observation period. In such cases, the duration of the aggregation was determined by adding all the time periods during which the aggregate was observed at the site. All vessels were topically fixed after the appropriate observation period. Under this protocol, arterioles in 14 mice were injured at both an upstream and a downstream site and observed for 5 minutes, arterioles in 14 mice were injured and observed for 10 minutes, and arterioles in 4 mice were injured and observed for 15 minutes. To complement these data, arterioles in 8 mice were also injured only at one site and fixed at a specific time. In addition to these 40 experimental mice, 4 mice were processed as sham controls. Additional control data were also provided by the examination of pial vessels (both arterioles and venules) lying adjacent to the injured pial arteriole. This experimental approach provided samples for electron microscopy that enabled us to evaluate the degree of endothelial injury in relation to the duration of platelet aggregation and in relation to morphological changes signifying platelet activation.

Electron Microscopy

Topical fixation with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer was used. In our experience, this provides optimal pial vessel fixation without dislodging the induced platelet aggregate. After topical fixation, the brain was carefully...
removed and immersed in the same fixative for 2 hours, then transferred into 0.1 mol/L phosphate buffer. Next, the cortical area underlying the craniotomy site was undercut to form a thin slab with intact surface vasculature. The arteriole of interest was identified with the aid of the map made during the in vivo observations. The slab was then placed in 1% buffered osmic acid for 2 hours. After this, it was stained en bloc, dehydrated in chilled ethanol and propylene oxide, and flat embedded in epoxy resin (Ted Pella Corp, Tustin, Calif) for sectioning with the ultramicrotome. Thick sections were cut parallel to the vessel’s long axis, stained with toluidine blue, and with the aid of the prepared map, examined by the light microscope to accurately localize the injury site and the related platelet aggregate. Once the site was identified, subserial thin sections were made, stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy.

**Endothelial Injury**

The degree of endothelial cell injury was graded on a scale from 0 to 5. Grade 5 was used for cases revealing endothelial cell denudation, and grade 4 designated those cases showing luminal membrane rupture. Grades 1 through 3 entailed a more subjective evaluation of the degree of endothelial injury as judged by vacuole formation, dilation of the rough endoplasmic reticulum (RER) and of mitochondria, cytoplasmic lucency, and cell swelling. In general, grade 1 was used for the most minor degree of endothelial injury, reflected by the presence of sporadic vacuolization accompanied in some cases by minor dilation of the RER and/or the mitochondria. Grade 2 corresponded to degrees of intermediate endothelial injury associated with an increased frequency of vacuolization as well as dilation of RER and mitochondria. At this stage, cells also exhibited some degree of swelling, and the cytoplasm showed patchy areas of lucency. Grade 3 included more severe degrees of endothelial damage associated with extensive vacuolization, disruption of RER and mitochondria, and marked endothelial swelling and cytoplasmic lucency. In grading these lesions, the evaluator did not know the length of observation period, duration of aggregation, or duration of laser exposure. When a vessel was evaluated, the degree of damage was awarded according to the area showing the greatest damage. This was always the wall beneath the formed aggregate.

**Platelet Aggregation**

In these same microvascular segments, we also evaluated the degree of platelet activation. Activation was associated with spheroidal forms showing granular centralization, the formation of pseudopods and/or degranulation associated with increased cytoplasmic lucency, and disappearance of most of the granular contents.

To evaluate more critically the relation between an injured endothelial segment and the platelet aggregate related to it, a rectangular grid scaled to an area 20 μm long and 15 μm high was placed over the electron micrographs, with the base of the rectangle centered over the point of maximal endothelial damage. This was always the vessel wall beneath the aggregate (near wall). Platelets included within this rectangular area were counted according to two parameters. One count included all the activated platelets, whether degranulated or not; the second included only the degranulated platelets.

The score of the injury was correlated with the counts of activated platelets, the counts of degranulated platelets, the duration of laser exposure, and the duration of the aggregate.

Additional correlations were calculated between the duration of exposure to the laser and the total number of activated platelets in the rectangular zone defined above. The number of degranulated platelets in this zone was also correlated with the duration of the laser. Correlations were also calculated for the relation between the duration of aggregation and the number of activated platelets.

**Statistics**

To perform these correlative evaluations, we used the nonparametric Spearman correlation coefficient and its statistical significance. We also used linear regression analysis calculating the regression coefficient ($r$) and its statistical significance ($P$). Since the results of the two tests were identical, we report here only the regression analysis.

**Results**

Careful transmission electron microscopic examinations of sham controls did not reveal any evidence of vascular abnormality (Fig 1a). Similarly, the vasculature lying adjacent to the injured pial arterioles revealed no abnormalities.

**Experimental Animals**

Despite variation in aggregate induction time, the degree of ultrastructural endothelial change observed at the onset of aggregation remained constant from one experimental animal to the next. However, once aggregation occurred and samples were taken at various times thereafter, a distinct progression of endothelial changes was observed that paralleled ultrastructural changes in the overlying platelets. At the onset of platelet aggregation and for a period of 5 minutes thereafter, those platelets in close proximity to the endothelium appeared discoid in shape, with their granular contents dispersed throughout the cytoplasm. The related endothelium showed modest change (grade 1), reflected in sporadic vacuolization and perinuclear membrane swelling. Occasionally, modest dilation of the RER and/or mitochondria was observed, with the investing media and adventitia showing no abnormalities (Fig 1b).

At the intermediate stages of aggregation (approximately 5 to 10 minutes after aggregate induction), activated platelets predominated. These consisted of pseudopod-forming and spheroidal forms with centralization of their granular contents. Paralleling this platelet change, endothelial injury increased (grade 2 or 3). There was an increase in both the frequency and degree of the endothelial lesions described above, together with the appearance of areas of cytoplasmic lucency. Again, the medial and adventitial layers did not show any abnormality (Fig 2).
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FIG 1. a, Electron micrograph reveals the wall of a pial arteriole taken from a sham control. Note normal endothelium (END), basal lamina (BL), and smooth muscle cells (SMC). b, Electron micrograph shows a segment of a pial arteriole in the early stages of endothelial injury. Note the presence of sporadic vacuoles (arrow) and minimally dilated rough endoplasmic reticulum (arrowhead). Discoid platelets (P) are present near the wall. Note that these changes occur without exposure of the underlying basal lamina or damage to smooth muscle cells.
FIG 2. Electron micrograph enlargement of an injured area of intermediate grade. Overlying it are activated rounded platelets (P) and/or platelets with pseudopods (Ps). Along the length of the endothelium, note areas of cytoplasmic lucency, vacuoles of unknown origin and variable size (arrows and *), and dilated rough endoplasmic reticulum (arrowheads) and mitochondria (M). These changes again occur without exposure of the underlying basal lamina (BL) or damage to smooth muscle cells.

At the advanced stages of aggregation (10 to 15 minutes after aggregation), the aggregate contained predominantly pseudopod-forming and degranulated forms. The underlying endothelium now showed marked cellular swelling and cytoplasmic lucency plus either luminal membrane rupture and/or endothelial denudation. Cases with rupture of the luminal membrane were assigned grade 4, and cases with denudation were assigned grade 5. In the related media, smooth muscle injury was occasionally seen. This smooth muscle change typically involved vacuolization, and such smooth muscle abnormality was seen only in relation to endothelium displaying luminal membrane rupture (Fig 3a) or denudation (Fig 3b).

The endothelium underlying the aggregate consistently showed significantly more endothelial injury than the contralateral noninvolved side, which showed only occasional lesions.

Statistical Correlations

A significant correlation was found between the number of activated platelets (spheroidal and pseudopod forms whether degranulated or not) and the degree of endothelial injury ($r=.43, P<.03$) (Fig 4). A much greater correlation was found when only the number of degranulated platelets was related to the degree of endothelial injury ($r=.83, P<.001$) (Fig 5). The correlation between the duration of the aggregate at the lesion site and the degree of endothelial injury was also significant ($r=.52, P<.01$) (Fig 6). A significant correlation was also found between the duration of the aggregation and the number of degranulated platelets at the site ($r=.56, P<.01$).

There was not even the suggestion of a correlation between the duration of laser exposure required to induce an aggregate and the degree of endothelial damage nor between duration of laser exposure and the number of activated or degranulated platelets.

Discussion

Our analysis revealed a significant correlation ($P<.01$) between the duration of aggregation and the underlying endothelial damage. A significant ($P<.05$) correlation was also found between the numbers of activated platelets (nondiscoid, degranulated or not) and endothelial damage. Finally, an even greater correlation ($P<.001$) existed between the number of degranulated platelets and the degree of underlying endothelial injury. Thus, it appears that platelet activation and especially degranulation are associated with endothelial injury.

There are two possible explanations for this association. First, it is possible that after the initial underlying
Fig 3.  a, Electron micrograph represents part of the wall of a pial arteriole exhibiting an advanced degree (grade 4) of endothelial injury. Note that the platelets adjacent to the wall exhibit degranulated forms (DP) in addition to spheroidal and pseudopod-forming forms. Note the marked endothelial swelling and lucency (*). The cell in the center of the field manifests luminal membrane rupture (arrows delineate the membrane remnants). b, Electron micrograph is part of a pial arteriolar wall exhibiting very advanced injury (grade 5) with endothelial denudation (arrows delineate boundaries of denuded zone). Note the presence of vacuolization in the smooth muscle layer beneath the denuded area (small arrow). The platelets adjacent to the vessel wall are mainly degranulated forms (DP).
injury that initiated local platelet aggregation or caused capture of passing platelet emboli, the endothelial injury progressed. As the injury progressed, it induced increased numbers of aggregating platelets and more severe shape change and degranulation in the overlying mass of platelets. The second possible explanation is that once initiated, the platelet aggregation, shape change, and degranulation progressed on their own and caused the underlying endothelium to become increasingly damaged.

A decision between these two “mirror image” hypotheses could be made if one could perform a study in which an endothelial lesion was made that would ordinarily initiate platelet adhesion/aggregation but in animals treated with a drug that inhibited platelet adhesion/aggregation. If, despite inhibited platelet aggregation, endothelial damage continued to progress at an undiminished rate, the presence of such progression would support the hypothesis that overlying platelet aggregation and especially platelet degranulation were not necessary for the progression of the damage in this light/dye model. Unfortunately, the interpretation of such a study would be ambiguous, for a reasons. First, any platelet inhibitor, for example a cyclooxygenase inhibitor, might also be directly affecting endothelial cells. The direction of such an effect on endothelial damage is not known. Second, exposure to the noxious insult, in our case light/dye, is sufficient to initiate damage but not so prolonged as to directly produce more severe injury. We are interested here in the progression of the damage after the noxious stimulus is removed. To be sure that an initial injury has occurred, we would have to continue to use initial platelet aggregation as our visible end point and then stop the noxious stimulus. However, if platelet aggregation were inhibited, this would require a more prolonged endothelial insult to initiate aggregation. By then, the insult itself might have produced more severe, and now perhaps even progressive, endothelial damage. Thus, such a study, using inhibitors of aggregation, would actually be biased in favor of finding severe damage before prolonged overlying aggregation or platelet degranulation had occurred. Thus, for the two reasons cited, such a study using inhibitors of platelet aggregation cannot really decide between our two competing hypotheses.

However, the merits of each hypothesis may be examined from the evidence at hand. Favoring the hypothesis that platelets caused progression of damage are the previous studies of others who suggested that platelets or platelet products can damage endothelium.3,10,20,25 Especially relevant are studies showing that platelet products may be toxic to endothelium.3,8,25 Against the hypothesis that the damage would have progressed on its own is the actual recovery of physiological function (recovery of endothelium-dependent relaxation) at the damaged site within an hour after injury in situations in which no platelet aggregate ever appeared.19 Moreover, in the present study, damage progressed after removal of the laser, and the degree of damage was unrelated to duration of the laser. In addition, there were several instances when an aggregate embolized shortly after formation and did not re-form. In these cases, even after 10 minutes, endothelial damage was only modest. Thus, laser damage sufficient to initiate aggregation did not progress in the absence of persistent aggregation. These facts all suggest that the initial laser insult was not the cause of the progression of endothelial injury. The only other factor present to account for progression is the overlying platelet aggregate. The significant correlation between the degree of endothelial damage and either the dura-

![Fig 4. Plot of the number of nondiscoid platelets (whether degranulated or not) in a 300-μm² area over the area of maximal endothelial injury against the degree of endothelial injury.](image_url)

![Fig 5. Plot of the number of degranulated forms in a 300-μm² area of maximal endothelial injury against the degree of endothelial injury.](image_url)

![Fig 6. Plot of the duration of aggregation before topical fixation against the degree of endothelial injury.](image_url)
tion of overlying aggregation or the number of overlying activated platelets could be interpreted to support either hypothesis. However, it is particularly relevant to note that the correlation between endothelial damage and the number of overlying activated platelets is much higher when only degranulated platelets are counted than when all activated platelets, degranulated or not, are counted. After shape change, the activated platelets are transformed into degranulated platelets on a one-to-one basis; each degranulated platelet comes from an activated platelet. Thus, if progressing endothelial damage caused progressive platelet activation with ensuing degranulation, the correlation between damage and the total number of activated platelets should not be greater than the correlation between damage and the number of degranulated platelets. Instead, the regression coefficient for damage and number of degranulated platelets was 0.83, whereas that for damage and total number of activated platelets (whether degranulated or not) was only 0.43. This difference would seem to support the hypothesis that degranulation was in some way a cause of the progression of the endothelial damage.

Although there may still be some room for doubt, we believe that the points summarized in the preceding discussion support the hypothesis that platelet aggregation, in this model, can contribute to progression of underlying endothelial damage. We do not assert that platelet aggregates are essential in all cases for endothelial damage to progress. Nevertheless, if platelet aggregates even on occasion can cause progression of underlying endothelial damage, this is an important consideration in understanding the pathogenesis of cerebrovascular disease.

For example, the sequence of progressive endothelial change we have described and its direct relation to the accompanying maturation of the platelet aggregate may be relevant to various cerebral circulatory disorders, particularly to transient ischemic attacks. Transient ischemic attacks are thought to result from platelet emboli. However, the transient symptoms of repeating transient ischemic attacks are often stereotypical. Although it is possible that streamlines of flow always carry emboli or activated platelets to the same place, another explanation is possible and is supported by our data. Of all the platelets in an embolic shower, only those passing a site of endothelial injury may adhere to that site. Initially, only a modest injury may be required to attract previously activated platelets to that site. The initial collection of adherent aggregating platelets may soon disintegrate and pass on. While present, however, they may damage or redamage the endothelium. The increased damage makes the site selectively attractive to activated platelets or emboli in the next wave coming from some upstream source. They therefore adhere selectively to the site of previous embolization, which then sustains more damage. With each successive wave of adhesion, symptoms repeat, since the same vessel or group of vessels is the site of adhesion/aggregation. Ultimately, instead of disintegrating and passing on, the adhering, aggregating platelets may lead to a longer-lasting thrombus and permanent neurological damage.

Precisely how the platelets might contribute to the progression of endothelial damage remains unclear. Although many investigators have suggested or identified platelet mediators that may cause endothelial injury, it remains controversial which of these may be operative in the pathogenesis of that injury. One in vitro study has implicated thromboxane A2 and, to a greater extent, serotonin. It is conceivable that one or a combination of these agents may be mediators of the continuing endothelial degeneration. Thromboxane mimetics and serotonin have frequently been applied topically to pial vessels to demonstrate the vasoactive properties of these mediators. No one appears to have performed electron microscopic studies to assess the endothelium for damage after such brief exposures. In vivo observations do not suggest damage. However, external application may not mimic local release by platelets adjacent to endothelium. Even when externally applied mediators reach the endothelium, they may not pass through it to reach the lumen and the luminal surface of the endothelium. It is at that surface that damage may be triggered by factors released from platelets.

Of added interest was the presence, in our study, of smooth muscle injury accompanying some of the instances of very severe endothelial damage. The basis of this injury is unknown.

If platelet products do, indeed, damage endothelium, then one might attempt to break the cycle of damage and redamage not only by therapies directed against platelet adhesion and aggregation but also by identifying and directing therapies against the endothelium-toxic agents released by the platelets. For this reason, it is important to identify more precisely the mediators involved and the mechanism by which they injure the endothelium.

Indeed, tests of serotonin blockers might, for example, appear to validate our interpretation of the data if such treatment greatly reduced the endothelial damage. However, inhibitors of serotonin might also interfere with platelet aggregation and, ultimately, with the release of other possibly toxic mediators. Thus, because of the interaction of platelet products with platelets themselves, an in vivo study identifying the toxic substances released by platelets will not be easy to carry out.

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References

The study by Said et al above examining the relation between platelet aggregation in the cerebral circulation of mice and the degree of endothelial damage provides data suggesting that processes involved in the aggregation of platelets could promote endothelial injury. Although it is quite difficult to eliminate an alternative interpretation of the data, as discussed by the authors of this study, that the observed degree of platelet activation is a consequence of the degree of endothelial injury, certain observations suggest that platelet-induced endothelial damage could be an important interaction in cerebral vascular pathophysiology.

The most convincing evidence in the present study supporting platelet activation as a cause of endothelial injury is the lack of a relationship between the duration of the noxious stimulus (laser/dye) required to initiate endothelial damage and platelet aggregation, and the progression of the damage. The damage progressed only if platelets remained over the site. The laser is no longer on during this period. Moreover, in the cerebral circulation, the laser light/dye treatment has been shown to temporally impair endothelium-dependent relaxation,1,2 as has been observed in other microcirculatory preparations,2 in a manner that recovers beginning within 1 hour after exposure.2 In the absence of platelet aggregation at the site of impaired endothelial function, the laser light/dye treatment itself does not appear to produce the endothelial cell injury observed in the present study during platelet aggregation. Although the data of Said et al do not rule out the possibility that the observed degree of platelet aggregation is a consequence of the degree of endothelial dysfunction set in motion by light/dye, the design of the study and previous work of this group strongly suggest that platelet activation/aggregation promotes endothelial cell injury.

Platelet aggregation as a source of endothelial injury, as discussed by Said et al, has been examined only in a limited manner. Since platelet-induced endothelial injury now appears to be a potentially important interaction in vascular pathophysiology, critical questions need to be examined in this area, such as identifying the mechanism through which platelets cause endothelial injury and how this process could contribute to thrombosis-associated vascular disorders, including transient ischemic attacks in the cerebral circulation.

Michael S. Wolin, PhD, Guest Editor
Department of Physiology
New York Medical College
Valhalla, NY

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S Said, W I Rosenblum, J T Povlishock and G H Nelson

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