An Assessment of Early Platelet Activity in Experimental Subarachnoid Hemorrhage and Middle Cerebral Artery Thrombosis in the Cat

BY IRA C. DENTON, JR., M.D., JAMES T. ROBERTSON, M.D., AND MARION DUGDALE, M.D.

Abstract:
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- There is increasing evidence that platelets play a key role in the development of arterial occlusive lesions and in the tissue response to them. Direct evidence for a platelet contribution in experimental vascular occlusive stroke and subarachnoid hemorrhage was sought. Platelet counts were done on jugular blood before and after production of the vascular lesion. Craniectomy had little or no effect but occlusion or puncture of the middle cerebral artery invariably produced a prompt and striking fall in the platelet count of venous blood draining the affected side. Some implications of this observation are discussed.

ADDITIONAL KEY WORDS
- cerebral vessel vasospasm
- cerebral infarction
- platelet, sequestration in brain
- vessel wall edema
- platelet, activated state

Introduction
- Various investigators have suggested that some of the untoward and frequently devastating events of stroke may be related to platelets.

A platelet is normally a disk-shaped structure containing a variety of granular elements. When "activated" it develops pseudopods, the main body becomes dense and contracted, and the granules appear indistinct. These characteristics of platelet morphology are readily recognized by phase microscopy. Activation can be induced by a variety of stimuli, such as contact with injured endothelium, adenosine diphosphate, thrombin, etc. In the formation of a thrombus, platelets initially adhere to an area of injury in the vessel lining. Other platelets then aggregate about this nidus to form a platelet thrombus.

The platelet mass may break up after a short period of time, may embolize, or may be gradually replaced by a fibrin thrombus. During this sequence of events, which is generally irreversible after fibrin begins to form in the platelet mass, a variety of intracellular contents is liberated.

Platelet activation and aggregation may prove damaging in several ways. Besides the obvious mechanical effects of platelet emboli on the microcirculation, Mustard has shown that the endothelium at the site of lodgment of a platelet aggregate is injured by the platelets or by substances released from them.1 One of these substances is a factor which greatly increases the permeability of the blood vessel wall,2 leading to edema formation. In addition, platelets release potent vasoconstrictor substances3,4 which have been shown to cause vasospasm in the cerebral arteries of animals.4

Only a few observations have been reported that relate these facts about platelets to the problems of stroke and intracranial bleeding. Honour and Russell5 observed the
formation of platelet thrombi after minimal mechanical injury to the cortical vessels of the living rabbit. Platelet emboli were released periodically from these thrombi, only to be replaced by new platelet aggregates. Bell et al. observed platelet emboli in the cortical vessels during development of experimental ischemic infarction and speculated that these were the cause of the edema and vasospasm that accompanied spread of the infarction. Gilroy et al., studying patients with acute thrombotic stroke, found an unusually high percentage of "activated" platelets in the systemic blood. However, no fall in the platelet count in the peripheral blood of such patients was noted by another observer.8

Various reports9–11 have suggested that substances derived from blood cause the vasospasm seen clinically after subarachnoid hemorrhage. Kapp et al.4 found that platelet extracts were far more active in this regard than were the other formed or fluid elements of the blood.

These scattered observations show that platelets could play a role in the pathology of stroke and have led some workers to suggest that the approach to clinical stroke, both preventive and therapeutic, may ultimately involve interference with platelet activity.12 To date there have been no studies actually demonstrating the sequestration of platelets in the brain affected by an acute vascular lesion. Such data would seem basic to any hypothesis implicating platelets in the pathogenesis of stroke. If sufficient platelets are trapped during evolution of the stroke, it should be possible to show a difference in the platelet count of blood entering the affected area of the brain and that draining from it. Moreover, the platelets draining from the lesion might well be in the "activated" forms if they had remained for a time within the area of ischemic injury. The present study was undertaken to find out if such a numerical difference could be demonstrated in cats with experimental subarachnoid hemorrhage or middle cerebral artery occlusion. Morphological observations about the platelets were made incidentally.

Methods
Common, apparently healthy cats procured the day before surgery and thus not "worked up" served as subjects. Following intrapleural nembutal anesthesia (25 mg/kg), the cats were intubated and allowed to breathe spontaneously. Using the operating microscope the procedure was carried out by the same surgeon under unsterile conditions.

PLATELET SAMPLING TECHNIQUE
A medium-bore polyethylene catheter was introduced into the superior vena cava by way of the left external jugular vein and positioned so that its tip sampled as nearly as possible the cerebral venous return from the side of the experimental hemorrhagic or thrombotic lesion. Preliminary studies revealed that other sampling sites including the jugular veins or more peripheral veins were either anatomically or technically unfeasible. Our catheter thus necessarily sampled an admixture of venous return from both cerebral and extracerebral territories. This, however, appeared acceptable since control animals (below) were available for comparison.

The catheter's patency was assured for the duration of the procedure by intermittent weak heparin (10,000 u/1000 ml Ringer's solution), irrigations amounting only to that quantity required to fill the catheter each time (0.1 ml). In sampling, an initial aspiration of blood was taken to clear the catheter, ruling out dilutional artifacts. Thereafter, a small aliquot sufficient for platelet counting was removed. The platelet counts were performed at arbitrary intervals, up to 200 minutes (table 1) by the same experienced technologist, under phase microscopy employing the method of Brecher and Cronkite, using one pipette and two chambers. This technique carries an error of approximately 10%.14 Platelet morphology was reviewed by one of us (M.D.). Proper catheter position was confirmed at post-mortem examination.

INTRACRANIAL PROCEDURES
Each cat was placed in one of three operative groups. The technologist counting platelets was not informed of which procedure had been done.

Group 1: Sham Operated Control Group
A right temporal retro-orbital craniectomy with dural incision and exposure of the middle cerebral artery at its bifurcation from the intracranial internal carotid artery16 was performed in eight cats. The artery was then manipulated but not punctured or clipped. Preliminary studies showed that about 3 ml of blood was lost during experimental subarachnoid hemorrhage. Accordingly, 3 ml of blood was removed from each control animal, in six from the superficial temporal artery, in one from the right common carotid and in one from a femoral vein. Thus, quantitatively and temporally the blood loss would compare to the Group 2 animals. Serial
platelet counts were taken preoperatively (baseline), immediately postoperatively and at selected intervals thereafter up to 200 minutes.

**Group 2: Subarachnoid Hemorrhage**
These six animals underwent the identical surgical procedure. Blood loss was kept to a minimum until the subarachnoid hemorrhage was produced by puncturing the internal carotid artery or middle cerebral artery with a #7-0 surgical suture needle. The following observations were made: (1) the subarachnoid hemorrhage spontaneously stopped within two to three minutes, (2) the quantity of subarachnoid hemorrhage measured between 3 to 5 ml, (3) subarachnoid staining uniformly occurred, but to a varying degree. The above-described platelet sampling procedures were used.

**Group 3: Middle Cerebral Artery Clipping**
The above craniectomy and arterial exposure was carried out with minimal blood loss and a small Mayfield clip applied to the middle cerebral artery of six animals. The same platelet sampling procedures were used. At the sacrifice of the animal, the placement of the clip was confirmed.

**Results**
Table 1 gives the actual platelet counts of each animal group at the described intervals, the baseline being the preoperative platelet count.

Table 2 gives the same data, but with the platelet counts expressed as percentages of the initial, preoperative value. These data are presented graphically in figure 1.

Of the eight animals of Group 1, two (Nos. one and three) showed no change in the platelet count during the period of observation and two showed only minimal and transient decreases (Nos. four and five). Two (Nos. two and six) had falls of about 30% but recovered before the end of the experiment.

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remaining two (Nos. seven and eight) showed slow decreases of about 20% without leveling off or recovering by the end of the observation period. In man, the usual response of the platelet mass to surgery or trauma is a platelet fall beginning from zero to two hours, lasting one to two days, followed by a rise to supranormal levels. In contrast, Group 2 animals displayed uniformly a precipitous fall below baseline values, and counts continued well below the baseline at 200 minutes with only one (No. six) appearing to rise again. In two animals (Nos. one and two) counts fell to 37% and 38% and were still decreasing at the end of the experiment. In two animals (Nos. three and five) counts fell to 65 and 70% and then made a partial recovery. Animal No. four showed a slow, steady fall throughout the period of observation. Animal No. six showed a 45% fall but made a partial recovery to 80% of the baseline value. The pattern followed by these animals is clearly different from that seen in the control group in both the rapid initial fall and failure to recover by 200 minutes.

In Group 3, an immediate, precipitous drop occurred, comparable to that noted in Group 2. Thereafter, a general rising trend ensued in all except one animal (No. four). At 200 minutes, however, values remained depressed below the baseline to a variable extent, ranging from 50% to 95%. Thus, this group also differed substantially from the control group. In both Groups 2 and 3 our technician observed densely contracted agranular platelet forms characteristic of the activated state. Her observations were confirmed by one of us (M.D.).

**Discussion**

Our data show that the platelet content of jugular blood is reduced after production of an ipsilateral arterial lesion. This can be explained only by assuming that some platelets remained for a time within the cerebral vessels. Moreover, the platelets leaving the brain show the morphological features associated with activation. This suggests that the platelets were not only sequestered within the brain but were also activated and, therefore, probably aggregated. What effect, if any, these sequestered platelets have on the development or progression of the brain lesion is yet to be demonstrated. It is conceivable that platelet emboli occlude collateral vessels, thereby augmenting the area of ischemia. It is also possible that the se-
TABLE 2
Percentage of Baseline Platelet Counts at Selected Intervals. Derived from table 1

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*Lab accident.

Quersted platelets release substances that affect vessel tone and permeability, leading to spasm and edema which further impair cerebral circulation. Should this prove to be the case, the prompt use of agents that decrease platelet reactivity could well have a beneficial effect on the developing brain lesion.

References

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