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SUMMARY Platelet aggregation was produced in pial arterioles by exposing them to appropriately filtered light from a mercury lamp, following intravascular injection of sodium fluorescein. The dye acted as a target for the radiant energy and initiated a sequence of events resulting in the platelet aggregation. The aggregates adhered to the vessel in which they first appeared. When a microneedle punctured the brain adjacent to a subsequently irradiated arteriole, platelet aggregation was inhibited, even though the arteriole was not touched by the microneedle. Inhibition was manifested by prolongation of the exposure time required for the light-dye stimulus to initiate an adherent aggregate and by lengthening the time required for the aggregate to grow and totally block erythrocyte flow in the affected vessel. It is suggested that a material(s) diffuses from the zone of brain puncture, to and through the arteriolar wall, with a resultant inhibition of aggregation. It is noted that the inhibiting effect is reduced as the distance between puncture and wall increases.

WE HAVE suggested that vascular responses to brain injury may have two components. The vessels themselves may be damaged and respond to their own injury, and undamaged vessels may respond to injury of the adjacent brain. We have studied the effects of one type of damage to the vessel itself. Then we have studied the effects of brain trauma on adjacent, undamaged vessels. In the present study we combined the two models in order to observe their interaction.

Our model for producing vascular injury involves a new technique which produces platelet aggregation in microvessels on the cerebral surface of living animals. A mercury lamp is used to illuminate these "pial vessels." This illumination, properly filtered, results in no visible damage unless sodium fluorescein is injected intravenously. The technique appears to mimic a previously established method, in which a laser beam, together with heat absorbing dye in the vascular lumen, induces local platelet aggregation presumably by producing thermal injury of the endothelium. The new technique employing sodium fluorescein, has the advantage of producing aggregates that fluoresce so that their first appearance is very easy to detect.

Following our studies with light and dye, we began a series of investigations which showed that platelet aggregates could be produced in, and would adhere to the wall of, a venule which was itself undamaged by penetrating injury to adjacent brain. This work has been reported in abstract form and will be the subject of a subsequent, detailed, report.

The model employing light and dye, produced aggregates in both arterioles and venules. The model employing brain injury, produced aggregates only in venules. If a tissue substance caused the latter by diffusing from the zone of brain damage through the wall of the undamaged venule, then failure of aggregation in arterioles might result for one of two reasons: either the arteriolar wall impeded diffusion of the substance, or the effect of the hypothetical diffusing substance was too weak to overcome the larger disaggregating forces produced by the more rapid flow of blood on the arteriolar side. If one or both of these hypotheses were correct, we predicted that platelet aggregation produced in arterioles by light and dye, would be accelerated by previous puncture of adjacent brain, since the latter would release a diffusible factor(s) whose effects in arterioles would be insufficient to produce aggregation by themselves, but whose action would be additive with that of the light and dye. Much to our surprise, if exposure to light and dye was preceded by puncture of adjacent brain, aggregation in arterioles was not accelerated; rather, aggregation was significantly inhibited, as described below.

Methods Male mice, ICR strain, were anesthetized with urethane and the surface of the brain exposed as previously described. The field was observed through a Leitz Ultropak microscope, employing an 11× objective and dipping cone. A drop of artificial cerebrospinal fluid was placed between the cone surface and the brain surface. Vessels were studied either with a tungsten lamp or with the aid of a 200 watt mercury lamp filtered by both heat and UV filters. The temperature in the field rises less than 0.5 degrees centigrade so that very long periods (over an hour) of observation resulted in no discernible damage. When sodium fluorescein (0.2 ml of a 2% solution in saline) is injected intravenously, and the mercury lamp is employed with a suitable, additional, excitation and barrier filter, fluorescing platelet aggregates appear, generally within 45 seconds, and adhere to the endothelium of arterioles and venules, but only at the site exposed to light. The platelet composition of the fluorescent aggregates has been confirmed by electron microscopy. When observed with ordinary illumination they look just like the “white bodies” described in the classical literature concerning platelet aggregation. In general, the “first” aggregate noted in this system is an aggregate adhering to the endothelium. This begins as a very small mass and grows. Pieces may break off, and other aggregates may appear adhering to other sites in the illuminated field. These are not quantified by our current

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system of measurement. The mercury lamp remains on during the entire period during which we observe the phenomena just described.

In the present study animals were alternated as follows: one animal was prepared as described above, an arteriole was preselected, and fluorescein injected. The observer switched from tungsten to mercury light and utilized a stopwatch to measure the period preceding the appearance of the first discernible platelet aggregate. As aggregates built up, time was monitored, until the aggregates blocked flow of blood in the preselected arteriole. The next animal was treated exactly as the first, but the brain adjacent to the preselected arteriole was punctured to a depth of 1–2 mm by a glass microneedle whose tip tapered from a tip of 50–70 μm diameter to a width of 300 μm, 2 mm above the tip. The needle was immediately withdrawn after puncture and the vessel was observed as described above. Fluorescein was injected and the field was exposed to the mercury lamp. The time to appearance of the first aggregate and the time elapsing prior to flow stoppage, were measured as in the preceding mouse. Since aggregation occurs earlier if exposure to light and dye begins closer to the time of craniotomy,2 great care was taken to see that a control (i.e. non-punctured animal) was injected with dye and exposed to the mercury lamp at precisely the same time following craniotomy as the punctured mouse immediately preceding it in the experimental series. In general, craniotomy preceded further study by approximately ten minutes.

Results

Four studies were performed. In each study the site of puncture was further from the arteriole than in the preceding study. The results are summarized in table 1, and show that brain puncture only at a mean distance of 171 μm from the arteriolar wall, significantly *lengthens* the time required for light and dye to induce platelet aggregation. This effect diminished to statistically insignificant levels as the distance between puncture and arteriolar wall increased, although the trend toward lengthening was still strongly present even with puncture distances as far as 610 μm from the wall.

In addition to time prior to appearance of the first aggregate, one can also measure the time required for aggregates to get larger and larger, until flow of erythrocytes ceases.2 The time to stop flow, though measured, has not been previously used by us because it is affected by the configuration of the vessels in the field, proximity of the preselected vessel to branch points etc., and in our hands is a parameter with much greater variability than time to first aggregate. Nevertheless, as seen in table 1, where statistically significant differences appeared with respect to this parameter, they also showed an inhibitory effect of brain puncture on platelet aggregation induced by light and dye. Thus in studies 1 and 3, it took significantly longer for aggregates to build up and stop flow in brain punctured mice, than it did in mice exposed to light and dye without preceding brain puncture. The same trend, though not statistically significant, appeared in studies 2 and 4.

As previously noted, brain puncture was associated with arteriolar spasm, though the arteriole in question was not touched by the needle.8 Spasm occurred in 60% of the animals in study 1 and 30% of the animals in study 2, but not in any of the animals in study 3 and 4. The narrowing occurred *prior* to the exposure to light and dye. When brain punctured animals were separated into two groups according to whether or not arteriolar narrowing had occurred, and each group was compared with its own controls, we found that the interval prior to aggregation was significantly prolonged in both the group with constricted arterioles and the group without constricted arterioles. Moreover, arteriolar constriction had no effect on the time required for aggregation to stop flow in the arteriole.

Finally, it should be noted that animals with punctured brains and control animals displayed no differences with respect to arterial blood gas pressures, pH, or body weight.

Discussion

These experiments were begun to observe the effects on cerebral vessels of combined injury to both the vessels and the adjacent parenchyma. The present set of experiments also test the hypothesis that platelet aggregating and/or vasotoxic materials were released from injured brain. We predicted that such substances would diffuse to and through adjacent arteriolar walls, and exacerbate the platelet aggregation produced when those arterioles were exposed to illumination from a mercury source, in conjunction with intravenous injection of sodium fluorescein. The data presented here does support a hypothesis suggesting that platelet-active or vasoactive materials are released from injured brain. But the results do not support the hypothesis as originally stated. Rather, they indicate that materials released from injured brain, can *interfere* with platelet aggregation and/or vascular damage leading to such aggregation. Thus, brain puncture lengthened the time it took for the noxious stimulus (light plus dye) to induce aggregation. Moreover, it took longer for these aggregates to build up and stop flow. The fact that these inhibiting effects ceased as the distance between puncture and vessel increased, is further evidence in support of the suggestion that

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**Table 1** Effect of Brain Puncture on Platelet Aggregation Induced by Light and Dye in Pial Arterioles

<table>
<thead>
<tr>
<th>Control Mice</th>
<th>Mice with Punctured Brain</th>
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<td>N</td>
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<td>Study 1</td>
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N = number of mice.

All other values are expressed as Mean ± Standard Deviation.

* p < .01; ** p < .05 compared with value for control mice, Student’s "t" test.

**Distance from center of puncture to arteriolar wall.**
a diffusible substance(s) accounts for our results.

The present findings, coupled with our previous work¹ suggest an extremely complex situation, in which multiple substances may be released from injured brain, to either produce or inhibit platelet aggregation and/or vessel injury. The available data does not permit us to predict when one or another of these factors may predominate. However, differences between arterioles and venules might explain some of the data. For example, a substance(s) stimulating aggregation may affect only venules because it does not penetrate the walls of arterioles, but a substance(s) inhibiting aggregation may have a different capacity to penetrate arteriolar walls and hence produce the effect reported herein.

We cannot rule out the possibility that the substance(s) in question originate in blood, since, at the very least, a minute amount of hemorrhage is produced at the spot where the needle penetrates the brain. It should be noted, however, that in these experiments, as in studies where brain puncture alone induces platelet aggregation, the effects of brain puncture are manifest even in the very large numbers of cases where hemorrhage is so slight that no red cells can be observed touching the vessel in question. If a vasoactive or platelet-active material does originate in blood, its effects in the present experiment do not appear explainable by flow of the material in the blood stream, since the arterioles in question are a considerable distance upstream from the site of injury.

We have previously considered that prostaglandins (PG) or thromboxanes (TX) are responsible for some of the effects we have observed. Aspirin, an inhibitor of PG or TX synthesis, inhibits platelet aggregation in both the light-dye² and the brain-puncture³ model. Indomethacin, another inhibitor of PG and TX synthesis, inhibits the platelet aggregation in the light-dye model. It is possible that brain injury releases PG's or TX's, having either aggregate enhancing or inhibiting effects. It would be of interest to selectively inhibit one without inhibiting the other, and recent developments in PG pharmacology may make this possible.

Acknowledgment
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References

Effects of combined parenchymal and vascular injury on platelet aggregation in pial arterioles of living mice: evidence for release of aggregate-inhibiting materials.

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