MANAGEMENT OF CEREBRAL VASOSPASM has been limited because of poor understanding of the pathogenesis of the disorder so that both morbidity and mortality remain high.1 There may be an early, short-lived phase occurring immediately following SAH and a subsequent phase that is prolonged or chronic.1 The acute phase may be primarily a laboratory observation rather than a consistent finding in man.2 The secondary vasoconstriction seen on the angiogram in the second week post hemorrhage seems clinically to be most important. Both phases of vasospasm are considered to result from an abnormal constriction of the muscular layers of the cerebral vessel.3 Whether the two phases are independent or interactive with respect to the clinical course has not been settled. There appears to be little doubt, either from animal studies or from human cases, that pathological changes occur in significant numbers of cerebral vessels after SAH.4,9 Whether intense vasoconstriction leads to structural changes or vice versa is conjectural. If morphological alterations account for or significantly contribute to the radiographic appearance of vascular constriction following SAH, correlated anatomical events within a large segment of the cerebral vascular network becomes of paramount importance. No studies have reported an indepth analysis of the propagation of structural alterations from the rupture site under such experimental conditions.

Norepinephrine (NE) is known to produce marked and acute vasoconstriction of cerebral arteries10 and is reported to be elevated in the blood, urine and cerebrospinal fluid (CSF) of patients suffering from cerebral hemorrhage and SAH.11-13 In addition, the injection of NE into the preponine cistern has been reported to produce biphasic vasoconstriction and result in structural alterations in the cerebral arteries of monkeys.14

The purpose of the present study was to compare the effect of vessel wall catecholamine depletion early in the course of SAH on the severity of the structural damage that occurs in later stages following SAH. Additional goals of this study were to ascertain variabilities in vessel NE concentration at various post-SAH time intervals, to note how such NE concentrations relate to the morphological status of the vessel, and to examine the propagation of structural alterations within the cerebral vascular network.

Materials and Methods
Twenty-six adult cats were divided into four groups. SAH was produced by rupture of the RMCA. Under general anesthesia with intramuscular ketamine (20 mg/kg), the head was immobilized in a stereotaxic instrument. After intraorbital exenteration a small cranietomy was performed adjacent to the right optic foramen exposing the RMCA. A suture-hook with thread made from a sharpened no. 27 needle was passed through the wall of the vessel. The dura was closed within the orbit and seven days were allowed for healing. Following a seven day healing period, cats were anesthetized again and the hook was pulled from the vessel wall resulting in a "closed space" SAH. To prevent wound infection, Keflin (50mg/kg/day) was administered intramuscularly for 5 days following the initial suture operation. Reserpination of cats was as follows: Animals were pretreated with a single injection of 0.08 mg/kg reserpine subcutaneously two days prior to suture operation and further maintained on 0.05 mg/kg reserpine every other day until sacrificed. Our studies showed that dosages above these levels, on a continuing basis, resulted in severe drug intoxication (diarrhea, weight loss, and lethargy, etc.). Group I (4 cats) served as controls. Two were used for light microscopic (LM) studies and two for catecholamine fluorescence studies. Group II (2 cats) was used to evaluate the effectiveness of reserpine in reducing vessel wall catecholamines. Following nine days of reserpinization, cats were sacrificed and fluorescence studies conducted. In Group III (10 cats), following SAH, cats were sacrificed (two/per/interval) at 1, 3, 10, 16 and 30 days. Material from five of these cats were studied for healing. Following a seven day healing period, cats were sacrificed and fluorescence studies conducted. In Group III (10 cats), following SAH, cats were sacrificed (two/per/interval) at 1, 3, 10, 16 and 30 days. Material from five of these cats were studied for histochemical analysis.* Additional goals of this study were to ascertain variabilities in vessel NE concentration at various post-SAH time intervals, to note how such NE concentrations relate to the morphological status of the vessel, and to examine the propagation of structural alterations within the cerebral vascular network.

*These studies are in compliance with AHA humane guidelines and further approved by the local University of Mississippi Medical Center animal review committee.
ANGIOPATHY OF SUBARACHNOID HEMORRHAGE/Yoshioka et al

with the light microscope, while the remaining five were used for catecholamine fluorescence studies. In Group IV (10 cats), animals were pretreated with reserpine prior to SAH and then maintained on reserpine (as described above) until sacrificed and studied as in Group III.

For LM study, brains were perfused with cold saline and 10% formalin, then removed from the cranial cavities and both middle cerebral arteries (MCA's) were dissected from their vascular beds. Vessels were fixed in 10% formalin and embedded in paraffin. Arterial cross sections (6μ thick) were stained with hematoxylin and eosin (HE). Whole brains and gross sections were studied macroscopically for evidence of infection and infarction.

For catecholamine fluorescence studies, vascular perfusion was done with isotonic cold saline. Following brain removal, both MCA's were removed and rinsed briefly in cold saline. The MCA of the cat consists of a "main trunk" approximately 8 mm long and a "branching segment" approximately 12 mm long. The main trunk of both MCA's were dehydrated in vacuum at −60°C for 2 hours, followed by one hour at room temperature. After drying, specimens were exposed to formaldehyde gas at +80°C for one hour, then embedded in paraffin at +60°C. Cross sections (10μ thick) were then placed on non-fluorescent slide glass and deparaffinized by the addition of xylene. Whole sectioned preparations were examined under a fluorescence microscope (Zeiss) equipped with a dark-field condensor and a Kodak Ektachrome 200. The exciting light was provided by an Osram HOB 200 mercury lamp and filtered through appropriate excitation-suppression filter combination (Zeiss 487705).

In order to correlate variations in pathological events and catecholamine fluorescence at the various post-hemorrhaged time intervals among the experimental groups, a method of grading both became necessary. Of all pathological changes observed, subintimal proliferation was the most obvious and consistent alteration. Additional pathological alterations appeared related to the degree of severity of subintimal proliferation. The present pathological grading system has been previously described and is based primarily upon the severity of subintimal proliferation, and other complementary changes. The earliest changes, Grade I, consisted of mild subintimal proliferation consisting of both endothelial cells and smooth muscle cells of 2-3 cell layers thick, mild splitting and corrugation of the internal elastic membrane, myonecrosis, and intramural hemorrhage. Grade II arteriopathy consisted primarily of subintimal proliferation being 3-8 cell layers thick, corrugation of the internal elastic membrane, and myofibrosis. Grade III, representing the most severe vessel changes, consisted of a combination of severe subintimal proliferation greater than 8 cells thick, myofibrosis, corrugation and disruption of the internal elastic membrane and severe shrinkage of the media of the vessel. For alterations in vessel catecholamine fluorescence, a distinction was made between normal (+ +), pronounced decrease (+), weak fluorescence detectable only in the freeze-dried cross sections (±), and total absence of fluorescence (−).

In order to evaluate the longitudinal spread of morphological changes, the following procedures were performed on both MCA's of only those animals sacrificed at the 30-day interval (Groups III and IV). Using the LM, study of serial cross sections (6μ thick) of the MCA's were performed in a proximal-distal direction. By noting the first appearance of vessel damage and its last, the number of serial sections multiplied by 6 and converted to mm allows approximation of spread of damage. Reconstruction of propagated proliferative changes were then made.

Results

Pathological Alterations and Catecholamine Fluorescence

The results are summarized in table 1.

In Group I (Controls), vessels appeared normal and consisted of a single layer of endothelial cells, an internal elastic lamina, several layers of smooth muscle cells and an adventitia (fig. 1). The presence of adrenergic transmitter in sympathetic nerves were represented by a bright yellow-green fluorescence network (+ +) within the vessels wall. The fluorescence was present in the adventitia and at the adventitial-medial border, but not within the media itself (figs. 2 & 3). Yellow auto-fluorescence of the internal elastic lamina and orange fluorescence of serotonin in blood platelets were also demonstrated, but were easily distinguishable from vessel wall catecholamine fluorescence (fig. 2).

In Group II (reserpinized-control), reserpine significantly reduced the intensity and number of visible fluorescent lines (+) in the adventitia of the MCA's of cats sacrificed 9 days after initial injection (fig. 4 & table 1).

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<td>Untreated</td>
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RMCA = right middle cerebral artery; LMCA = left middle cerebral artery; SAH = subarachnoid hemorrhage; § = normal fluorescence; † = pronounced decrease of fluorescence; †† = weak fluorescence only in the sectioned vessel; § = complete loss of fluorescence; †† = medial fluorescence; G1_{2-3} = graded vessel alterations (see materials and methods for details); G1 = least severe; G2 = moderate severe; G3 = most severe.
In Group III (subarachnoid hemorrhaged), cats tended to ignore food and water for a few days and left hemiparesis developed immediately in two. This remained unchanged in one cat sacrificed 3 days following SAH, while the other cat sacrificed 16 days following SAH gradually recovered over an 8 day period. By 1 and 3 days post-SAH, the RMCA showed Grade I alterations. Such alterations were confined to areas near the site of rupture at 1 day, but had extended further, both proximal and distal, from this site by 3 days. The RMCA of the cat sacrificed 1 day post-SAH showed a complete loss of fluorescence (—) in both the whole stretched and cross sectioned preparations. By 3 days, the RMCA showed weak fluorescence only in the sectioned vessel (±) (fig. 5). By 10 and 16 days post-SAH, the RMCA showed Grade II pathology (fig. 6). Such alterations extended further longitudinally from the site of rupture when compared to 1 and 3 days. At 10 days, this vessel showed weak fluorescence only in the sectioned preparation (±), and by 16 days fluorescence had increased (+), but still remained below control levels. At this later time period (16 days), in addition to weak adventitial fluorescence, green fluorescent spots were observed in the media of the RMCA (fig. 7). By 30 days, the RMCA showed Grade III alterations and catecholamine fluorescence (+) remained below normal (fig. 8 & 9). No medial fluorescence was observed at this later period. In the LMCA (contralateral to side of rupture), arteries showed only Grade I pathology (not including myonecrosis and intramural hemorrhage) at all post-SAH periods (table 1). Fluorescence at 1 and 3 days in the LMCA was remarkably decreased, but had returned to normal by 10 days (See table 1). At all post-SAH time periods, catecholamine fluorescence could not be demonstrated in areas of subintimal proliferation in either MCA. In Group IV (reserpinized-subarachnoid hemorrhaged), all animals showed miotic pupils about two hours following the initial injection of reserpine and this sign continued until they were sacrificed. Some cats suffered from mild diarrhea and exhibited depressed spontaneous movements. Following SAH, one cat immediately developed left hemiparesis but had recovered by 3 days. At 1, 3, 10 and 16 days post-SAH, the RMCA's showed only Grade I alterations (table 1). The artery showed complete loss of fluorescence (—) for 10 days post-hemorrhage and only weak fluorescences could be demonstrated in sectioned vessels (±) by 16 days. As in Group III, fluorescence could be demonstrated in the media of the RMCA at 16
days. By 30 days, the RMCA showed Grade II pathology with no notable change in fluorescence (fig. 10 and table 1). No medial fluorescences was observed at this time period. The LMCA's showed Grade I alterations (excluding myonecrosis and intramural hemorrhage) at all post-hemorrhagic periods. In this vessel following 10 days post-hemorrhage, fluorescence could only be demonstrated in sectioned vessels (+). By 16 and 30 days, fluorescence increased (++) but still remained below normal (See table 1). As in Group III, catecholamine fluorescence was not observed in areas of subintimal proliferation.

Propagation of Vessel Alterations

The results are shown in figure 11. In the cat, the entire 20 mm segment of the MCA's could be successfully removed. Using the previously described surgical approach, the hook was placed more proximal than distal in the main trunk of the RMCA.

Vessel Rupture

Figure 11-A represents a reconstruction of the propagation of subintimal proliferation in the RMCA 30 days following SAH. At the site of rupture, proliferation was very severe, resulting in almost total occlusion of the lumen. Such proliferation was continuous for approximately 2 mm, spreading equally proximally and distally from the site of rupture. Proximal to rupture, the continuous proliferation became milder and discontinuous for approximately 2 mm. Distal to the site of rupture, continuous proliferation also became milder and discontinuous for approximately 4 mm. Therefore, the entire length of the main trunk of the RMCA showed some degree of vessel damage. When the branching segment of the same vessel was examined, mild proliferative subintimal changes, being and discontinuous, were observed throughout its full ex-
tent. Figure 11-B represents the propagation of proliferation in the LMCA (contralateral to ruptured side). In the main trunk, proliferation was mild, spotty and discontinuous. Such changes were seen at intervals along the longitudinal aspect of the vessel for approximately 5 mm of the 8 mm segment. The proliferation seemed to occur more frequently in distal aspects of the trunk than in proximal areas. When the branching segment of the LMCA was studied, mild and focal proliferation very similar to that observed in the RMCA could be demonstrated throughout the entire 12 mm segment (fig. 11-B).

Reserpine Pretreated — Vessel Rupture

Figures 11C and 11-D represent the spread of subintimal proliferation in the RMCA and LMCA at 30 days post-SAH in cats pretreated with reserpine. At the site of rupture (RMCA), smooth muscle proliferation was continuous for approximately 0.6 mm. Distal to the point of rupture, mild but discontinuous proliferation skipped along the main trunk and into the branching segment (fig. 11-C). The frequency and severity of proliferation along the longitudinal aspect of the vessel was less when compared to its untreated counterpart (11-A). Proximal to the point of rupture, no proliferation could be demonstrated. The LMCA showed mild proliferation at various intervals along the longitudinal aspect of both the main trunk and branching segment (11-D).

Gross Cerebral Pathology

Softening of the cerebral cortex in the areas of distribution of the RMCA was observed in cats of Group III sacrificed at 3, 16 and 30 days following SAH. Two of them (3 and 16 days cats) developed left hemiparesis in their post-SAH course. Infarction was most pronounced along the course of the main trunk of the vessels, which correlated well with the pathological conditions of the vessel wall. Infarctions were not observed in cats of other groups.

Discussion

Experimentally induced acute vasospasm may have little in common with the disorder which occurs in man after aneurysm rupture. Wilkins (1980), in reviewing the angiograms of patients whose aneurysm ruptured during the examination found no convincing evidence of immediate vasospasm with SAH. Agents known to produce in vivo and in vitro acute vasospasm do not appear to promote chronic spasm. Also, drugs and chemical agents known to prevent or reverse acute laboratory vasospasm have no such influence on chronic vasospasm seen in man. Not uncommonly chronic vasospasm may persist for weeks but virtually all of the known spasmogenic agents are inactivated quickly and survive only briefly in free form in biological fluids. Pathophysiological studies in our laboratory indicate that acute vasospasm may be a physiologically reversible mechanism while delayed spasm may be pathologically based.

While numerous substances normally present in blood have been shown to cause acute vasospasm, the evidence implicating NE release in secondary vasocostriction is substantive. Following SAH, massive overactivity of the sympathetic nervous system has been consistently found. Furthermore, when applied directly in large concentrations, it is capable of causing morphological alterations in the cerebral artery. Both cardiac necrosis and intramyocardial hemorrhage have been produced in laboratory animals subjected to SAH. Pretreatment with reserpine (a NE depletor at the vesicle level) virtually assures prevention of these findings. In a study of 77 patients who died following SAH, Smith et al reported a significant correlation between angiographic constriction and pathological alterations of cerebral arteries. In this series, 6 patients were receiving reserpine when their aneurysm ruptured. Five of these showed no vessel alterations while one showed only mild changes. In dogs, constriction of the basilar artery does not occur when blood taken from dogs pretreated with reserpine is injected into the subarachnoid space, but...
does occur when untreated blood is used. There is much less early spasm in sympathectomized dogs than in controls, but the degree of late early spasm seen in the two groups appears identical. Some have concluded that the vasoconstrictor substance released by sympathetics may not be NE but that some other agent in blood acts at the alpha adrenergic receptor site.

In addition to reserpine's effect on vascular wall catecholamines, other known actions could bear upon the results of this study. Serotonin (5-HT) is known to cause severe and acute vasoconstriction, proliferation of fibroblasts and the contraction of myofibroblasts. Platelets, which are known to aggregate upon the intimal surface of vessels following either injury, rupture or induced spasm, contain virtually all of the 5-HT in blood. Reserpine prevents the storage of platelet 5-HT, along with several other vasoactive amines. Reserpine treatment also reduces the concentration of 5-HT in adrenergic nerve fibers and ganglia and may cause a release of NE and histamine from platelets. The ameliorative effect of reserpine pretreatment in this study, thus, may be via its effect on platelet serotonin.

The contraction of normal vascular smooth muscle is tied to the concentration of intracellular free calcium ions (Ca++). Many of the known vasoconstrictors induce vessel contraction by increasing intracellular free Ca++. Reserpine administration leads to depletion of molar Ca++ from extracranial vessels in normal dogs, rabbits, and rats and from the myocardiun of several strains of mice. There is good evidence to indicate a defect in Ca++ transport in smooth muscle cells of cerebral arteries following induced and prolonged vasospasm. If mechanical or ischemic vessel trauma results in Ca++ overloadng, reserpine, by decreasing the availability of this ion, or perhaps by countering the Ca++ influx, could modify acute vessel constriction and the later morphologic alterations perhaps attributable to it. Finally, reserpine, via its effect on blood pressure could have modified the amount of blood that escaped into the subarachnoid space upon rupture of the MCA. Both the volume of subarachnoid blood and perhaps the extent of its intramural and extramural dissection may be important factors in the angiopathy seen after SAH.

In the present study, SAH caused immediate and complete, but transient, loss of adventitial fluorescence of the adrenergic nerve plexi supplying the ruptured artery. Concentrations remained well below normal over a 30 day post-SAH period. This acute depletion of NE seen after SAH is in agreement with the works of others although Lobato et al. found normal levels within 2-3 weeks after hemorrhage. The acute decrease in adventitial fluorescence following SAH was accompanied by a later increase in medullary fluorescence, the significance and source of which remains unclear. Normally, as NE is released from nerve ending it binds briefly onto the receptors of smooth muscle cells. The majority is then quickly taken up again into the nerve ending. Subarachnoid blood may block the uptake of free NE into adrenergic vesicles leaving excessive extracellular NE to be taken up by vascular smooth muscle cells. This amine is known to accumulate in smooth muscle of various organs overloaded with NE.

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Figure 11. Density of geometric shapes located along intimal layer of diagram of RMCA indicates the presence and intensity of subintimal proliferation. Arrows indicate point of vessel rupture.
The angiopathy of subarachnoid hemorrhage I. Role of vessel wall catecholamines.
J Yoshioka, B R Clower and R R Smith

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