Platelet Aggregation in the Cerebral Microcirculation
Effect of Aspirin and Other Agents

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SUMMARY After a certain period of time filtered ultraviolet light produces platelet aggregation in microvessels on the cerebral surface of the mouse, but only when sodium fluorescein is first injected intravascularly to provide a light-absorbing, heat-generating target. The platelet aggregates fluoresce. They occur only in the illuminated field and adhere to arteriolar and venular walls. Vasoconstriction is not detected prior to or up to 30 seconds after aggregation. Electron microscopy reveals damaged endothelium and undamaged red cells, as well as aggregates consisting almost exclusively of platelets in varying stages of aggregation, pseudopod formation, and degranulation. The time between onset of the noxious stimulus and appearance of the first aggregate can be measured as the vessels are observed microscopically. This “time of aggregation” is prolonged by pentobarbital as opposed to urethane anesthesia, and also is related to time elapsed after craniotomy. We also found that aspirin and indomethacin significantly prolong time to first aggregate, but only on the arteriolar side of the circulation. This is so even though the composition of the aggregates is the same on both the arteriolar and venular sides. Heparin has no effect.

PLATELET aggregates can be produced in the microvessels on the cerebral surface by damaging these vessels mechanically or by suffusing them with ADP.1-4 Several years ago5 we discovered that aggregates were produced in these vessels if the vessels were exposed to a mercury lamp; we also found that aspirin and indomethacin significantly prolong time to first aggregate, but only on the arteriolar side of the circulation. This is so even though the composition of the aggregates is the same on both the arteriolar and venular sides. Heparin has no effect.

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METHODS

MICROSCOPE AND LIGHT

A 200-watt mercury lamp was used to induce damage and to provide light for microscopic examination of the vessels after damage. When observations with some other light source were desirable, a tungsten lamp was substi-
tuted by shifting a slide in the dual lamp mounting. The light was focused as incident light, through a Leitz Ultrapak microscope. An 11× objective was used. The light intensity was measured in the focal plane with a silicon diode detector calibrated against a standard established by the Bureau of Standards. The light was filtered with two Leitz (KG-1) heat filters plus two Leitz dichroic filters that filter both heat and ultraviolet (UV) rays. Finally a BG-12 (blue) filter and appropriate neutral density filters were also interposed in the light beam to give an intensity of 14 × 10⁶ µW/cm² (±2.5 %). This value was checked daily.

Generally, the observations were then made with 10× ocular and dipping cone (i.e., immersion attachment). In one study, observations were made via closed circuit television (TV) microscopy, and an image-splitter and strip chart recorder were used to measure diameter.13 A series of stopwatches was used to measure the time between onset of trauma and time to first aggregate. Time to first aggregate was defined as the appearance of fluorescent aggregates adherent to the vessel wall. In practice, aggregates could not be recognized prior to this point. An arteriole and a venule were present in each field, so that onset of platelet aggregation could be measured in each. The onset of trauma was defined as the instant that the observer switched from tungsten to the filtered mercury light. This was 5–10 seconds after intravenous (tail vein) injection of 0.2 ml of 2% sodium fluorescein. Appropriate barrier filters caused the light emitted by the excited fluorescein to appear bright green or yellow-green. The damaging light was left on to determine how long it would take for flow to be stopped entirely by buildup of aggregates; however, this parameter proved less useful in discriminating drug effects and will not be referred to further. It should be noted that no damage was inflicted by the mercury light, filtered in the manner described above, unless fluorescein was infused into the blood stream. This was established not only in several dozen control experiments during the present study, but also in a vast number of experiments over the past 5 years, in which this system of illumination has been our standard equipment for long-term observation of pial vessels by TV microscopy. It is assumed that the dye absorbs light and thereby acts as a heat-generating target, the heat then damaging the vessel. This assumption is identical to that made for the well accepted laser model of microvascular damage which employs a different dye as a heat-generating target.6,8,11

**ELECTRON MICROSCOPY**

After inducing aggregation in the previously exposed vessels, osmic acid was dripped on the vessels. The mouse was then killed and the blackened area was excised, minced, and placed in identical fixative. After fixation, routine dehydration and embedding were accomplished and 1-μm sections were prepared, as well as thin sections on grids for observation with an electron microscope.

**PREPARATION OF VASCULAR BED**

Male, ICR (Dublin Farms) mice were anesthetized with sodium pentobarbital or urethane (see Results). Tracheotomy and craniotomy were performed, the dura was stripped, and a drop of artificial cerebrospinal fluid (CSF) (Elliott's solution14) was placed on the exposed site. Because the results were affected by the time elapsing between surgery and onset of vascular damage, scrupulous attention was given to keeping this interval the same for all mice in a given experiment. Drug-treated mice were alternated with control mice for study on any given day.

**DRUGS**

Aspirin (acetylsalicylic acid, USP) was dissolved (3 mg/ml) in distilled water plus sodium bicarbonate to give a solution of pH 7.38 ± 0.70. The control was bicarbonate-buffered artificial CSF14 at the same pH. These solutions were injected intraperitoneally (ip). Indomethacin was dissolved in saline with pH adjusted to between 10 and 11 with NaOH and injected ip. The concentration of indomethacin was approximately 5 mg/ml after pH adjustment. NaOH solutions at the same pH served as the control. For studies of the effects of heparin, sodium heparin (150 U/mg) was dissolved in saline and 10 mg/kg were injected ip. Equal volumes of saline served as the control. All solutions were prepared fresh, daily.

**GASES**

Arterial Pco 2, Po 2, and pH were measured in control and experimental mice at the end of each experiment with an Instrumentations Laboratories Ultramicro blood gas analyzer.

**BLOOD PRESSURE**

A tail cuff and pulse transducer recorded systolic blood pressure when required.15,17

**Results**

When pial vessels of dye-injected mice were exposed to UV light for 20–30 seconds, aggregation of brightly fluorescent material was noted, adherent to the vessel wall, first in the venule, then in the arteriole. The faster response on the venular side was highly significant statistically and is obvious in all the data displayed below. As exposure to light continued the aggregates built up from the wall toward the center of the lumen. The buildup was much more rapid in the arteriole, where complete closure of the lumen generally occurred rapidly from one or two aggregates on opposite walls. Light microscopy revealed aggregates to be amorphous material staining like platelets.

**ELECTRON MICROSCOPY**

As revealed in Figure 1 the aggregates consisted of platelet masses, which often showed pseudopods or degranulated zones. Rarely, endothelial damage was observed beneath a platelet (Figs. 2 and 3). Leukocytes were infrequent and fibrin was rare. Red cells in the vicinity of platelet aggregates showed no damage. Control preparations from mice injected with fluorescein, but exposed only to tungsten light, showed no alterations.

**EFFECTS OF ANESTHETIC**

In these experiments, exposure to filtered UV light occurred within 5 minutes after craniotomy. Twenty mice
Figure 1 An arteriole is shown containing a mass of aggregated platelets. Some are degranulated or display pseudopods without granules (arrow). Original magnification 13,000x.

were anesthetized with urethane, (2 mg/g, ip) and sodium pentobarbital (6 mg/100 g), with more needed as required. The time from exposure to first aggregate was measured and found to be significantly longer in the pentobarbital group, both in arterioles and venules (Table 1). The arterial Pco₂ and pH were also significantly different in the two groups, the pentobarbital-treated mice having higher Pco₂ (40 ± 13 mm Hg vs. 57 ± 13; \( P < 0.02 \)) and lower pH (7.19 ± 0.10 vs. 7.12 ± 0.08; \( P < 0.02 \)).

RELATIONSHIP OF BLOOD PRESSURE TO FIRST AGGREGATION

Twenty mice were anesthetized with pentobarbital and the systolic pressure was measured immediately before exposure of pial vessels to filtered UV light. No correlation (\( r = 0.03 \)) appeared between blood pressure (BP) and time to first aggregate. The mean BP for the group was 93 ± 17 (mean ± so) mm Hg and the time to first aggregate was 48 ± 18 seconds in arterioles and 29 ± 6 seconds in venules. The experiment was performed within 15 minutes after craniotomy.

RELATIONSHIP OF TIME AFTER CRANIOTOMY TO TIME OF FIRST AGGREGATION

Twenty mice were anesthetized with pentobarbital; 10 were tested 5 minutes after surgery and 10 were tested 15 minutes after surgery. The latter displayed a significant increment in the time between exposure to filtered UV light and first aggregation observed in arterioles. An effect on venular aggregation time was not observed (Table 2).

EFFECTS OF ASPIRIN

In these experiments urethane was the anesthetic. Aspirin significantly prolonged the time required to initiate aggregation, but the effect was seen only in the arterioles and not in the venules. This is shown in Table 3. Mice tested 30 minutes after aspirin or control were operated on
35 minutes before testing. Mice tested 60 minutes after aspirin or control were operated on 65 minutes before the test. The diameters of the arterioles and venules were essentially the same as those in the experiments described above. The blood pH of the aspirin-treated mice was usually significantly higher than that of the controls, and the arterial Pco₂ was lower, apparently because of the effect of aspirin as a respiratory stimulant. However, the effects of aspirin were not correlated with pH (r = 0.26) and the effect of aspirin on aggregation was observed even in experiments in which pH and Pco₂ were like those of the control group. For example, an experiment was performed in which the vessels of 20 controls and 20 aspirin-treated mice were exposed to the filtered mercury lamp for only 10 seconds out of every 2 minutes. In the controls aggregation was first observed in arterioles after a total exposure to the mercury lamp of 39 ± 8 seconds, and in the venules after a total exposure of 17 ± 2 seconds, values comparable to those observed with continuous exposure to the lamp. Aspirin, 25 mg/kg, given 60 minutes before initial exposure to the lamp, significantly prolonged aggregation time in the arterioles (46 ± 9 seconds compared to 39 ± 8 seconds, P < 0.03) but the blood Pco₂ and pH were comparable to the values in the controls (39 ± 14 mm Hg vs. 38 ± 9 mm Hg, and 7.28 ± 0.01 vs. 7.25 ± 0.06).

EFFECTS OF INDOMETHACIN

Indomethacin significantly prolonged the time to first aggregation, but like aspirin, indomethacin only was effective on the arteriolar side of the circulation (Table 4). In each of the experiments illustrated the blood gases and pH

**FIGURE 2** A platelet is adherent to the wall of a venule. The endothelium is denuded at the point of adhesion and the underlying basement membrane (arrow) is exposed. Original magnification 45,000x.
EFFECT OF HEPARIN

Twenty mice were given heparin (10 mg/kg, ip) and compared with 20 controls given only diluent. The heparin had no effect on time to first aggregate in either arterioles or venules. The heparin was given 30 minutes before testing.

EFFECTS OF INJURY ON ARTERIOLAR DIAMETER

In 10 mice anesthetized with urethane, the diameter of arterioles was measured with a TV screen and image-splitter. Measurements were made for 5 minutes prior to injection of sodium fluorescein, and in all instances a steady baseline was observed; that is, diameter did not change and, of course, no platelet aggregates were noted. After injection of fluorescein the vessels were observed until platelet aggregation was noted. Attempts to monitor diameter beyond this time were generally unsuccessful after an additional 30 seconds, because aggregation buildup made it difficult to distinguish the true boundaries of the vessel wall. This was true even though the aggregates fluoresce and the wall does not. The situation is much worse if tungsten light and direct microscopic observation are used, because the platelet aggregates then appear as white bodies that provide even less differentiation between lumen (including aggregate) and vessel wall.

The 10 arterioles measured 34 ± 6 (mean ± SD) μm in the control period and 35 ± 6 μm at the time of platelet

Figure 3 A mass of platelets is adjacent to endothelium which appears attenuated but not definitely denuded (open arrow). Fibrin bundles (solid arrow) are found between platelets. Fibrin was an extremely rare component of the aggregates. Original magnification 45,000×.
aggregation. This difference is not significant, nor were significant changes in diameter noted between onset of trauma and time of aggregation, or in the short interval of reliable measurement following aggregation.

**Discussion**

Platelet aggregation in the vessels within the subarachnoid space on the cerebral surface has not been studied extensively. In a few investigations, the larger surface arterioles (100–200 μm) were pinched with varying degrees of force and platelet aggregation was observed at the site of "major" trauma. Similar platelet aggregates were also observed after local injury with cold, heat, or chloroform. More recently Gautier et al. have used the pinching technique on larger cerebral surface vessels. Mechanical trauma has also been utilized in studies of platelet aggregation in other vascular beds such as mesentery, but the principal tool for producing platelet thrombi in vivo has been the laser. In the laser studies vessels under 100 μm in diameter have been studied, usually in rabbit ear chambers, mesentary of various animals, or hamster cheek pouch. The results of experiments described in the present paper may be compared with those of the previous workers, suggesting that our general observations are not dependent on peculiarities of the vascular bed we employed to elicit aggregates.

**MORPHOLOGICAL CONSIDERATION**

Electron microscopy has shown that the thrombi formed in our experiments were overwhelmingly composed of platelets, as were the thrombi produced by pinching or by laser. The degree of endothelial damage after exposure to a laser beam seems dependent on the strength of the laser and the presence or absence of a dye as a heat target. With a ruby laser and no dye, endothelial damage was present but modest or moderate and RBC were severely damaged. In fact, with such a laser the platelet aggregates may be induced primarily by substances released by injured RBC forming the nidus of the aggregate. On the other hand a He-Ne laser combined with an energy-absorbing dye produces much more severe endothelial damage with exposure of underlying basement membrane and also produces RBC damage apparently as severe as that produced by the ruby laser, if electron microscopic criteria are used. By the same criteria our filtered UV light coupled with sodium fluorescein produces no RBC damage and modest to moderate endothelial damage. We assume that it is the damaged endothelium which is critical for the localization of aggregates in our study, because aggregates do not adhere to vessels outside the illuminated field, and aggregation is not potentiated in a second field by prior irradiation and aggregate formation in the first field. Hovig et al. also assume that endothelial injury accounts for aggregate adhesion to the vessel wall, hence aggregate localization, in the laser model.

**DIFFERENTIAL SPEED OF RESPONSE IN ARTERIOLES AND VENULES**

Our data indicate that aggregation occurs first in the arterioles, then in the venules. Since our light beam incor-

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### Table 1: Effect of Pentobarbital on Platelet Aggregation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pentobarbital (n = 20)</th>
<th>Urethane (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of arterioles (μm)</td>
<td>32 ± 7</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>Time to first aggregate, arterioles (sec)</td>
<td>33 ± 7</td>
<td>40 ± 10*</td>
</tr>
<tr>
<td>Diameter of venules (μm)</td>
<td>32 ± 6</td>
<td>32 ± 9</td>
</tr>
<tr>
<td>Time to first aggregate, venules (sec)</td>
<td>22 ± 5</td>
<td>30 ± 5*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD; *P < 0.05, urethane vs. pentobarbital.

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### Table 3: Effect of Aspirin on Platelet Aggregation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Arteriole</th>
<th>Venule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin, 25 mg/kg, ip, given 60 min before test (n = 20)</td>
<td>43 ± 10*</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>Control (n = 20)</td>
<td>36 ± 6</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Aspirin, 100 mg/kg, ip, given 60 min before test (n = 10)</td>
<td>102 ± 62*</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>41 ± 7</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Aspirin, 100 mg/kg, ip, given 30 min before test (n = 10)</td>
<td>52 ± 17*</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>36 ± 5</td>
<td>20 ± 4</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD; *P < 0.01 compared to appropriate control.

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### Table 4: Effect of Indomethacin on Platelet Aggregation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dose</th>
<th>Arthuriole</th>
<th>Venule</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg/kg (n = 10)</td>
<td>70 ± 8*</td>
<td>20 ± 4</td>
<td></td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>37 ± 7</td>
<td>20 ± 4</td>
<td></td>
</tr>
<tr>
<td>12.5 mg/kg (n = 5)</td>
<td>6 ± 17*</td>
<td>17 ± 3</td>
<td></td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>39 ± 10</td>
<td>18 ± 2</td>
<td></td>
</tr>
<tr>
<td>6.25 mg/kg (n = 10)</td>
<td>34 ± 6</td>
<td>17 ± 3</td>
<td></td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>30 ± 5</td>
<td>16 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD; *P < 0.05, 5 minutes vs. 15 minutes.

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* Significant difference from appropriate control (P < 0.01).

† Significantly different from appropriate control (P < 0.05).
porates the entire microscopic field, our method permits simultaneous observation of arteriole and venule. In hundreds of observations, aggregation never occurred in the former without first occurring in the latter. Callahan et al.28 found that aggregates occurred more readily in venules than in arterioles following electrical injury of vessels in hamster cheek pouch. Kochen and Baez27 made similar observations after laser injury of vessels in rat mesentery. This result has been explained by the high flow velocity on the arterial side,28 the idea being that high flow velocity may inhibit platelets from sticking to arteriolar endothelium. However, Begent and Born21 showed that aggregates actually grow faster as velocity increases, the growth being related to the numbers of aggregates passing a spot in a given period of time. These apparently contradictory ideas may be reconciled by further reference to the data and remarks of Begent and Born, who also found that above a given velocity (about 400 μm/sec) the growth rate of aggregates declined and above 3,000 μm/sec they failed to form. In the pial vasculature, as in other vascular beds, RBC velocity near the arteriolar wall is higher than that near the venular wall.22 Thus in the pial microvasculature, as elsewhere, the time to first recognizable aggregate may be a velocity-dependent phenomenon.

On the other hand, Arfors and Bergqvist23 have shown that platelet aggregability is significantly higher on the arterial side of the circulation and that this is not a velocity-dependent phenomenon. By aggregability they mean sticking platelets as a percent of available platelets. Perhaps this parameter tests some factor or factors other than, or in addition to, the factors determining either time to first aggregate or growth rate of an aggregate.

EFFECTS OF ANESTHETIC

Our data show that time to first aggregate is prolonged in the presence of pentobarbital, compared to urethane. We cannot say whether this is due to an effect of the anesthetic on endothelium or platelets, or both. The effect may be due to the respiratory acidosis displayed by the pentobarbital-treated mice as compared to the urethane-treated mice. However, we have never been able to demonstrate a significant correlation between time to first aggregate and either arterial PCO₂ or pH. Others have shown a depression of aggregate growth rate or defective platelet aggregation in vivo and in vitro24 following use of pentobarbital anesthetic, and in one study impaired growth rate with consequent diminution in embolization was demonstrated in pial vessels, when pentobarbital was compared with urethane.3

EFFECTS OF OTHER DRUGS AND LOCALIZATION OF DRUG EFFECT

Perhaps the most striking fact in our data, and one not readily found in the available literature, is a preferential effect of certain drugs on the arteriolar side of the circulation. These data are especially noteworthy since the drugs in question are aspirin and indomethacin, both inhibitors of prostaglandin synthesis and both known to be potent inhibitors of platelet aggregation in vitro, with the former being actively considered in the therapy or prophylaxis of cerebrovascular accidents. Traditionally platelets have been thought to play a lesser role in venous thrombosis than in arterial thrombosis and thus it might be expected that anti-aggregation drugs would be less effective on venous thrombosis. Conversely it was expected that anticoagulants (i.e., antifibrin clot) would be especially efficacious on venous lesions.26 In fact, few studies have claimed successful treatment of venous lesions with drugs whose major mode of action was thought to be directed against platelet aggregation.26 In our model, platelets are virtually the sole element in the thrombus, regardless of its location in arteriole or venule. Consequently, it is particularly important to note that aspirin and indomethacin were selectively effective on arteriolar aggregates. This implies that there may be factors other than the relative numbers of platelets in a thrombus to account for the difficulties in establishing the efficacy of anti-aggregating drugs on venous thrombosis.

With respect to the protective effects of aspirin and indomethacin, results of the opposite of ours appear to have been demonstrated by Arfors et al.27 They showed only an acceleration of clotting after aspirin, and this only on the venous side. Arfors et al.28 have in their study decreased the time to formation of a hemostatic plug in the venules, thereby decreasing rather than enhancing the difference between plug formation time in arterioles and veins. We are unaware of other in vivo studies of indomethacin.

On the other hand, several other workers have confirmed our observation that aspirin does inhibit thrombosis in vivo.8,29 In fact, Gautier et al.4 demonstrated a protective effect of aspirin on platelet aggregation in pial vessels. Wiederman9 made the interesting observation that aspirin may be protective only in models producing significant endothelial injury and not in models in which injured RBC released agents responsible for the aggregates. This would confirm the importance of endothelial injury in our own model.

We have no explanation for the preferential effect of aspirin and indomethacin or arteriolar aggregates in our model. Since aggregates of platelets adhering to the vessel wall are more rapidly produced on the venous side, it may be that the factors responsible for initial aggregation simply overwhelm the capacity of anti-aggregating drugs to alter initial aggregation in the venules. We do not know whether the effect we observed is demonstrable in other vascular beds. In fact, Kovač et al.8 showed protection by aspirin only on the venous side of the mesenteric bed. It is of interest that a consistent difference between arteriolar and venular endothelium has been demonstrated in virtually all organs of the body, including brain. Thus fibrinolysis by aspirin only on the venous side of the mesenteric bed. It is of interest that a consistent difference between arteriolar and venular endothelium has been demonstrated in virtually all organs of the body, including brain. Thus fibrinolysis by aspirin only on the venous side of the mesenteric bed. It is of interest that...
Alternatively, one might offer a less conventional hypothesis and speculate that drugs had an effect on the vessel wall itself, and that the protective effect of drugs was therefore related to the behavior of the walls with which they interacted. However, by considering chemical differences between arteriolar and venular endothelium, we do not mean to discount the importance of flow velocity, as discussed in an earlier paragraph. If slower flow makes adhesion or aggregation, or both, easier on the venous side this in itself could make a protective drug action on the platelet more difficult to demonstrate on the venous side.

Heparin had no effect in our experiments. This was expected since the major effect of heparin is on clotting factors (i.e., anticoagulation) rather than on platelet aggregation per se. In fact, Aftors et al.,10 Honour and Mitchell,2 and Born and Philp3 also failed to demonstrate an effect of heparin on either laser-induced or mechanically induced aggregates in rabbit ear chambers or rabbit pial vessels.

Whether we view these drug effects in the conventional manner, as arising from drug-platelet interaction, or in a less conventional manner, as arising from interaction of drug and vessel wall in the literature, it is important to reiterate that the drug effects demonstrated here are in conformity with several reports in the literature. This is true for the neutral effects of heparin and for the protective effects of aspirin and indomethacin discussed above, as well as for the effects of pentobarbital discussed earlier. Since our data are consonant with much of the literature and represents tests of drugs with widely varying structure and with presumably different biochemical modes of action, it seems permissible to attribute our results to the same factors that operate in other models, although the precise nature of these factors is unknown. It seems highly unlikely, in view of the consonance of our data, using several drugs, with the data of others, that the effects seen here are produced by some special mechanism peculiar to the use of fluorescein. In this regard it should be noted that fluorescein by itself failed to alter platelet morphology (see Results), nor did it alter the platelet count. Thus, we have no reason to believe that drugs were acting on a fluorescein-altered platelet.

We should also note that we have not used in vitro tests to establish the efficacy of the drugs in this study, since their efficacy is well known. Moreover, many workers have pointed out that in vitro results do not necessarily mimic in vivo data with respect to enhancement or inhibition of platelet aggregation. Certainly, if an action of drugs on the vessel wall is to be considered, in vitro systems may not provide an adequate basis for drug screening.

With respect to the dose of aspirin given here, we must note that the effects of aspirin may be dependent on its acetyl group, and this group may be cleaved by hydrolysis prior to absorption of the volume administered via intraperitoneal injection. This may occur particularly if the aspirin is administered at neutral pH as it was here. Thus, if our results are dependent on the action of unhydrolyzed acetylsalicylic acid, then other routes of administration or other solvents might permit us to demonstrate an effect at lower dosages than those used here. However, we cannot be certain that the acetyl group is responsible for the effects we observe, especially if any of these effects are related to an interaction of aspirin with the vessel wall rather than with the platelet. Although data are accumulating which support a role for the acetyl group in the inhibition of platelet aggregation, there are relatively few data to establish a role for this group in the anti-inflammatory actions of aspirin. Consequently, any speculation concerning the interrelation of dosage to blood levels of unhydrolyzed aspirin must await further elucidation of the possible effect of aspirin on vessel walls, and the mode of aspirin’s action as an anti-inflammatory drug.

**PLATELET AGGREGATION AND VASCULAR DIAMETER**

Our study failed to demonstrate vasospasm either before or concurrent with platelet aggregation. This is contrary to the findings of workers who were able to observe buildup of platelet aggregates at sites of previously induced arteriolar constriction while constriction was still present or immediately prior to the appearance of constriction.2,8,21 In two of these studies hemorrhage was not part of the model. As noted in Results, observational difficulties precluded measurements of diameter beyond the 1st minute of platelet aggregation. In the laser-dye model, spasm was most pronounced after the 1st minute. On the other hand, pronounced spasm would have been noted in our studies beyond the 1st postaggregate minute even though precise measurements of diameter were no longer possible. In view of the possibility of confusing vessel wall with adherent platelet aggregates and thereby erroneously concluding that spasm is present,12 we feel that the presence of spasm in vessels with platelet aggregates should be accepted with caution.
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