Pial Microcirculation in Subarachnoid Hemorrhage

BY D. A. HERZ, M.D., S. BAEZ, M.D., AND K. SHULMAN, M.D.

Abstract:
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It has been known for many years that rupture of intracranial aneurysms is associated with angiographically demonstrable cerebral vasospasm,\(^1\)-\(^3\) which can be confirmed visually at the operating table.\(^4\) Similar spasm has been noted in patients with traumatic subarachnoid hemorrhage,\(^4\)-\(^6\) and is seen less frequently with arteriovenous malformations and other disorders.\(^5\) It is clear, however, that blood experimentally introduced into the subarachnoid space by a variety of methods produces spasm of large intracranial arteries.\(^7\)-\(^11\) Vasospasm has been a deterrent to the surgical therapy of intracranial aneurysms, being associated with profound neurological deficits and death.\(^1\),\(^2\),\(^12\)-\(^17\) Such a grave clinical problem has led to many laboratory investigations on the pathophysiology of blood-induced large vessel spasm.\(^7\),\(^9\),\(^11\),\(^18\)-\(^25\) The regulation of blood supply to cerebral cortical tissue capillary beds is not exclusively a function of these large arteries, and is at least partially relegated to the precapillary resistance and distributing vessels of the pial microcirculation.\(^26\)-\(^28\) Although attention has been directed toward investigation of the cerebral microcirculation in various physiological and pathological conditions,\(^29\)-\(^40\) studies concerning cerebral microarterial trauma and subarachnoid hemorrhage are neither numerous nor conclusive.\(^28\),\(^29\),\(^37\) It therefore seemed appropriate to systematically examine, by direct in vivo microscopy, pial microcirculatory changes in these states.

Methods
Forty-five young guinea pigs of both sexes, weighing 400 to 500 gm, were anesthetized with pentobarbital (40 mg per kilogram i.m.). The animals were usually not re-injected with pentobarbital after surgery, so that they were under light anesthesia at the time when their cerebral cortical vessels were studied. Using the Zeiss operating microscope a tracheostomy, right common carotid cannulation, and left parietal craniectomy were done in each animal. The dura was excised along with the underlying thin, adherent arachnoid.

As diagrammed in figure 1 observations of pial microvessels were made using a Bausch and Lomb Stereozoom 7 trinocular microscope at 35X to 112X. Measurements of total vascular diameter were made with a Vickers monocular image splitter, equipped with a potentiometer, and adapted for recording on a Grass polygraph. The linearity (0.1% error) of this system has been established.\(^41\) Calibration of the millimeter-ruled recording paper on the polygraph was done at three selected magnifications, using a Zeiss stage micrometer. Measurements (n 21) at 35X of 130 \(\mu\)m units in the scale gave a standard error (SE) of \(\pm 1.07 \mu\)m. The SE was \(\pm 0.42 \mu\)m when the measurements were made at 77X of 50 \(\mu\)m units (n 35), and the SE was \(\pm 0.3 \mu\)m with 10 \(\mu\)m units (n 15) at 112X.

A continuous drip of physiological artificial cerebrospinal fluid\(^42\) (ACSF) at 37° ± 2°C was used to irrigate the tissue. ACSF temperature measurements were made with a Yellow Springs microthermistor probe #511, and a Tele-Thermometer recorder. This contained the following electrolyte concentrations in mEq/liter: Na\(^+\): 151
to 156; Cl: 135 to 140; HCO₃⁻: 20–22; K⁺: 3.4 to 3.6; Mg²⁺: 1.2 to 1.4; Ca²⁺: 2.1 to 2.3. ACSF was prepared daily from stock solutions, and brought to pH 7.3 by bubbling CO₂ through the mixture, after which it was immediately covered with mineral oil. Electrolyte concentrations were periodically checked by flame photometry. It was found that the addition of urea and glucose did not improve vascular reactivity but did cloud the irrigation fluid, and therefore they were excluded.

Adequate incident illumination was provided by two fiberoptic sources directed at the cerebral cortex tangentially to minimize glare (fig. 1). All vascular measurements were accompanied by determinations of systemic arterial blood gases (Gas Monitor, Radiometer, Copenhagen) and blood pressure (Statham transducer and Grass Polygraph) to ascertain that changes in vascular caliber were not due to concomitant changes in these parameters. Rectal temperature was maintained at 37° ± 2°C, using either heating lamps or an Air Curtain Incubator (Sage Co.).

GROUP 1: MICROTRAUMA
In 13 guinea pigs consecutive segments of pial microvessels were punctured with 5 μm to 10 μm tip, hand-pulled glass microneedles (fig. 1, fig. 2 — left). These were mounted on a Leitz manual micromanipulator. The smallest cortical surface penetrating arterioles were punctured first; after hemostasis had been established, micropuncture of the larger vessels followed. In order to prevent heavy perivascular clotting and/or brisk hemorrhage from obscuring a vessel under scrutiny, the speed of the perfusion fluid was titrated to the point where extravasating blood was just diluted sufficiently to permit resolution of the vessel. Measurements of changes in caliber were made at the closest possible area adjacent to each of a total of 20 micropunctures.

GROUP 2: TRANSIENT APPLICATION OF HEPARINIZED BLOOD
In 17 guinea pigs homologous heparinized blood was topically applied to the brain in 0.2 cc volumes. With a slow microdrip the blood would be sufficiently diluted for vascular measurement within 10 to 15 seconds. After vessels regained control size, a five to ten-minute waiting period was allowed before the next application of blood. In five of these 17 animal preparations, after heparinized blood-induced vasoconstriction had been demonstrated, the brain was covered for five minutes with a cotton pledget soaked in ACSF, containing 10 μg per milliliter of phenoxybenzamine (Smith, Klein and French), and maintained at 37° ± 2°C. Ten more heparinized blood applications were then made to each target vessel. In five other guinea pigs the brain was identically covered with propranolol-soaked (10 μg per milliliter) pledgets, after which nine additional applications of blood were made to five vessels. The receptor blockers used were diluted in the same ACSF used to bathe the cortex, so that vascular responses following their application could not be attributed to extraneous changes in the perivascular milieu.

GROUP 3: PROLONGED APPLICATION OF AUTOLOGOUS BLOOD
In 15 guinea pigs three to five drops of autologous blood were allowed to clot on the cortical surface. The clot was
Left: A pial artery (arrow) was photographed prior to being subjected to micromanipulation. Right: Following severe microtrauma, without vascular rupture, there was considerable vasoconstriction, with some degree of propagation to dependent arterioles.

then carefully removed with minimal mechanical trauma to the underlying tissue. In this way the pia surrounding many vessels became lightly stained with clotted blood that could not be washed off by the ACSF microdrip but did allow resolution of the external aspect of the vascular walls (fig. 2 — middle). In nine animals phenoxybenzamine-soaked cotton pledgets were applied, as above, and in six others propranolol was used. A total of 15 small penetrating arterioles were measured before and after the application of these drugs, under a slow ACSF microdrip.

**Results**

Before beginning each experiment the anatomy and flow characteristics of exposed cortical surfaces were noted, and microphotographs were taken in selected experiments. As previously reported,^{23} interarteriolar and intervenular connections were seen, but no arteriovenous shunts. There were no true endothelial capillaries seen, the penetrating vessels dipping into the cortical substance presumably branching beneath. Scattered venous capillaries were observed emerging from the cortex to join pial venules. Oscillating flow occurred in the interarteriolar connections, that is, the direction of flow would reverse from time to time.

When such vessels branched blood could sometimes be seen flowing toward these branches from either direction.

**GROUP 1: MICROTRAUMA**

Prior to puncture the effect of microneedle mechanical stimulation upon pial microvessels was often noted (fig. 3). It was found that trauma without rupture could produce slight or severe vasoconstrictions, which could be localized or propagated, for transient or prolonged periods. The degree, extent, and duration of constriction were directly dependent upon the degree and duration of manipulation.

The vessels subjected to micropuncture constricted an average of 44.3%. They fell into three subgroups according to size (table 1). In the first subgroup there were nine small penetrating arterioles (24 ± 2 µm o.d.). When punctured, a 36.6% vasoconstriction was measured both up and downstream from the site of injury, and sludging or stasis of red cells, plasma skimming and/or minor hemorrhage resulted, usually lasting about one minute. Concomitant narrowing of upstream arterioles was slight unless they became bathed in blood.

**TABLE 1**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Animal no.</th>
<th>Vessel no.</th>
<th>Total vessel diameter (µ)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>After Injury</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>9</td>
<td>24 ± 2</td>
<td>15 ± 1.4</td>
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<tr>
<td>2</td>
<td>5</td>
<td>48 ± 1.5</td>
<td></td>
<td>20 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>129 ± 12.8</td>
<td>68 ± 10.9</td>
<td></td>
</tr>
</tbody>
</table>

*Micropuncture done with the use of 5 to 10 µ tip glass microneedles.
†† = dilation; --- = constriction.
Mean and SE of measured vessel diameter.
Pooled data on 20 micropunctures in 13 different animals.
In the second subgroup five larger arterioles (48 ± 1.5 μm o.d.) constricted 58.6%. Vasoconstriction was noted distally downstream in these vessels and their branching penetrating arterioles, but was slight in the small upstream parent arteries. Brisk hemorrhage usually occurred, lasting three to five minutes. Sometimes blood flowed toward the rent from both directions; other times flow became sluggish or static downstream. A partially translucent presumably fibrin-platelet clot, along with vasoconstrictive narrowing of the defect, eventually would result in cessation of hemorrhage.

The third subgroup of six small arteries (129 ± 12.8 μm) hemorrhaged profusely and constricted 45.7% when punctured. If trauma occurred in the mid-point of such vessels, rapid flow toward the rent usually was seen from both directions. Vasospasm would be propagated proximally and distally, but not to the more distant penetrating arterioles. When these small arteries were traumatized near points where they branched, downstream flow in smaller vessels usually slowed and the vasoconstriction was propagated to smaller arterioles and the penetrating vessels. There was usually concomitant pallor of the surrounding cortex, and darkening and sludging of venular and venous blood. Once hemostasis had developed, small repeat hemorrhages would sometimes occur until an efficient clot had developed, and/or the vessel had locally constricted sufficiently to stop flow. After a 15 to 30-minute interval massive cortical swelling would occur. White thrombi could then be seen occluding or traveling sluggishly through arterioles and emerging venules.

GROUP 2: TRANSIENT APPLICATION OF HEPARINIZED BLOOD

Study here was limited to the precapillary smaller vessels (table 2). In 42 experiments on 17 vessels (28 ± 1.9 μm) a 33.2% constriction was observed lasting up to one minute. In ten instances pretreatment with topical phenoxybenzamine, followed by repeat application of heparinized blood, failed to result in significant vasoconstriction (paired t test, P = 0.01). In some instances the application of phenoxybenzamine itself produced moderate vasodilation. In nine instances, after pretreatment with propranolol, repeat application of heparinized blood produced an 8% vasoconstriction, which was not statistically significant (paired t test, P = 0.01). No particular change in vascular caliber was noted in response to propranolol alone.

GROUP 3: PROLONGED APPLICATION OF AUTOLOGOUS BLOOD

Clotted blood in continuous contact with pial vessels caused intense vasoconstriction seeming to be of greater degree than that noted following usual microtrauma, or during transient application of heparinized blood (fig. 2—middle). In most precapillary vessels sludging of red cells and plasma skimming were seen. A small number of vessels would remain open, however, exhibiting more rapid flow. In some arterioles and venules occasional white thrombi were observed. By contrast, cellular flow in larger vessels was not necessarily slowed. Treatment with topical phenoxybenzamine (table 3, fig. 2—right) produced a 98.9% vasodilation in nine precapillary arterioles, and was accompanied by a return of brisk flow. Treatment with topical propranolol did not produce significant vasodilation in six precapillary arterioles (paired t test, P = 0.01).

Because of light anesthesia the animals tended to hyperventilate, maintaining a measured PCO₂ of 25 mm Hg (SE ± 3). The Po₂ was 105 mm Hg (SE ± 10) and metabolic acidosis, attributed to surgery, with pH 7.37 (SE ± 0.04) prevailed. Systolic blood pressures usually ranged from 85 to 95; however, this is similar to that obtained in five awake guinea pigs who had no further surgery than femoral or carotid cannulation under ketamine anesthesia.

**Discussion**

The microvascular spasm noted following vascular microtrauma in the present study is similar to that

**Table 2**

| Group 2: Prevention of Blood-Induced Microvascular Spasm by Phenoxybenzamine and Propranolol |
|---|---|---|---|---|
| **Condition** | **Animal no.** | **Vessel no.** | **Total vessel diameter (μ)** | **Change (%,P)** |
| **Control** | | | | |
| Untreated | 17 | 42 | 28 ± 1.9 | 19 ± 1.2 | -33.2 |
| Phenoxybenzamine† | 5 | 10 | 34 ± 1.6 | 34 ± 2 | -0.2 |
| Propranolol‡ | 5 | 9 | 24 ± 2.7 | 22 ± 2.3 | -8 |

*+=dilation; −=constriction.†Vessel exposed to topical phenoxybenzamine, 10 μg per milliliter of solution five minutes before blood.
‡Vessel exposed to topical propranolol, 10 μg per milliliter of solution five minutes before blood.

Mean and SE of measured vessel diameter.
PIAL MICROCIRCULATION IN SUBARACHNOID HEMORRHAGE

TABLE 3

<table>
<thead>
<tr>
<th>Condition</th>
<th>Animal no.</th>
<th>Vessel no.</th>
<th>Arteriole diameter (μ)</th>
<th>Control*</th>
<th>After blockade</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxybenzamine†</td>
<td>9</td>
<td>9</td>
<td>11 ± 1.6</td>
<td>22 ± 1.9</td>
<td>98.9</td>
<td></td>
</tr>
<tr>
<td>Propranolol‡</td>
<td>6</td>
<td>7</td>
<td>11 ± 1.8</td>
<td>11 ± 2</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

*Vessels previously constricted by topical autologous blood.
†Phenoxybenzamine: 10 μg per milliliter of solution, topical. Application resulted in a significant reversal of vasoconstriction.
‡Propranolol: 10 μg per milliliter of solution, topical. Application resulted in no significant reversal of vasoconstriction.

Clotted autologous blood in prolonged contact with pial precapillary arterioles produced intense vasoconstriction.

found in the microcirculation of experimentally constricted primate brains. This may be due to: (1) a neurogenic reflex, (2) smooth muscle contraction resulting from mechanical stimulation, (3) the direct chemical effects of blood, or (4) a combination of these factors. It is known that pial arteries are richly supplied with both adrenergic and cholinergic nerve fibers, while innervation is sparse in precapillary arterioles. In addition, stimulation of cervical sympathetics has been shown to reduce small artery caliber and blood flow. There is strong evidence, however, that the response of pial vessels to sympathetic stimuli is smaller than that of vessels in most other parts of the body. It has been suggested that the autonomic nerves may modulate the effects of more potent stimuli, rather than being the major factor in regulation of cerebrovascular tone. Because of this, and the anatomical distribution of vascular innervation, it cannot be assumed that traumatically induced microvascular constriction is primarily due to a neurogenic reflex. Mechanical stimulation, without vessel rupture, produced only localized, moderate, and temporary constriction in large cerebral arteries studied experimentally. In the present study, the degree and duration of mechanically induced microvascular spasm were related to the severity of trauma, suggesting that the mechanical stimulus per se may indeed be a cause of vasospasm induced by micropuncture (fig. 3). It is also on record that gradients of electrical stimulation of the pial vessel wall result in gradients of constriction. The consistent, prolonged, and powerful constrictive effect of heparinized fresh homologous blood applied to untraumatized vessels would suggest, on the other hand, that chemical mechanisms also have an important effect. The fact that micropuncture with hemorrhage produced an average greater vasoconstriction (44.3%) than topical application of blood alone (33.2%) is indicative of a chemo-mechanical mechanism.

The chemical effect of blood also has been demonstrated in large vessels, and even in sympathectomized animals. Known vasoactive components of blood have been similarly studied; however, the agent or agents responsible for large vessel spasm remain undetermined. In vitro studies by Nielsen et al. have shown that isolated cats' middle cerebral artery strips constrict in response to serotonin, histamine, and acetylcholine, plus alpha and beta adrenergic drugs. In their experiments these effects also could be pharmacologically blocked. Reversal of blood-induced large vessel spasm has been reported in vivo, using alpha blockade. In vivo studies on the microcirculation have shown that alpha adrenergic stimulation with norepinephrine in higher doses than required to activate vessels elsewhere in the body produced only a moderate vasoconstrictor effect in pial vessels. These results, however, are not consistently reproducible. A more powerful effect can be elicited by topical 0.2 M BaCl2, and this effect can be inhibited by the use of a beta adrenergic blocker. Perivascular pH changes, in physiological ranges, also exert a noticeable effect, and the suggestion has been made that this may be potassium dependent. Changes in arterial Pco2 have a strong influence, which is more noticeable in the precapillary resistance vessels. It is the general consensus that (given a constant systemic blood pressure) changes in respiratory gases, producing secondary changes in local pH, are important local physiological parameters participating in the regulation of cerebrovascular caliber and cerebral blood flow.

The cortical microvessels are certainly responsive to the known vasoactive humoral substances discussed; however, the concentrations required to activate these vessels are much greater than those required to activate microvessels in other tissue beds. The substance or group of substances capable of producing microvascular spasm in subarachnoid hemorrhage remain unknown but probably at least partially act on receptors which can be influenced by alpha adrenergic blockers. It is clear that such spasm can be prevented and reversed by the topical use of an alpha blocker (tables 2 and 3, fig. 2 — middle and right). Furthermore, effective topical application...
of such a drug can be accomplished without adversely reducing blood pressure and pulse (which could be of future clinical import). The role of beta adrenergic blockers is less clear. In the present study topical pretreatment with the beta blocker, propranolol, did not of itself produce vasoconstriction, as might be expected. This agent prevented blood-induced vasoconstriction, perhaps due to its side effect as a local anesthetic.

In experimental cerebrovascular studies such as the present one, larger animals (dogs, cats and monkeys) are customarily used. Rosenblum and Zweifach pointed out the ease of studying a relatively larger percentage of anatomically similar cortical surface by using a smaller mammal, such as the mouse. In the present study it was considered desirable to similarly observe a relatively large area, yet be able to have vessels of arterial, arteriolar, and precapillary caliber within one low power field. While even main vessels in mice were too small, those of guinea pigs fit all criteria mentioned. These animals are as inexpensive as rats, easier to handle, and more amenable to blood samplings. They conveniently fit on a lucite plate, specially equipped with a headholder and aperture for a tracheostomy tube, mounted on a modified microscope stage for the purposes of micromanipulation, illumination and focusing.

In the clinical situation angiographically demonstrable constriction of large-sized and medium-sized cerebral arteries in patients with subarachnoid hemorrhage has not been consistently correlated with a reduction in hemispheric and regional blood flow. Such patients may show reduced flow without spasm on angiography, or normal flow despite the presence of spasm. It would appear that the disturbance in vascular dynamics in such cases at least partially occurs between the macroscopically visible constricted main arteries and the venous system, i.e., the vessels of the intervening microcirculation, which are not visible without high magnification. The present results may substantiate such a hypothesis. Blood-induced spasm of large arteries of guinea pigs did not necessarily impede visible cellular flow within them. However, the medium-sized (48 ± 1.5 μm o.d.) pial arterioles and smaller (24 ± 2 μm o.d.) penetrating arterioles, constricting similarly, exhibited sludging and/or stasis of cellular elements, white thrombi, and plasma skimming, accompanied by cortical pallor and darkening of venous blood. These microcirculatory changes are similar to those described in experimental strokes resulting from middle cerebral artery occlusion in cats and monkeys.

If the microvascular spasm observed in the present experiments occurs in patients with subarachnoid hemorrhage due to aneurysms, and if reduction of cerebral blood flow associated with experimental pial microvascular spasm can be extrapolated to the clinical situation, such patients should uniformly manifest a decrease in regional blood flow. The fact that this is not necessarily seen in clinical studies is possibly related to the extent to which spasm is propagated downstream and to the distribution of subarachnoid blood. If hemorrhage is confined to the area of a large artery at the base of the brain there will be little or no reduction of flow in that vessel until the lumen narrows 70% to 90% to reach a critically small size. Such severe narrowing is rarely seen angiographically in a patient with subarachnoid hemorrhage. To reduce cerebral blood flow the hemorrhage may have to extend over the convexity so that pial surface microvessels are also constricted. When such convexity distribution of blood occurs it is not necessarily uniform and therefore may be associated with small, isolated, patchy infarcts. Scintillation counters detecting blood flow over a relatively large region may simultaneously detect discrete separate regions of normally perfused, overperfused, and underperfused brain, and would not necessarily register an overall reduction in flow. In addition, luxury perfusion, represented by those few microvessels remaining open, may also partly account for normal or increased flow in infarcted regions. In patients with radioisotopic evidence of decreased blood flow, but no spasm on large vessel angiography, it is possible that convexity microvascular spasm has occurred, but is not detectable by macroscopic radiographic techniques.

Luxury perfusion represents a physiological arteriovenous shunt. Anatomical arteriovenous shunts below the cortical surface, if present, also may shift considerable volumes of blood in the face of surface microvascular spasm. The overall effect of physiological and anatomical shunts resulting from hemorrhagic or traumatic microvascular spasm would be for blood to be flowing around, through, and past an area of cerebrum, bypassing the capillary beds, and precluding tissue exchange of metabolites. Then there would not necessarily be an isotopically detectable reduction in regional or hemispheric blood flow. It is conceivable that this could even result in a third rapid compartment of flow, as seen in children with head trauma.

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