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A PRIMATE MODEL OF CHRONIC CEREBRAL VASOSPASM: DEVELOPMENT AND APPLICATION IN THE TREATMENT OF SUBARACHNOID HEMORRHAGE IN HUMANS

by

FRANCISCO JAVIER ESPINOSA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "A PRIMATE MODEL OF CHRONIC CEREBRAL VASOSPASM: DEVELOPMENT AND APPLICATION IN THE TREATMENT OF SUBARACHNOID HEMORRHAGE IN HUMANS", submitted by FRANCISCO JAVIER ESPINOSA in partial fulfilment of the requirements for the degree of Doctor of Philosophy in EXPERIMENTAL SURGERY.

Co-Supervisors

(External Examiner)

Date: October 29th, 1984

This thesis is dedicated to

my parents, Luis and Teresa,

and

to my wife Gina and daughter Jessica

ABSTRACT

Subarachnoid hemorrhage (SAH), which may be complicated by cerebral vasospasm (VSP), is fatal or severely disabling in about 70% of cases (179).

A model was created in monkeys for studies of the pathogenesis of the VSP as a basis for attempts to devise rational therapies. The present work consisted of three studies (A, B, and C).

STUDY A

Cerebral vasospasm was created by inducing SAH in 25 monkeys, and correlation was sought between the size of the hemorrhage seen on the first CT scan with the severity and time-course of VSP for up to 21 days. Eighteen monkeys were studied for at least 48 hr. Investigations included neurological examination, determination of cerebral blood flow (CBF), computed tomography (CT) scans, and measurement of vessel caliber on angiograms.

The incidence of VSP was significantly higher on Days 0, 7, and 14 ($p < 0.001$, < 0.01, and < 0.05, respectively), and reduction in vessel caliber was significantly greater at those times (Day 7, $p < 0.02$; Days 0 and 14, $p < 0.05$) in the large-SAH group than in the small/medium-SAH group.

Conclusions: 1. The time-course of the VSP was very similar to that after SAH in humans. 2. The size of the SAH seen on the initial CT scan correlated with the severity of VSP apparent radiographically for up to 14 days.

This model had two major disadvantages:

1. There was a high incidence of subdural hematomas, necessitating large numbers of animals for study.
2. In most cases, the VSP was localized to the pericallosal artery (the artery closest to the site of blood injection and to the collection of blood visible on CT scans); therefore, no obvious neurologic deficits developed.

STUDY B (Pilot Study)

Two new approaches for the creation of a better model of chronic VSP were investigated.

In Group 1, in eight monkeys, blood was injected under direct vision into the subarachnoid space through a right frontolateral craniectomy.

In Group 2, in two monkeys, the subarachnoid space was widely opened at the right sylvian fissure, exposing the right side of the circle of Willis and sphenoidal middle cerebral artery, and a hematoma (1.5 to 5 ml) was placed against the arteries. Isolation of the segment of the circle of Willis and placement of the hematoma provided a model of VSP closer to that in man.

STUDY C

Having developed a reliable method to induce chronic cerebral VSP after SAH in primates, a double-blind trial of nimodipine was conducted after inducing SAH in 30 monkeys. Using microsurgical techniques, frontotemporal craniectomy was performed and the subarachnoid cisterns were opened unilaterally. CSF was drained, and a fresh hematoma from an average of 7 ml of autologous blood was carefully placed against the

major anterior circulation arteries on one side. The monkeys, allocated randomly to two groups of 15, received placebo, or nimodipine (1 mg/kg), every 8 hr for 7 to 14 days after the SAH. Indices determined before and after SAH were as for Study A, together with assessment of pathological changes in cerebral-vessel walls on light and electron microscopy.

In the placebo group, delayed ischemic neurologic deficit developed in one monkey 4 days post-SAH and was present at death on Day 14. No such deficit occurred in the nimodipine group. Significant VSP (31-100% reduction in vessel caliber) developed in 26 (87%) of the 30 animals. VSP was significantly more common ($p < 0.0001$) and more severe ($p < 0.01$) on the clot side than on the non-clot side on Day 7, and was less or absent on Day 14 just before death. VSP was more common overall in the placebo group, and on Days 7 and 14 its incidence was significantly higher ($p < 0.05$) in this group than in the nimodipine group. However, the average percentage reduction in caliber of the maximally constricted vessel in each monkey was not significantly different in the two groups. The pathologic changes in vessels that were in spasm are described in detail.

PREFACE

It is estimated that as many as 5 million persons in North America harbor saccular cerebral aneurysms, 90% of which may rupture to produce a SAH. With an annual incidence of 12 per 100,000 population, in the U.S.A. about 28,000 aneurysms rupture each year (11,179). Aneurysmal SAH carries a very poor prognosis: about 40-50% of the patients die within 2 weeks as a result of their first bleeding (8-13,179,196). In the 50-60% who survive the acute stage, cerebral VSP may develop in about 25-37% - the reported figures range from 21% to 66% (16,18,196, 198,203). In 10-17% of these cases, neurologic deterioration (including death) will occur 1-2 wk after the hemorrhage. Clearly, the 60-70% morbidity and mortality rates (8,11,20,21,179) demonstrate much room for improvement.

Therapeutic strategies of potential benefit include improved diagnosis (awareness of warning signs) and early referral to a neurosurgical center (55), prevention of rebleeding, treatment of cerebral VSP, and improved surgical results. One approach for the prevention of rebleeding, and perhaps of VSP, is early operation (17,19,304,305). At present, however, there is no treatment that can reliably prevent the deterioration in neurological status that results from VSP (19,32,305). Numerous pharmacological agents have been tried to relieve the VSP (44), usually unsuccessfully; the treatment of VSP remains uncertain and unsatisfactory (16,44,45).

The inadequacy of current therapies reflects our lack of knowledge of the condition, an ignorance that stems largely from the absence until

recently of an experimental model of chronic VSP that could mimic the clinical syndrome (46-48,52,53). The present studies, in monkeys, were designed to create a model of chronic VSP by inducing a large SAH, then to test the hypothesis that the size of the SAH as apparent on CT scans is critical to the development of severe chronic VSP, and finally to investigate the efficacy of one pharmacologic agent in preventing VSP after SAH. The agent chosen was nimodipine, which antagonises calcium entrance to cerebral smooth-muscle cells and thus might be expected to prevent or reduce the severity of chronic cerebral VSP after SAH (49-51).

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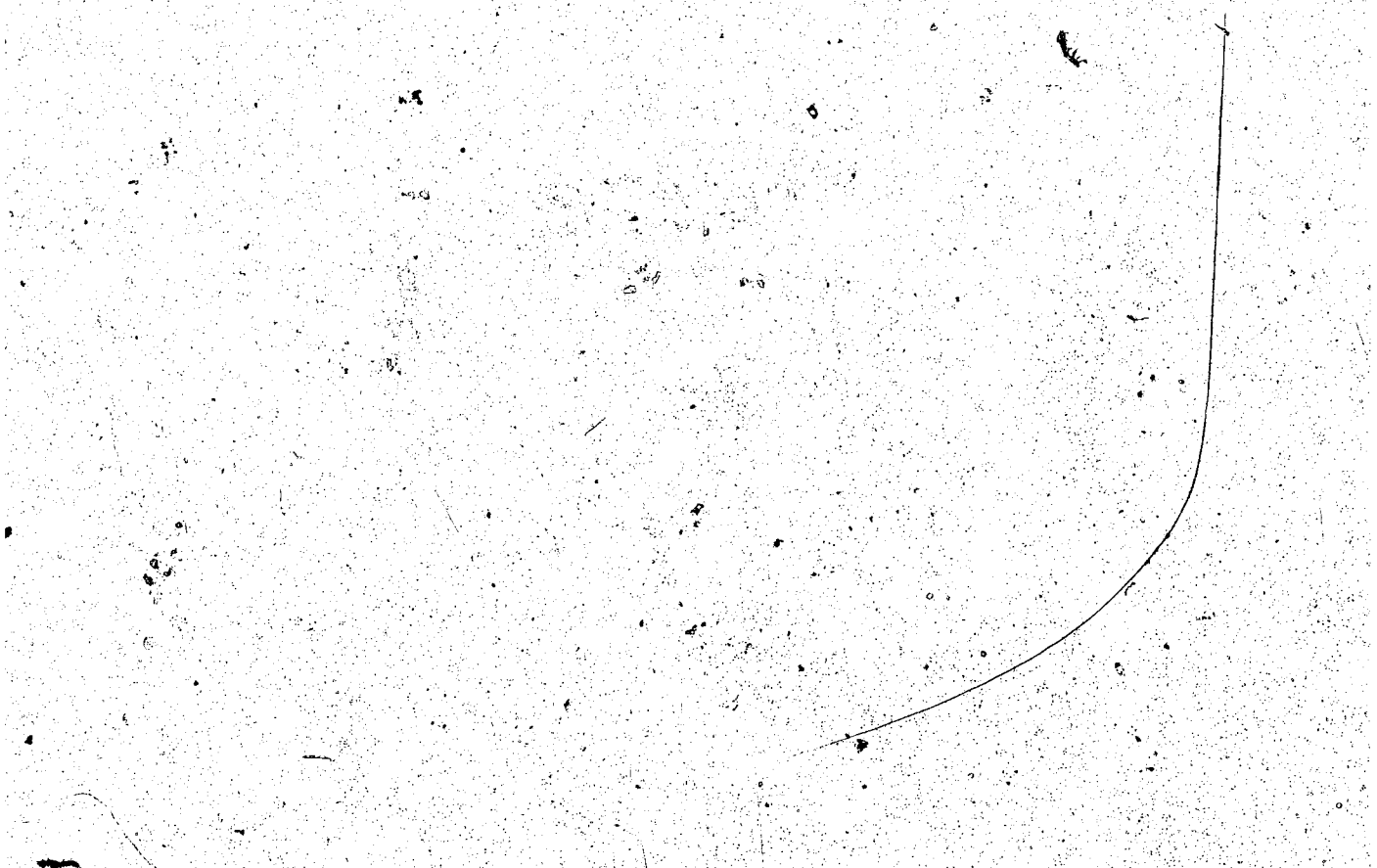

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CHAPTER ONE

THE PROBLEM

I. CEREBRAL ANEURYSMS AND SUBARACHNOID HEMORRHAGE: HISTORICAL REVIEW[1]

The first recorded description of what appears to be a subarachnoid hemorrhage is in the Old Testament, 2-Kings 4:18-20: "And when the child was grown, it fell on a day, that he went out to his father to the reapers. And he said unto his father, My head, my head. And he said to a lad, Carry him to his mother. And ... he sat on her knees till noon, and then died."

Morgagni of Padua in 1583 was probably the first to describe a dilation of cerebral vessels at necropsy, but the first precise description of an intracranial aneurysm, of the carotid artery in the cavernous sinus, was by Biumi of Milan in 1778. Blackall, in 1813, first described the symptoms and clinical signs of intracranial hemorrhage. The patient, a 20-year-old woman, complained of headache and photophobia, and necropsy revealed a pea-sized aneurysm at the distal end of the basilar artery. Serres, in 1819, diagnosed a ruptured aneurysm in a 59-year-old woman, basing his pre-necropsy opinion on the sequence of neurological signs after the acute event; the cause of death proved to be an egg-sized aneurysm of the basilar artery. It is interesting that these two first clinico-pathological descriptions of cerebral aneurysms were made on female patients harboring a basilar

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[1] Much of this review was compiled from references no. 1, 2, 4, 11, 15, 20, 22-25, 54.

artery aneurysm; at present, the known incidence of such aneurysms is very low - 3-4%, compared with 90-95% in the anterior cerebral circulation (2,4,11).

Clinical Diagnosis

In 1859, in reviewing 62 reported cases of aneurysms of the cerebral vessels, Gull emphasized their recognition at necropsy rather than during life, and it was not until 1864 that accurate clinical diagnosis began to be made with any regularity. In that year Hutchinson made a clinical diagnosis of intracranial aneurysm, and in 1875 he verified this when the patient died (from a pelvic abscess): necropsy revealed an aneurysm within the inner part of the middle fossa.

There was a major advance in the diagnosis of SAH in 1891, when Quinke introduced the lumbar puncture. A further stimulus to the clinical diagnosis of intracranial aneurysms occurred in 1923, when Symonds reported five cases diagnosed during life and concluded that ruptured aneurysms are initially indicated by SAH with symptoms and signs suggestive of a tumor at the base of the brain. The first extensive review was by McDonald and Korb, in 1939, who assembled 1125 cases of saccular aneurysm from 407 reports published up to the end of 1937; all had been verified at necropsy or operation.

Cerebral angiography, introduced by Moniz in 1927, proved to be the most significant advance in establishing the diagnosis of cerebral aneurysm. Moniz used an open surgical method for introducing contrast medium into the carotid artery, and Loman and Myerson were the pioneers in the development of percutaneous carotid angiography, first reported in 1936.

The introduction of cerebral computed tomography (CT) in 1972 revolutionized the approach to the investigation of intracranial pathology in the practice of neurology and neurosurgery. English engineer G.N. Hounsfield, a computer expert working in the Central Research Laboratories of EMI, directed his attention to the problem of reconstructive transmission X-ray scanning. In fact, the mathematics had been 'worked out' by Radon in 1917 and again, independently, by Bracewell in 1936 (316). Hounsfield's work, which was independent of the others', began in 1967; it would bring him the 1979 Nobel Prize in Medicine. This he shared with physicist A.M. Cormack who, in 1963 and 1964, described mathematics related to the reconstruction of a cross-sectional radiographic image that could be applied to tomography (157).

Nonsurgical Therapy

Until the nineteenth century, therapy consisted of bed-rest supplemented with medication thought to be appropriate; for example, in 1761 Morgagni suggested using ergotamine, potassium iodide, strychnine, and galvanic current.

Conservative therapy was the only treatment until it was found, in 1809, that occluding the cervical carotid artery could relieve symptoms and signs of an unruptured aneurysm or prevent rebleeding from a ruptured one. This treatment was not very widely practised, but it is of interest that Vanzetti, in 1858, reported the cure of two carotid aneurysms in the cavernous sinus by prolonged digital compression of the ipsilateral carotid artery. No further innovations were introduced until about 17 years ago.

Antifibrinolysins

Gibbs, in 1967, followed by Mullen in 1968 and Norlen in 1969, introduced the idea of using antifibrinolytic agents to prevent natural lysis of the clot sealing a ruptured aneurysm. An NIH Co-operative Study completed in 1975 indicated that antifibrinolytic therapy reduced the rate of early rebleeding (occurring within 14 days) by nearly half, from 20.9% (with bed-rest only) to 11%.

Extracranial Surgical Occlusion

The first surgical therapy for cerebral aneurysms was unilateral ligation of the common carotid artery in the neck; this was used successfully by Travers, in 1809, to treat a carotid cavernous fistula that was causing unilateral pulsating exophthalmos. In 1902 Sir Victor Horsley reported successful treatment of saccular aneurysms found at operation by unilateral occlusion of the common carotid. Unilateral ligation of the internal carotid was first used by Turner in 1926 and by Dott a few years later, and the bilateral maneuver was introduced by Hamby and Gardner in 1932; the latter, which was performed in two stages, was reserved for the treatment of refractory aneurysms of the carotid artery in the cavernous sinus.

Dandy was the pioneer who showed, in 1944, that unilateral but not bilateral occlusion of the vertebral artery in the neck could be safely used to treat infratentorial aneurysms. In 1950, however, French and Haines pointed out that unilateral occlusion of a vertebral artery could be fatal.

Intracranial Surgery

The first attempt at intracranial surgery to treat an aneurysm was by intracranial and cervical occlusion of the internal carotid artery by Zeller in 1911; unfortunately the ligature was accidentally torn, and the patient died. Similarly, Cushing's first two attempts at intracranial surgical treatment of aneurysms, both of them on the internal carotid artery, resulted in death: in 1917 he ligated the artery after an aneurysm on its wall had burst at operation; and in 1926, having tapped a sac he believed was an intracerebral cyst, he occluded it with fine strips of fresh muscle fed through the puncture.

Successful surgical treatment of an intracranial aneurysm was achieved a few years later. In 1931, Dott, in Edinburgh, wrapped muscle around the saccular aneurysm; and 5 years later, Tönnis, in Germany, split the corpus callosum and applied muscle to cover an aneurysm that the then-new technique of angiography had revealed in the anterior communicating artery. In 1937, McConnell, in Ireland, opened an aneurysmal sac and packed it with muscle, and on 27th March of the same year Dandy initiated clip-occlusion. Dandy applied the clip to the neck of an aneurysm at the junction of the right internal carotid and posterior communicating artery; the patient recovered from his third-nerve palsy.

Surgery in Modern Times

Modern surgical treatment of intracranial aneurysms began after World War II. Dandy's classical monograph in 1944 provided the impetus, and between 1945 and 1954 new operative techniques were developed by Norlen in Sweden, Krayenbühl in Zurich, Scoville, Hamby and Mayfield in

the U.S.A., Falconer and McKissock in London, and Gillingham in Edinburgh. Improved retractors and clips, and investment methods for aneurysms with broader, complex necks, were developed. Great advances were made, despite the poor illumination and restricted vision that complicated the operation.

But with the advent of improved diagnostic and treatment methods came recognition of a new development: severe chronic cerebral vasospasm. This was exemplified in patients who, having appeared well for hours or days after the hemorrhage or postoperatively, experienced states of confusion, stupor and coma, with major neurological deficit and in some cases death. The delayed neurologic deterioration was shown to be associated with severe arterial narrowing apparent on cerebral angiography: almost invariably the arteries that were in severe spasm were in cerebral areas appropriate to the clinical syndrome. Ecker, in particular, emphasized the problems of spasm in the late 1940s, and in 1951 he and Rienmenschneider described the angiographic appearances of diffuse vasospasm in cerebral arteries after SAH. In 1965 Echlin demonstrated severe spasm of the basilar artery after its surgical exposure and direct application of fresh blood in monkeys, and in 1968 Simeone and co-workers reported for the first time that diffuse prolonged VSP could occur in animals. In that same year Brawley presented evidence in dogs that VSP may be both acute and recurrent (biphasic) after avulsion of the anterior cerebral artery and that an injection of fresh arterial blood into the subarachnoid space causes acute and prolonged VSP. However, after such experimental induction of SAH, in all animal species studied, clinical manifestations were minimal or absent. Thus, although

the findings in experimental animals increased our knowledge of VSP they could not be extrapolated to VSP in man.

In 1956 Botterell (153) instituted a system of clinical grading to guide the selection of patients likely to benefit from surgical therapy. This system, which was expanded by Hunt and Hess (154) in 1968, was an important advance: now, surgical results could be correlated with the neurological status at first medical attention - a major influence in clinical outcome.

The greatest technical advance in vascular neurosurgery was in the 1960s, when the introduction of the operating microscope provided for the first time excellent magnification and illumination of the operative field. In 1965 Pool published the first accounts on the use of the operating microscope in intracranial aneurysm surgery. Yasargil (155) advanced the state of the art on microsurgical techniques for cerebral aneurysms, and reported a significant decrease in mortality rates. Fox (54) demonstrated how the microscope improved intracranial surgery for aneurysms during 14 years of practice: initially from 1964 to 1975, the mortality in 95 cases operated on without a microscope was 15%, and then from 1975 to 1978 in 81 cases operated on with microsurgical technique it was reduced to 7%. Improved anesthesia and surgical skills, however, could also contribute to the progress made by Fox.

II. INCIDENCE, PREVALENCE, MORBIDITY AND MORTALITY OF SUBARACHNOID HEMORRHAGE FROM RUPTURED ANEURYSM

Intracranial aneurysms can be classified as saccular, atherosclerotic, inflammatory, traumatic or dissecting aneurysms, or as microaneurysms (3-5). The present review relates only to saccular or berry aneurysms, which account for 66% to 90% of all cerebral aneurysms (4).

The incidence of saccular aneurysms in the general population has been estimated as about 1 to 4%, but it varies depending upon the population characteristics and the nature of the study (i.e., all-systems necropsy or a search for cerebral aneurysms). Thus, reported figures range from 0.2% to 16.0% (2-4,20,21). Spontaneous rupture of a saccular aneurysm is the commonest cause of nontraumatic SAH, being responsible for about 50% (6,7).

As might be expected, the geographical incidence of aneurysmal SAH varies considerably. In Rhodesia, a 1982 report stated that only 3.5/100,000 population are encountered annually (7). In Rochester, Minnesota, the same incidence during a 30-year study from 1945 to 1975 remained remarkably constant at 11 (8); in Finland it was said to be at least 10/100,000 population (26), and a more recent study quoted an incidence of 19.4 in central Finland (173). It has been estimated (11,20) that approximately 3,000 aneurysms rupture each year in Canada (12/100,000 population) and as many as 28,000 in the USA (12/100,000 population) (179). The highest incidence (25/100,000 population/year) is in Japan, where 28,000 new cases of SAH are reported annually (7).

The reported figures may represent only part of the actual incidence of SAH among the general population. During a 2-year study in

New York, cases at the City Morgue that were the result of unrecognized or untreated SAH equalled about 33% of the number of cases seen at the New York University Medical Center (20).

Aneurysmal SAH is highly lethal. Overall, 8% to 15% of the patients die within 24 hr, before they can receive medical attention. The mortality rate rises to 20-24% by 48 hr, to 44-56% by 14 days, and to 66% by 1-2 months after the rupture (8,-13). The mortality of aneurysmal SAH is directly related to the patients' neurological status at first medical attention (9,12,13). Phillips et al. (8), who studied the association between the patients' neurological status on admission to hospital and the mortality rate by 10 days post-SAH, recorded 10% mortality among patients graded 1 or 2 (asymptomatic or with headache and nuchal rigidity), 28% in grade 3 (focal deficit), and 80% among those of poorer neurological status (grade 4 or 5, major deficit or moribund). Winn et al. (14) studied the same association but with mortality rates by 6 months post-SAH: in patients graded 1 on admission, mortality was 15%; in those graded 2 it was 30%, for grade 3 the mortality increased to 54%, and for grade 4 to 65%; and all patients graded 5 had died. In general, in North America the 1-year survival rate is 47% for patients in good neurological state (grade 1) when first seen by a physician, compared with only 8% for those in neurological grade 5. Ropper and Zervas (198), however, studying the 1-year outcome of 112 good-risk SAH patients, observed a management mortality of only 11%, although functional recovery was very poor: only 46% of the patients recovered completely, 25% were emotionally impaired, and only 44% were able to return to their previous (or comparable) jobs; 20% obtained lesser employment, and 24% were unable to work.

The complications most commonly responsible for the rising mortality rate after the acute stage are cerebral ischemia, rebleeding, and hydrocephalus. The ischemia results from severe VSP, which develops in about 25-37% of cases within 2 weeks; rebleeding, if untreated, kills up to 30% of patients within a decade of their first SAH; and hydrocephalus may prove lethal by about 3 weeks post-SAH if no therapy is given to the patient.

III. CURRENT CONCEPTS OF CEREBRAL ANEURYSMS

The pathogenesis of cerebral berry aneurysms is a subject of continuing debate. To understand the controversy over the origin of these sacs requires knowledge of the morphology of cerebral arteries and aneurysms.

Microscopic Anatomy of Cerebral Vessels

All of the extracerebral intracranial arteries have a similar structure and are composed of the three coaxial coats: the tunica intima, lined by a layer of endothelial cells; the tunica media, or muscularis and elastin fibers; and a collagenous tunica adventitia. An internal elastica separates the intima from the media, but the external elastic lamina that characterizes extracranial arteries is absent (56). Around bifurcations of arteries, the intima may have focal thickenings at the lateral angles, face, dorsum, and apex. The muscularis media may cease abruptly at the apex (medial defects of Forbus), leaving a gap consisting only of intima, elastic lamina, and adventitia; these defects may be present at birth but in many cases develop postnatally (57). It has been suggested that the medial defects constitute a physiological mechanism to maintain blood flow during vasoconstriction; however, this explanation is inconsistent with their distribution, some forks having no defects and others having a defect at the lateral angle rather than at the apex. The most plausible explanation is that they act as a raphe between the muscle of the adjoining arterial wall at sites where the direction of vasoconstriction is in two very different or almost opposing directions (Fig. 1). This thesis is substantiated by the

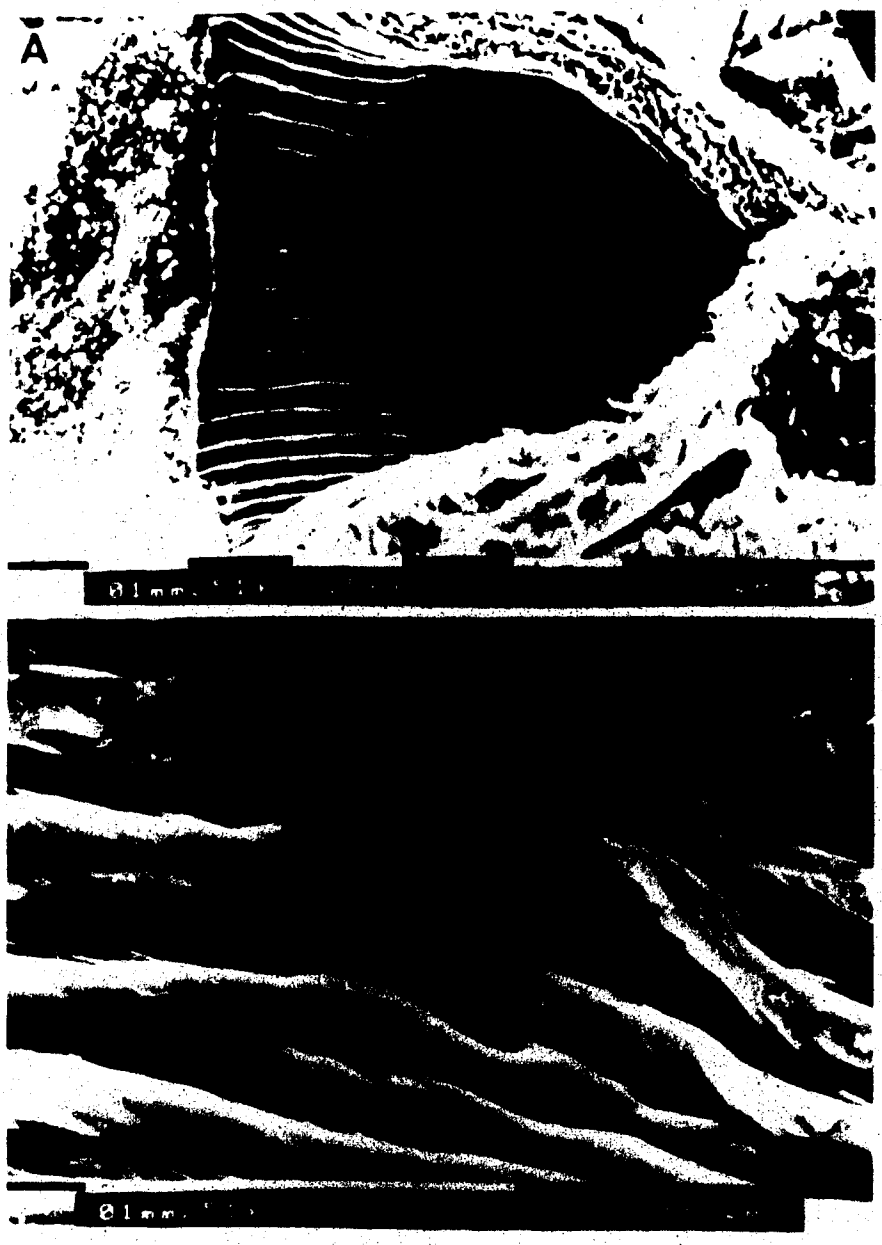


FIGURE 1

A: Scanning electron micrographs of the luminal surface of a middle cerebral artery and daughter branch. X 156.

B: At higher magnification (X 625), note corrugations of the endothelium (due to contraction of media) on both vessels - but absence at the apex or bifurcation, probably due to lack of media in this region (medial defect of Forbus).

observation that medial defects have a known predilection for acute angles, whether apical or lateral (57).

The internal elastic lamina of human cerebral arteries consists of two layers; the luminal surface is a fibrous mesh that transforms into a sponge-like layer, and the outer layer, which is adjacent to the media, forms a solid mass. This internal elastic lamina contains fenestrations (Fig. 2) which are present at all ages (they have been described in newborns and in adults up to 90 years of age). Campbell and Roach (58), who measured these 'windows' in the internal elastic lamina at the bifurcation of human cerebral arteries, recorded two very different sets of figures (means $\pm$ SEM). In 'normal' internal elastica, the mean diameter was 2.1 $\pm$ 0.13 µm, the number of fenestrations was 4518 $\pm$ 397 per mm^2, and the area of internal elastic lamina that contained normal fenestrations averaged 1.8 $\pm$ 0.16%. In 13 of the 28 specimens they studied, there were enlarged (abnormal) fenestrations near the apex of the bifurcation: their mean diameter was 7.0 $\pm$ 0.34 µm (significantly greater than the normal), their number was less (2606 $\pm$ 284/mm^2), and the area containing them was significantly greater (15.0 $\pm$ 1.1%). Campbell and associates proposed that the enlarged fenestrations represent a weakness in the internal elastic lamina at the apex of the bifurcation, a defect that may contribute to the initiation of microaneurysms. They also opined that the internal elastic lamina at the apex of bifurcations may have a fragmentation or gap, together with lumpy degradation, both of which increase with age and are presumed due to hemodynamic factors (58,59).

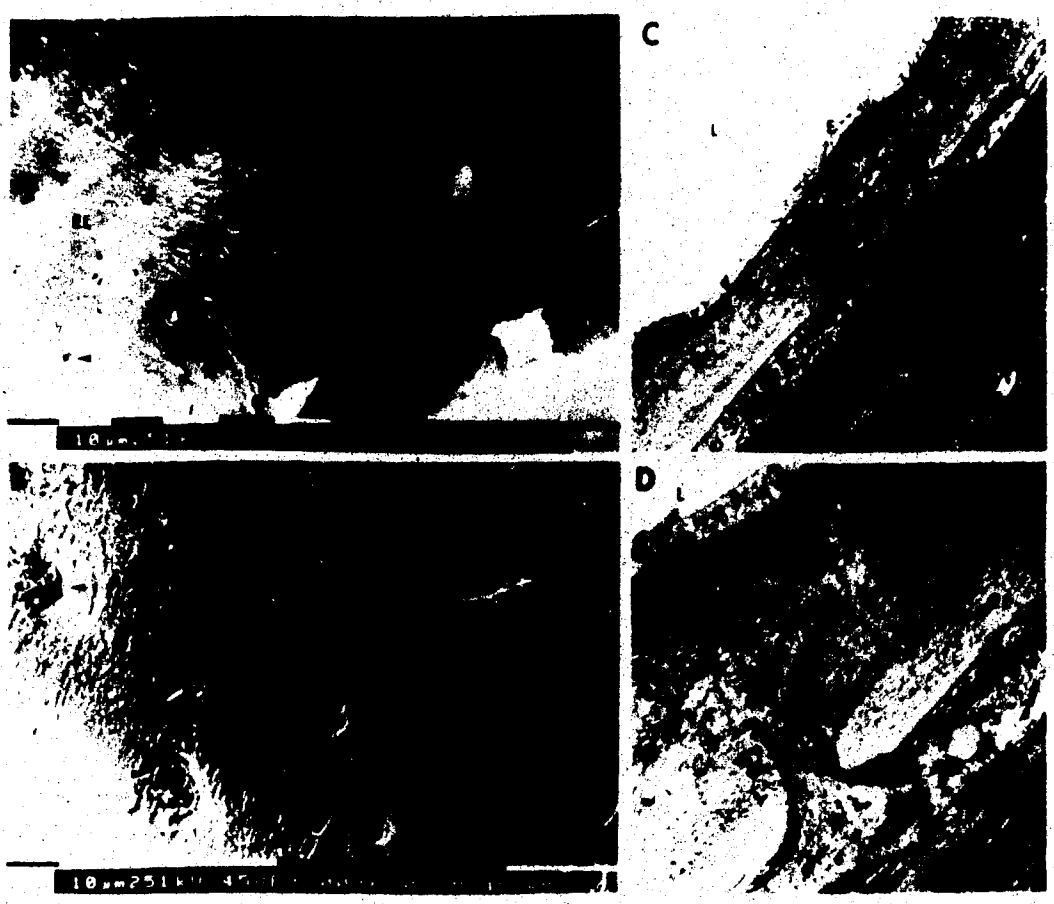


FIGURE 2

Scanning (left) and transmission (right) electron micrographs of internal elastic lamina (EL) of a middle cerebral artery. Note the normal fenestrations (arrow heads), 1.25 to 3 μm in diameter, that allow communication between the tunica media (M) and the basal membrane beneath the endothelium (E). L = lumen; A X 1150; B X 4580; C X 5500; D X 17,500.

All of these features of the internal elastica are considered of major importance in the formation of berry aneurysms.

Optical Microscopy of Saccular Aneurysms

Most saccular aneurysms occur at the apices of a major arterial bifurcation or at the origin of small branches from such arteries; they do not occur on peripheral branches of cerebral or cerebellar arteries or on small intracerebral vessels (60). The aneurysmal sac is characterized by absence of the normal layers of the vessel wall (56,60-62); it is devoid of media, which usually ceases abruptly proximal to the neck but in some cases extends for short distances into it. At about the same point, the internal elastic lamina is absent or exists as fragmentary remnants. In some places the intima appears normal, but in many others it contains subintimal proliferations consisting of smooth-muscle fibers and elastica. Atherosclerotic changes are usually confined to the intimal pads at the entrance of the sac; the adventitia serves as the outer layer of the sac.

The thickness of the sac wall varies: small aneurysms are thin-walled and most large ones have thicker walls, but parts of the neck and sac may vary in size. Thickened walls have a laminated appearance; the multi-layered capsule is composed of fibrous laminae interspersed with deposits of hemosiderin, cholesterol, and foam cells. The outer aspect of the wall consists of loose fibrous tissue; this is infiltrated with leukocytes and lymphocytes, and phagocytes filled with hemosiderin, if hemorrhage has occurred. Thrombosis, in varying degrees of organization, is a common finding in large aneurysms (56,60,62).

In long-standing aneurysms, most of the wall may be calcified or may evidence arteriosclerosis similar to that in cerebral arteries (62,63). Rupture is identifiable microscopically as a break in the wall, usually at the dome of the aneurysm. Vasa vasorum commonly invade the outer wall (Fig. 3), occasionally extending into the intima if this has undergone severe proliferation (63,64).

Electron Microscopy (EM) of Saccular Aneurysms

Scanning EM of the luminal surface of surgically-obtained intracranial saccular aneurysms (65) and the experimentally induced cerebral aneurysms (66) reveals regressive changes of any endothelial cells still present; e.g., balloon-like protrusions, crater-like defects, and bridges composed of cytoplasm (Fig. 4). Gaps are present at junctions of these endothelial cells, with many leukocytes and platelets adherent. The endothelium varies: usually, near the fissures of the aneurysmal fundus, it is missing; at the aneurysmal body it has an irregular pattern of small convolutions and intervening gullies; and near the neck of the aneurysm it is fairly well preserved. The adventitia may remain unchanged (65).

On transmission EM, the most significant findings are: 1. splitting of the elastic lamina in the sac; 2. no elastica at the site of rupture, this having been replaced by collagenous tissue, and subendothelial thickening of the basement membrane; 3. scanty, sclerotic smooth-muscle cells, separated by dystrophic basement membranes, cell debris, and other cells with autophagic vacuoles; and 4. vasa vasorum invading the outer wall and internal elastic lamina (60,64). The

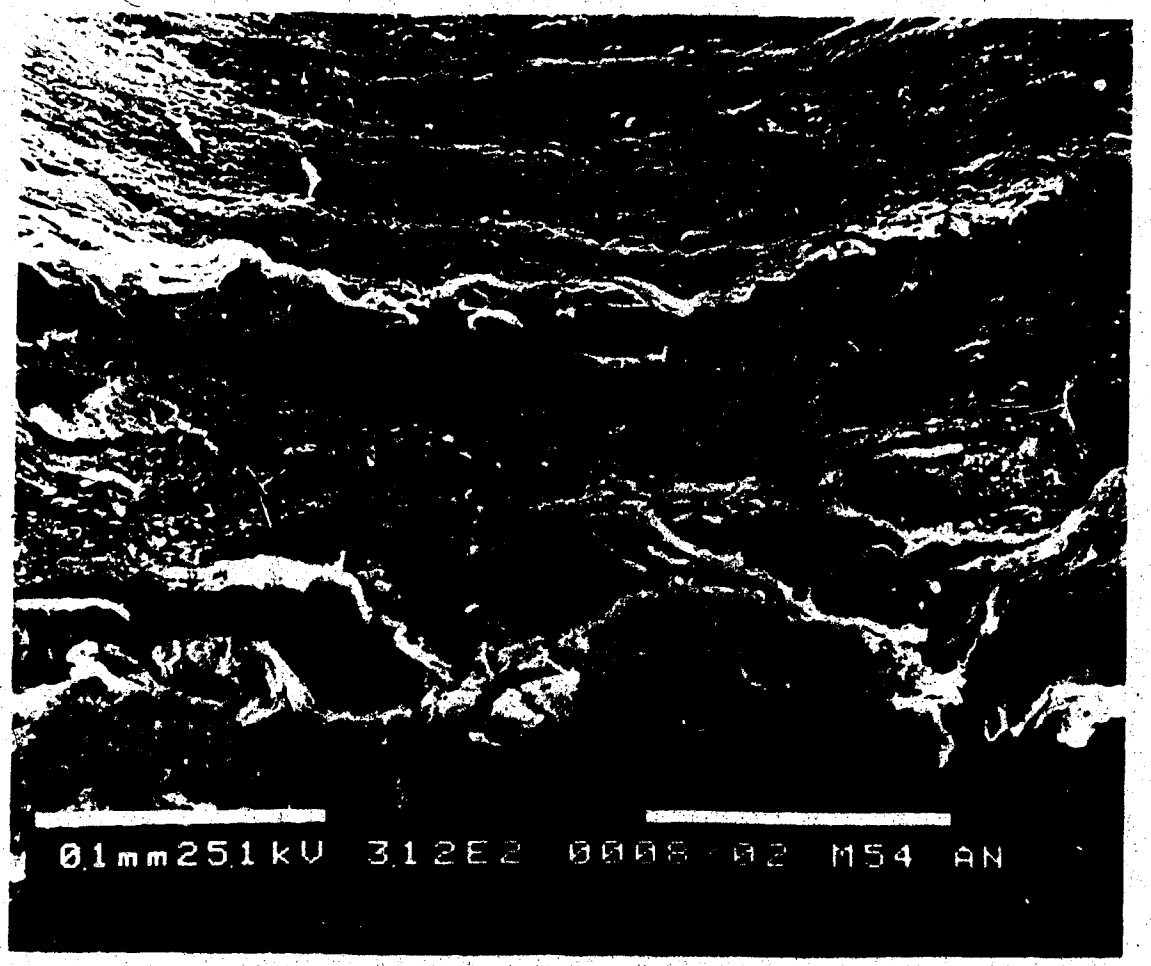


FIGURE 3

Scanning electron micrograph of the cut edge of aneurysmal wall, showing vasa vasorum (arrows) invading the tunica adventitia. X 312.

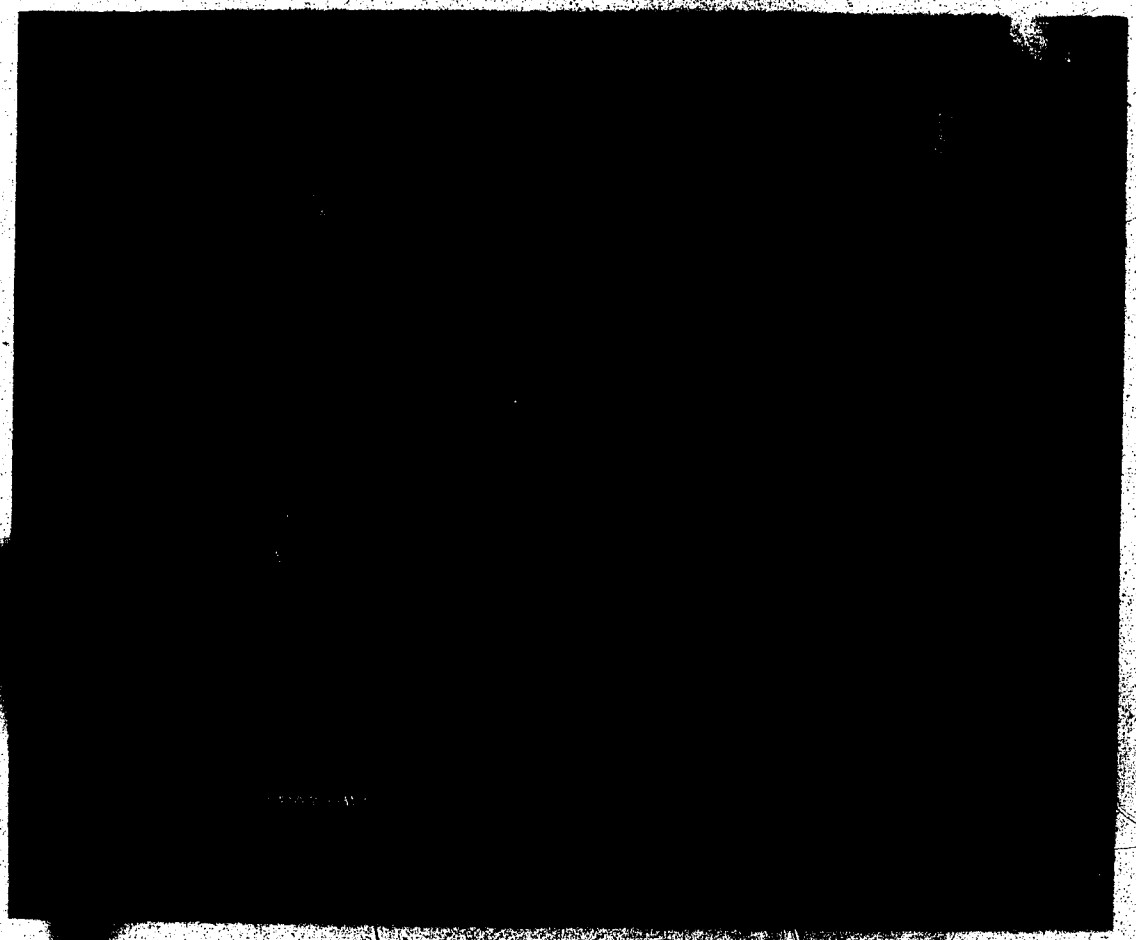


FIGURE 4

Scanning electron micrograph of the luminal surface of an aneurysm, Showing crater-like deficits, balloon-like protrusions, and disrupted endothelium. X 1200.

endothelial cells contain intracytoplasmic vacuoles that are empty or are full of lipid material. Within the connective tissue some well-preserved fibroblasts with star-like prolongations are seen, and the cytoplasm of muscular fibers contains swollen mitochondria and dilated endoplasmic reticulum.

Pathogenesis of Saccular Aneurysms

Three main theories have been advanced to explain the origin of cerebral berry aneurysms. One postulates a congenital defect of the media at the apex of arterial bifurcations, another that degenerative changes within the vessel walls damage the internal elatica, allowing the formation of saccular aneurysms, and the third theory holds that congenital defects and degenerative changes act together in the formation of aneurysms (7,56,68).

Medial-defect Theory

This theory, propounded by Eppinger and Forbus, holds that aneurysms orginate from an inborn defect in the elastic properties of the arterial wall. The defect is said to be a result of lack of muscularis lamina, together with degeneration of the elastic membrane due to its overstretching in response to hemodymamic stresses at the apex of bifurcations (56,68).

Degeneration Theory

Many investigators do not consider absence of the media as the primary factor in the development of aneurysms: this medial defect is said to be present in 80% of the cerebral arteries of normal subjects and of patients with saccular aneurysms (56). Moreover, areas lacking muscularis can tolerate high intraluminal pressures (up to 600 mm Hg)

without bulging. Stehbens (57) theorized that the medial defects act as a raphe between the muscle of adjoining arterial wall at sites where vasoconstriction is in opposite direction, and suggested that "medial raphe" would be a more appropriate term. He also found that the prevalence of medial defects increases with age, thus establishing that not all are congenital.

Medial defects occur at bifurcations of small and large arteries, whereas most aneurysms occur on large arterial bifurcations. Stehbens (57) described three types of "pre-aneurysmal lesions" found in a study of 454 cerebral arterial forks; there were 7 funnel-shaped dilations, 29 areas of thinning, and 14 microscopic evaginations. Funnel-shaped dilations occur at apical angles (these are the infundibulae seen on angiography); the media is absent or attenuated, the internal lamina is degenerated or absent, and the adventitia is attenuated. In these dilations, unlike Forbus's medial defects, there is no abrupt discontinuity of the media and the adventitia is not thickened. Areas of thinning are found at the apex and adjacent to the distal side of the branches of large bifurcations. Grossly, the wall is thin, with attenuation of the adventitia, absence of the media and internal elastica, and slight thickening of the intima. The lesions bulge when perfused at a pressure of 30 cm H_2O. Small or microscopic evaginations are early aneurysmal changes visible only under the microscope. The intima bulges out through a medial defect or at its junction with normal tissue; usually there is associated degeneration of the elastica but in some cases the internal lamina is intact.

This degeneration theory is supported by experimental results (66,69-73): saccular aneurysms have been induced in rats by causing

connective-tissue abnormalities that weaken the arterial wall, and then increasing hemodynamic stress (71,73).

Combination Theory

This theory, adopted by Carmichael, suggests that arterial degeneration and congenital defects play roles of varying importance in the development of cerebral aneurysms (68), with constant systemic hypertension and impingement of the blood stream as additional factors (73).

Location of Aneurysms

About 90% to 95% of saccular aneurysms are on the anterior part of the circle of Willis. The four commonest sites, in order of frequency, are: 1. in the region of the anterior communicating artery (28-34%); 2. at the origin of the posterior communicating artery from the internal carotid artery (25-26%); 3. at the bifurcation of the sphenoidal segment of the middle cerebral artery (12-17%); and 4. at the bifurcation of the internal carotid artery into anterior and middle cerebral arteries (4-5%). The incidence of vertebrobasilar aneurysms is about 3-4% and that of cerebellar aneurysms is 1% (2,4,11). Multiple aneurysms are clinically diagnosed in only 15-20% of the cases; 47% of all double aneurysms are located contralaterally (2). Pathological analysis, however, have revealed an incidence of multiple aneurysms as high as 50%. Explanation for this discrepancy among clinical or pathological studies derives from the 60-70% sensitivity of cerebral angiography in detecting the cerebral aneurysm; incidental multiple aneurysms are found by chance occasionally at surgery (156).

Factors Predisposing to Aneurysm Formation

Age and Gender

The incidence of cerebral aneurysms is highest during the 4th, 5th, and 6th decades of life, peaking between the ages of 55 and 60 years (2,3,5,6,8,11). Saccular aneurysms are rare in childhood; therefore, they are considered acquired anomalies (5,6,8).

Overall, cerebral aneurysms are more common in women than in men, in a ratio of f:m 3:2 (6,8,11). Under the age of 40 it is more common in men than in women (m:f 2.7:1), but thereafter the sex-related ratio is reversed. The f:m ratio rises from 3:1 at 60-69 years to nearly 10:1 in the eighth decade (5,6,11).

Multiple aneurysms, also, are more common in females than in males (7). In familial cases of aneurysm there is both a predominance of females (f:m, 1.7:1) and a relatively young age at diagnosis (mean, 39 years), indicating the possibility of an inherited defect of the blood vessels that renders females more susceptible to aneurysm formation (7). The influence of steroidal hormones on collagen proliferation may enhance the formation and impair the repair of aneurysms (56,74); however, the sex-related differences do not correlate with either the population distribution or the prevalence of hypertension by sex or by age.

Heritability

Autosomal dominant inheritance of intracranial berry aneurysms has been suggested in some families. Evans et al. (75) described six affected family members in three generations, in a pattern that strongly suggested dominant inheritance, and Fox and Ko (76) found 6 proven cases among 13 siblings, 11 of whom were studied. Intracranial aneurysms have been reported in identical twins (77). Multiple familial cerebral

aneurysms are much rarer than single ones (77,78), and the frequency of aneurysms at identical sites in two relatives is more than twice that expected by chance (78,79). Also, aneurysms tend to rupture at a younger age in familial than in non-familial cases (78,79).

Hypertension

Essential hypertension is one of the most frequently reported medical conditions in patients with ruptured intracranial aneurysms (80). Because hypertension increases hemodynamic stress and accelerates atherosclerosis, a saccular aneurysm is more likely to develop from a defect in the media and internal elastica, and is more liable to grow and rupture, in a hypertensive person (56). In a retrospective study of nonfatal and 64 fatal cases of ruptured berry aneurysm, Franks (81) found hypertension commoner in the fatal group; also, the incidence of multiple sacs was higher and the survival rate after two hemorrhages was poorer in the hypertensive than the normotensive patients. He concluded that hypertension may contribute to both development and rupture of aneurysms. Andrews and Spiegel (79), comparing 212 aneurysm patients with an age-matched population, found systolic pressure significantly higher in the female patients in the age groups 18 to 54 years; under 55, both male and female hypertensive patients were twice as likely as the normal population to have multiple aneurysms; and for females, but not males, increasing age and higher systolic and diastolic pressures all correlated with an increasing number of lesions. Systolic and diastolic hypertension are significantly more prevalent in association with aneurysms in men in the third through sixth decades and in women in the fourth through sixth decades (29). Furthermore, secondary hypertension caused by polycystic kidney disease and aortic coarctation is

associated with an increased incidence of cerebral berry aneurysms (56,76,78,82).

Although some investigators have found no correlation between hypertension and saccular aneurysms (79), clinical experience (56,59,79) and experimental data (66,69-71,73) support the concept that hypertension is conducive to the development of berry aneurysms and may influence their growth and rupture.

Vascular Disorders

Masuzawa et al. (83) reported two women with berry aneurysm who had Takayasu's disease. Arteritis was not apparent in the intracranial vessels, and no pathological changes were found in the vessels adjacent to the aneurysm, leading the authors to conclude that the sac had resulted from high arterial pressure or hemodynamic stress through the patent brachiocephalic vessels. Intracranial aneurysms have been reported in association with absence of one or both internal carotid arteries (73, 84,85), solitary pericallosal artery (86), and cerebral arteriovenous malformations (87,88); in such cases the aneurysm may develop as a result of altered hemodynamics (84,87).

Other Diseases

Aneurysms have been associated with several other disorders, especially those affecting connective tissue. The latter include the Ehlers-Danlos syndrome (56,57,82,89), polycystic kidneys (56,82,89), fibromuscular arterial hyperplasias (82,89,90), fibromuscular dysplasia of intracranial arteries (91); moyamoya disease (56,92), type-III collagen deficiency (57), cerebral transmural angiitis as a complication of systemic lupus erythematosus (93), giant-cell arteritis (94), meningiomas and gliomas (95), and pituitary tumors (96).

The incidence of aneurysm co-existent with pituitary adenoma is significantly higher (7.4%) than in the general population, an association whose cause remains obscure. It may be that the prolonged high level of serum growth hormone in acromegaly produces arteriosclerotic and degenerative changes in the arterial wall, and that hypertension - which is common in acromegalics - may affect the arterial wall and predispose to the formation of an aneurysm (96,97).

Although none of these associations has been proved greater than in age-matched control subjects, the unusual location of the aneurysm suggests an association in some cases, especially with aortic coarctation and polycystic renal disease. The increased incidence of cerebral aneurysms may also relate to the high incidence of hypertension in these conditions (56).

Cigarette-smoking and Alcoholism

Bell and Symon's retrospective study (74) of 208 patients with ruptured cerebral aneurysms showed a highly significant excess of cigarette-smokers among both men and women and indicated that continued smoking increases the risk of SAH by a factor of 3.9 for men and 3.7 for women.

Hillbam and Kaste (98), in Finland, studied 75 consecutive patients aged 15 to 55 years who had verified aneurysmal SAH. In 25% of cases, the patients had drunk large quantities of alcohol in the 24 hours before the bleeding; this incidence of alcoholic intoxication was two to four times higher in the males and three to five times higher in the females than in the general Finnish population of the same age and sex. Experimental data indicated that acute alcoholic intoxication may cause brief vasodilation and cerebral hyperemia, and that prolonged intoxi-

cation may increase cerebral blood flow when alcohol-withdrawal symptoms appear. The authors concluded that occasional alcoholic intoxication may heighten the risk of aneurysmal SAH.

Hemodynamics in the Formation and Progress of Aneurysms

Hemodynamic Stress

Berry aneurysms arise at a branching site on the parent artery. Two major factors conducive to their formation are the effects of impingement of the axial stream on the apex of bifurcations and of pulsatile flow. The hydrostatic pressures exerted upon arterial bifurcations are greater than the pressure exerted at the orifices of branches and lateral angles (56,67,89,99). The aneurysms arise at a curve in the artery; these curves, by producing local alterations in intravascular hemodynamics, exert unusual stress on apical regions - which receive the greatest force of the pulse wave. Further evidence of the influence of hemodynamic stress on the origin of saccular aneurysm is the fact that virtually all of these sacs point in the direction the blood would go if the curve at that site were not present. Their dome or fundus points in the direction of maximal hemodynamic thrust in the pre-aneurysmal segment of the parent artery - at least until the aneurysm enlarges, when the direction of the fundus may change if it encounters obstacles. It is rare to find an aneurysm on the concave side of an arterial curve or pointing in a direction opposite to that of flow in the parent artery (100).

Hashimoto et al. (73) demonstrated in rats that increased hemodynamic stress is causally related to the development of induced cerebral aneurysm. The animals were made hypertensive and were fed

3-aminoproprionitrile (BAPN), which causes fragility of vessel walls. Despite the severe hypertension and BAPN, cerebral aneurysms did not develop in the group in which the carotid arteries were not ligated. In the other two groups (one or both common carotid arteries ligated), cerebral aneurysms developed on the vessels in which hemodynamic stress was increased.

Turbulence

Another principle of hydraulics - turbulence, which occurs when the flow of fluids inside a tube exceeds a critical velocity - plays an important role in the growth and rupture of the sac. Turbulence can make the vessel wall vibrate, and vibration of an arterial wall at low frequency (up to 200 cycles/sec) can be highly destructive to structural components (56,101). Thus, turbulence at arterial bifurcations can weaken aneurysmal walls, stimulating growth of the sac. The weakening may precede histological changes.

Pulsatile Flow and Rupture

Jain, using a latex model of aneurysms, studied mechanisms of aneurysmal rupture (102). When an aneurysm was subjected to pulsatile flow, it pulsated visibly and intraluminal pressures proximal to the site oscillated but distal pressures fluctuated little. When a constricting clamp was applied about 10 cm proximal to the aneurysm, the pressure rose proximally but remained unchanged distally and the aneurysm pulsated until the proximal vessel was stenosed to a diameter of 1 mm. When two aneurysms were inserted into the system, pulsation was less in the distal than in the proximal sac; when the pulsating flow was increased the proximal aneurysm ruptured, and when overall pressure was raised (by obstructing the outflow tube) both lesions ruptured

simultaneously. Thus, in summary, Jain showed in this model that repeated stretching of the aneurysmal wall weakened it, leading to its rupture; an aneurysm could be protected by changing from pulsatile to nonpulsatile flow, by constricting the feeding vessel; and when there were two aneurysms on the circuit, pulsation in the distal one was damped by the proximal aneurysm.

Reviewing the literature, Jain (102) found parallel clinical situations in relation to ruptured multiple aneurysms: of 18 cases of two aneurysms on the same vessel or one on a parent vessel and one on its branch, in 12 cases the proximal sac had ruptured and in the other 6 the distal one had ruptured and the proximal one was thrombosed. Differences in size of multiple aneurysms may affect flow characteristics; for example, a small aneurysm proximally may not damp pulsation in a larger one distally. Also, a sudden marked rise in blood pressure may rupture multiple aneurysms simultaneously. In a study in humans (56), intraaneurysmal pressures were pulsatile and comparable to systemic pressures; after carotid ligation, they were reduced by two-thirds but some remained pulsatile.

Spontaneous Aneurysmal Thrombosis

The shape and size of an aneurysm seem to influence whether the sac will rupture or will enlarge and thrombose. Most aneurysms rupture, becoming manifest in a sudden, dramatic, catastrophic event. A few go on to become giants, exceeding 2.5 cm in diameter.

Roach studied fluctuations in flow and velocity in aneurysm models (103). In dogs, she identified the aortic trifurcation and tied the tail artery at various distances before its bifurcation; short aneurysms (< 1 tube diameter) were unaltered after 2 weeks' experiments, medium-

sized aneurysms (2-2.5 tube diameters) ruptured, and long ones (> 4 tube diameters) thrombosed. These findings agree with clinical experience: most giant aneurysms in patients are partly thrombosed when found (56, 60,63,99,103). Then, in a plastic model of the trifurcation and distal vessels in dogs, Roach demonstrated a stagnant zone in the distal part of aneurysms: no flow occurred beyond 2.5 tube diameters of an aneurysm's origin, thus predisposing to thrombosis distally.

Extramural Factors in Aneurysmal Growth and Rupture

Suzuki and Ohara (104) suggested that bleeding from an aneurysm is stopped soon after the rupture by both the CSF's ability to accelerate clot formation and the spasm of cerebral blood vessels, allowing formation of a protective layer. The newly formed defensive layer may be fairly weak in the early stage, so that further ruptures may occur at this site. However, rebleeding after 3 weeks is rare; Suzuki and Ohara thought this may be due to reinforcement of the newly formed fibrin-layer wall by arachnoid-like tissue and capillary proliferation. These capillaries are subjected to continuous pressure from the pulsatile aneurysm. If, as a result, they rupture, slight bleeds at the aneurysmal wall will induce formation of another fibrin layer; i.e., the aneurysm will grow as the wall is thickened by repeated hemorrhages and their repair and absorption. If, however, the capillary hemorrhages are severe, they will create a weak point that is a potential site for further rupture.

Pressures and resistance in the environment of an aneurysm may influence its growth and rupture. Carotid-cavernous and carotid-ophthalmic aneurysms tend to become giant; the changes of rupture are reduced by the extramural protection afforded by the dura of the caver-

nous sinus and the anterior clinoid process respectively. Rebleeding is more likely if the intracranial pressure (ICP; recorded epidurally) is below 400 mm H_2O; it stops when the epidural pressure approaches the systemic diastolic pressure (56). Decompression measures such as spinal tap, mannitol infusion, and ventriculostomy can precipitate further bleedings by altering the tamponade effect of raised ICP on a weakened aneurysmal wall.

Induction of Cerebral Berry Aneurysms in Animals

Studies of aneurysms induced by suturing a segment of a vein to an artery provide information concerning hemodynamics but give little information about the pathogenesis of idiopathic cerebral berry aneurysms (63,105). However, intracranial aneurysms induced in rats and monkeys that are grossly and microscopically similar to berry aneurysms in humans may be useful for study of the development and pathophysiology of this disorder and in attempts to devise therapy (66,106).

Hashimoto et al. (66,69,70,73), in experiments in rats, increased hemodynamic stress on experimentally fragile cerebral arteries; saccular cerebral aneurysms resulted. The rats were rendered hypertensive with deoxycorticosterone and high-salt diet, unilateral nephrectomy, and ligation of one or both of the common carotid arteries, and the arterial fragility was induced by feeding BAPN to the animals. BAPN causes defects in the cross-link formation between elastin and collagen, and thus connective-tissue abnormalities. Nagata et al. (71) induced an even higher incidence of cerebral aneurysms in rats, and claimed that this higher incidence was due to the induction of hypertension by renal infarction. They stated that hypertension plays a primary role in the

development of experimental cerebral aneurysms. Suzuki et al. (5), however, using an experimental model similar to Hashimoto et al.'s, found no absolute relationship between the development of aneurysms and the degree of hypertension. Their attempts to induce cerebral aneurysms in a larger species (adult dogs) resulted in death of 8 of the 10 animals from toxic effects of the BAPN. More recently, Espinosa et al. (67) reported the first cerebral aneurysm (carotid-ophthalmic) to develop in a non-human primate after accidental occlusion of the ipsilateral internal carotid artery. An essential feature in this sporadic case was the occlusion of the internal carotid artery two months before aneurysmal development (Figs. 5 and 6). This finding agrees with Hashimoto's rat model (66,69,70,73); it is perhaps the increase in hemodynamic stress that causes aneurysms to develop. If this case could be reduplicated, it would make the closest model to human intracranial aneurysms.

FIGURE 5

Lateral cerebral angiograms of monkey 54 at the time of control studies (C), immediately after occlusion of the left internal carotid artery (-2M) and 2 mo afterwards (2M). C: No aneurysm is seen at the ophthalmic:carotid junction (arrowhead). 2M: An aneurysm is evident 2 mo after the occlusion (arrow) of the internal carotid artery.

FIGURE 6

Oblique (top) and anteroposterior cerebral angiograms of monkey 54, showing development of an aneurysm of the left carotid:ophthalmic junction (arrow), 2 months (2M) after occlusion of the ipsilateral internal carotid artery.

No aneurysm is seen on the control angiogram (C). Severe vasospasm developed acutely (O), and was moderate 1 hour (1H) post-SAH. No vasospasm was seen on Day 3, but is again moderate on Day 7 and mild on Day 13.

IV. CURRENT CONCEPTS OF CEREBRAL ARTERIAL SPASM AND ITS TREATMENT

Significance, Incidence, and Pathophysiology of Vasospasm

Half of the 60% of patients who survive the acute stage of SAH from ruptured aneurysm experience delayed neurological deterioration caused by one or more of a wide variety of problems, including rebleeding, vasospasm, hydrocephalus, and postoperative complications (179,196). The principal cause of delayed morbidity and mortality after the initial event in this syndrome is cerebral ischemia from VSP (179,196-198). The VSP results from thick hematomas in the basal cisterns, most commonly from a ruptured aneurysm (135,320) but occasionally associated with SAH from rupture of an arteriovenous malformation (199), trauma (200,201, 293,294), or surgery (17). Severe symptomatic VSP occurs in about 25-37% of patients who suffer SAH from ruptured aneurysm, causing permanent neurological deficit or death in 7-17% (50,179,196,198,203).

Vasospasm develops between days 4 and 12 after the hemorrhage, the peak incidence occurring between days 6 and 8, and lasts for up to 2 weeks (165,179,204). There is a significant association between severe symptomatic VSP and decreased CBF, the initial neurological status post-SAH, delayed neurological deterioration, final non-surgical and surgical outcome, whether VSP occurs pre- and/or postoperatively, and findings at necropsy (37,196-198,205,206,208,209,261). In studies of 21 patients who died as a result of SAH from a saccular aneurysm, without surgical treatment, Graham et al. (261) found a significant relationship between the presence and degree of angiographic VSP and ischemic brain damage; 85% of the hemispheres with vessels in spasm had ischemic damage in the

distribution of the affected vessel, whereas only 18% of the hemispheres without infarction evidenced mild VSP.

The most reliable way to predict severe VSP is from the size of the SAH on a CT scan within 3 to 4 days after the event (290-292). Kistler et al. (291), in a prospective study of 41 patients with aneurysmal SAH, predicted the development or absence of severe VSP with a high degree of accuracy, based on CT appearances: this complication developed in 20 of 22 patients in whom CT showed thick layers of blood in the subarachnoid space, and was absent in 14 of 19 evidencing no blood or a diffuse SAH. These authors attributed the false-positives and false-negatives to inadequacy of the CT technique. Silver et al. (292), also, reported that the SAH pattern on the CT scan correlated with the presence/absence of VSP and the development of hydrocephalus, the latter being associated with intraventricular hemorrhage.

Cerebral arterial spasm develops gradually over hours or days, with hyponatremia and hypovolemia often preceding the clinical syndrome (196). Vasospasm is commonly manifested by an early reduction in consciousness, and fever (plateau wave between 38° and 39°C), followed by focal symptoms and signs in the distribution of the compromised vessel (33,165,206-210). The syndrome may remain unchanged or resolve within a few days, but it may progress to cause permanent neurological disability or, if associated with diffuse severe VSP, may kill the patient (33,179,197).

Pathogenesis of Cerebral Arterial Spasm

Chronic VSP is an abnormal sustained contraction of the cerebral arteries in response to vasogenic substances in the subarachnoid space.

Because it is nearly always associated with SAH from a ruptured aneurysm, it has been postulated that the blood that coagulates in the basal cisterns is the causal agent (211). Many substances have been suggested as mediators of VSP, including serotonin (217,238,256), norepinephrine (215,217,257), prostaglandins (186,187,196), angiotensin (222), histamines (216), hemoglobin (128,212-214,234,296), lipid hydroperoxides (158) and fibrinogen-degrading products (220,221,258), but no one agent has been identified as responsible for VSP. Another hypothesis holds that VSP is due to increased sensitivity of the cerebral arteries to norepinephrine and serotonin (223,224).

Boullin et al. (225) and Okwuasaba et al. (226) investigated hemorrhagic CSF from patients with ruptured aneurysm or other CNS disease and from subjects with no known cerebral disorder; they could not identify the vasoconstrictor substance but eliminated serotonin, histamine, norepinephrine, epinephrine, potassium, acetylcholine and angiotensin I as the cause of the chronic VSP. More recently, in studies with canine basilar artery in vitro, Sasaki et al. (158) determined the vasoconstrictor activity of hemorrhagic CSF from patients with SAH. Dithiothreitol and dithioerythritol (both, 10^{-4}M), which reduce disulfide bonds, reversed contractions induced by the hemorrhagic CSF, on average by 40% and 61% respectively; the same molar concentration of 5,5'-dithiobis-(2-nitrobenzoic acid), an agent that oxidizes sulfhydryl groups, reversed the inhibitory effect of the reducing agents; and no significant reduction in VSP was achieved with antagonists of neurotransmitters - methysergide (10^{-10}M), mepyramine (10^{-7}M), phenoxybenzamine (10^{-5}M), propranolol (10^{-6}M) and atropine (10^{-6}M). Sasaki et al. (158) concluded that prostaglandins, hemoglobin, and lipid hydroper-

oxides are probably the vasoconstrictor agents in hemorrhagic CSF, and excluded serotonin, histamine, norepinephrine and acetylcholine. Voldby et al. (256) reported a similar range of concentrations of 5-HT in CSF from SAH patients and controls (between < 2 and 5 nmoles/L), and found no correlation between this concentration and the severity of VSP, the CSF pressure, or the clinical grade of the severity of SAH.

A more recent hypothesis (165) is that chronic VSP results from the combination of the vasoconstrictor effects of oxyhemoglobin (oxyHb), lipid peroxides, and prostaglandins, together with reduced or even absent synthesis of the vasodilator prostacyclin. Lysis and phagocytosis of erythrocytes are the chief mechanisms by which blood is removed from the subarachnoid space, clearing away about 75% of the red cells (163). After SAH, blood lysis starts within 24 hours and peaks during Days 5 to 7 (162,163) - coincident with maximal severity of VSP. In studies in dogs, intact erythrocytes were inactive whereas hemolyzed red cells altered the tension of isolated basilar artery (126,164). The vasogenic activity of incubated erythrocytes increases within 36 hours, peaks on Day 3, and then is maintained for at least 14 days (125,126); the contractile activity of the hemolysates is dose-dependent on the concentration of oxyHb (125,126,164,165). When oxyHb is released into the subarachnoid space it undergoes spontaneous oxygenation to methemoglobin (metHb); this releases superoxide anions, which damage the cell membrane by peroxidation of fatty acids (125,161,162,165). In a 7-day study of dogs, 2 mg of lipid hydroperoxide (15-hydroperoxy arachidonic acid; 15-HPAA) injected into the subarachnoid space caused biphasic spasm: the initial phase lasted 10 hours, and the second, which started by Day 2 or 3, lasted for up to the end of the study (161). High

concentrations of peroxide have been found in the CSF of patients with SAH, correlating with VSP appearances on angiography (162).

In addition to causing VSP, lipid hydroperoxide of 15-HPAA induces severe degeneration of endothelial cells and myonecrosis of the tunica media (161). Sasaki et al. (159) postulated that these pathological changes in the arterial wall are probably due to the toxic action of free radicals released during lipid peroxidation, even in the absence of VSP: although VSP did not develop after the induction of SAH in dogs treated with OKY-1581, which inhibits thromboxane (Tx), endothelial damage and myonecrosis occurred in both treated and untreated animals, and OKY-1581 given to healthy dogs (no SAH) gave rise to no side effects that could explain the changes in the arterial wall. Other investigators (143) have observed that these changes may occur in the arterial wall when only sustained contraction is evident. In VSP induced with norepinephrine or blood, during the early phase the contraction affects mainly the endothelial cells, causing them to swell and disrupting the tight junctions; later, when the VSP has become chronic, proliferation of myointimal cells can occur, together with vacuolization and necrosis of smooth-muscle cells and alterations in the pattern of innervation of the adventitia (53,110,138,145,299). The tunica adventitia may become markedly fibrosed, and may be invaded by inflammatory cells especially if VSP is due to SAH (138,227).

Free radicals may not have vasocontractive activity (298). Their main effect appears to be damage to the vascular endothelium and tunica media (125,159,161,162,165,166). Prostacyclin (PGI_2) is the predominant prostaglandin synthesized in healthy vascular endothelium at least in dogs and humans (160,167,168). This agent inhibits platelet aggregation

and is one of the most potent vasodilators known to man (167). After SAH, however, if the endothelium is damaged the synthesis of PGI_2 is greatly diminished and its concentration in the arterial wall decreases to a critical level, rendering the vessel unprotected from vasoconstrictors (160-165).

Hemoglobin, also, has vasoconstrictor effects, although the activity of 'purified' Hb is only one-third to half that of the hemolysate (127,164). In addition, Hb is one of the main cofactors required for cyclo-oxygenase to convert arachidonic acid to cyclic endoperoxides (PGG_2, PGH_2), the precursors of both vasoconstrictor and vasodilator prostaglandins. Several studies have documented the capacity of the arterial wall to synthesize prostaglandins. Hagen et al. (171) reported that bovine cerebral arteries incubated with 0.1 μCi (1-^{14}C)-arachidonic acid for 3 hours synthesized prostaglandins $F_{1\alpha}$, $F_{2\alpha}$, D_2 and TxB_2; the average concentrations at 1 hour were 196 ng for PGE_2 and 172 ng for $PGF_{2\alpha}$. When the endothelium is damaged, the metabolism of arachidonic acid in the arterial wall probably shifts toward the synthesis of vasoconstrictor prostaglandins (169,172,176). Sasaki et al. (160) reported that normal canine cerebral arteries transformed ^{14}C-arachidonic acid to both 6-keto-prostaglandin ($PGF_{1\alpha}$), a spontaneous metabolite of prostacyclin, and small amounts of $PGF_{2\alpha}$, but no other vasoconstrictor prostaglandins. Exposure of the specimens to blood for 3 - 8 days decreased the synthesis of prostacyclin but did not change the synthesis of vasoconstrictor prostaglandins from control levels. The angiograms indicated rather mild VSP; the authors did not state whether there was any association between the degree of VSP and the concentration of prostaglandins. Decreased PGI_2 in the absence of vasoconstrictor prosta-

glandins probably results in mild or minimal VSP in the early stage. Maeda et al. (170), who recorded the biosynthetic activity of prostaglandins in canine vascular arteries for 6 days, found that normal arteries synthesized $PGF_{1\alpha}$ (most abundant), $F_{2\alpha}$, E_2 and D_2. After the induction of SAH (8 - 12 ml of blood injected into the chiasmatic cistern), there was no significant change in the rate of synthesis of prostaglandins by the spastic arteries initially but on Days 2 and 6 the synthesis of PGE_2 was significantly increased and that of $F_{1\alpha}$ and $F_{2\alpha}$ was decreased.

The finding of increased concentrations of the vasopressor prostaglandins $F_{2\alpha}$ and E_2 in the CSF of patients with SAH (174) led Fukumori et al. to suggest that their increased synthesis is probably responsible for the VSP. Walker et al. (175), also, measured the concentrations of vasopressor prostaglandins. In the CSF of patients with SAH the increases were greatest in PGE_2 and $F_{2\alpha}$ on Day 8, and in dogs subjected to SAH the concentrations of PGE_2 (most abundant), $F_{2\alpha}$, 6-oxo-$F_{2\alpha}$ and thromboxane B_2 were increased; in vitro, the synthesis of prostaglandins in whole cortex and choroid plexus was unaltered by exposure to blood, but in cerebral vessels thus exposed the synthesis of PGE_2 and 6-oxo-$F_{1\alpha}$ was decreased. La Torre et al. (177) measured the concentration of $PGF_{2\alpha}$ in the CSF of four groups of patients:

Group 1 (n = 15) No CNS disorder. Mean $F_{2\alpha}$ concn., 38 $\pm$ 6 pg/ml.

Group 2 (n = 6) Meningo-encephalitis, meningioma, or malignant brain tumor. Mean $F_{2\alpha}$, 196 $\pm$ 42 pg/ml.

Group 3 (n = 4) Nonaneurysmal SAH (postoperative, hypertensive, or pituitary apoplexy). Mean $F_{2\alpha}$, 406 $\pm$ 92 pg/ml.

Group 4 (n = 8) Aneurysmal SAH. Mean $F_{2\alpha}$, 656 $\pm$ 81 pg/ml.

The authors found no association between cerebral VSP and the PG-F_2 concentrations in the CSF, probably because few CSF samples were obtained on Days 2 to 12, especially Days 6 to 8 (when VSP is maximal).

Although prostacyclin can reverse vasoconstriction in a dose-dependent manner when applied in concentrations of 10^{-10} to 10^{-6}M, it produces constriction when the concentration is 10^{-5}M or greater (169, 176,184,186). In vitro, prostacyclin abolishes the contraction induced by many agonists, including prostaglandins TxA_2, F_2 , D_2, E_2, H_2, norepinephrine, 5-HT, angiotensin, and even that induced by bloody CSF from patients (169,173,181-183,191). In vivo, prostacyclin given iv in baboons causes severe, prolonged dilation of cerebral arteries (176) and, in dogs, marked hypertension, severe tachycardia with cardiac arrhythmias, and decreased myocardial and cerebral blood flows (194). In experimental SAH, prostacyclin is not as effective in vivo as in vitro: in dogs (190), when injected into the vertebral artery it failed to reverse acute VSP (24 hr); and in cats (184), iv injection of prostacyclin (25 - 75 ng/kg/min) or indomethacin (4 mg/kg) on day 5, singly or in combination, did not relieve the chronic vasospasm and did not increase CBF but caused marked hypotension. Quintana et al. (192), using PGs to treat vasospasm induced by the injection of oxyHb into the subarachnoid space in cats, reported significant dilation of spastic arteries and no significant side effects. After SAH, decreased synthesis of prostacyclin and increased production of vasoconstrictor PGs (F_2 and E_2) result in severe VSP; and, if platelets aggregate and release thromboxane A_2 at the site of endothelial damage, vasospasm may be exacerbated (172-177).

Sano and co-workers (125,158,165) have postulated that delayed arterial spasm is most likely due to the action of free radicals released during auto-oxidation of oxyHb to metHb, after hemolysis. Lipid hydroperoxides inhibit the synthesis of prostacyclin and damage the vascular endothelium. Peroxidation of linoleic and arachidonic acid is increased in SAH, facilitated by the action of Hb. This increases the synthesis of endoperoxides, which thus become the source of vasoconstrictor prostaglandins, whose enzymatic system remains intact. Vasospasm probably results from the interaction of oxyHb, lipid peroxides, and prostaglandins, together with a deficit in the synthesis of prostacyclin (158,180-188). The most potent vasoconstrictor prostaglandin is thromboxane A_2, followed by $F_{2\alpha}$, E_2, and endoperoxide H_2 (165,174,189, 193). Prostaglandins act upon smooth-muscle receptors that are not blocked by atropine, methysergide, mepyramine, propranolol and oxybenzamine or hexomethonium (193). There are at least five receptors for prostaglandins, including those for PGs D, E, F, I and the thromboxanes. These agents exert their effects via adenylate- or guanylate-cyclase systems (cAMP, cGMP). A rise in cGMP increases the concentration of intracellular calcium by releasing it from organelles and facilitating the ingress of extracellular calcium. Relaxation is mediated by cAMP, which activates a specific protein kinase; this stimulates the energy-requiring system responsible for the binding of calcium in cellular organelles, and reduces the intracellular concentration of calcium to less than 1×10^{-6}M.

Physiology of Smooth-muscle contraction

Many questions concerning the mechanisms of smooth-muscle contraction remain unanswered. However, the physiology of stimulus/contraction coupling merits a brief review because it serves as a framework for consideration of therapy. The exact nature of membrane-based transduction events producing smooth-muscle contraction following calcium mobilization is unknown and the precise nature of calcium channels in vascular smooth muscle has not been clarified. Exactly how intracellular calcium is released, sequestered, and removed, also, is unclear (228).

Calcium in body fluids exists in two forms - free and bound to protein. The concentration of free calcium is equal in both CSF and blood; however, the total concentration of calcium is greater in blood than in CSF, because the former contains more protein and thus more bound calcium. The concentration of free calcium in these fluids is 1.15×10^{-3}M, and that of intracellular calcium in contact with myofilaments (activator calcium) is 1.8×10^{-7}M at the beginning of contraction, 1×10^{-6}M for half-maximal contraction, and 1×10^{-5}M for maximal contraction (231). In the presence of activator calcium, the adenosine triphosphatase (ATPase) associated with these contractor proteins transforms ATP to ADP and releases the energy required for contraction of the myofilaments. It is the concentration of free calcium at the actin and myosin filaments that determines the degree of contraction at any given moment; therefore, by regulating the amount of intracellular free calcium, smooth-muscle cells regulate the amount of contraction. There are two sources of this activator calcium: extracellular, and bound intracellular calcium. Both the influx of

calcium into smooth-muscle cells and its intracellular release result in smooth-muscle contraction.

The interior of a smooth-muscle cell is separated from the extracellular space by an ion-impermeable bilayer phospholipid membrane which is studded with intrinsic membrane proteins that act as ion-conducting channels. Blockade of a given channel is selective, not just for individual classes of ion channels but for specific states of the given type of channel (317). Thus, the specific action of a calcium antagonist may reflect a preferential interaction between a drug and a specific type of ion-channel protein or the drug's action on a specific region of the membrane phospholipid in contact with that protein.

Contractions induced by depolarization in response to high concentrations of potassium are dependent upon extracellular calcium, and the tonic contractile phase induced by alpha-adrenergic stimulation also reflects the availability of extracellular calcium. It has therefore been assumed that calcium ingress causes smooth-muscle contraction in response to both norepinephrine and a high potassium concentration. This assumption has been complicated by the findings that norepinephrine-induced contractions can occur in completely depolarized smooth muscle and that norepinephrine can activate arterial smooth muscle without causing depolarization. As increased influx of ^{45}Ca has been demonstrated in response to both norepinephrine and high-potassium activation, it has been concluded that calcium can enter a cell via two types of channels: potential-dependent channels (PDCs), that are operated by membrane depolarization, and receptor-operated channels (ROCs).

The hypothesis of multiple calcium channels has been proved by van Breemen and colleagues (229), who showed that a given dose of the calcium antagonist verapamil can close channels sensitive to high potassium concentrations while keeping the norepinephrine-sensitive ones fully operational. This differing sensitivity indicates either two sets of channels or two activating processes impinging on the same channel. When muscle is in the resting state, neither the PDCs nor the ROCs are open but the membrane is still permeable to calcium. As this passive leak is insensitive to calcium blockers, the resting-state calcium leak may be a third pathway of entry for calcium into the cell (229); this probably relates to the stretch-dependent channel recently reported (230).

Bevan (230), studying the biphasic response of isolated blood vessels, noted two types of smooth-muscle intrinsic tone: the rhythmic tone, an activity that originates from a pacemaker cell within the vessel wall and is associated with calcium-induced action potentials that propagate along the vessel, and thus is sensitive to calcium-channel blockers; and maintained tone, which is not associated with calcium action potentials. Maintained myogenic tone is absent in large systemic blood vessels but it is present in small peripheral arteries, including the intracranial ones. Maintained tone is dependent upon extracellular calcium but independent of innervation, prostaglandins, and angiotensin; the more a vessel is stretched, the more it contracts - up to a certain limit, and then this ability is lost. Stretch-dependent channels are temperature-sensitive, and appear to be different from PDCs and ROCs. At temperatures below 34°C, tone is absent; but as temperature is raised above this level, the amount of tone increases (230). It

is of interest that high body temperature (as in malignant hyperthermia) eliminates the calcium requirement for myosin/actin interaction in skeletal muscle (195). Rousseaux et al. (210) noted correlation between hyperthermia (38 - 39°C), SAH, and severe symptomatic vasospasm, in up to 88% of their patients. It is possible that hyperthermia contributes to the increased smooth-muscle tone associated with SAH from ruptured aneurysm.

According to Silver and Stull (232), four molecules of calcium bind to four divalent metal-binding sites on the protein calmodulin to form an active calcium-calmodulin complex, which in turns binds to inactive myosin light-chain kinase (MLCK) to form a catalytically active holoenzyme. Activated MCLK catalyzes phosphorylation of the light-chain subunit of myosin (P-light chain), which in turn stimulates actin/myosin interactions; actomyosin ATPase activity increases concurrently. Intracellular calcium creates closed bridges between actin and myosin, and thus gives rise to contraction and increased ATPase activity. ATPase and tension increase in parallel when the intracellular concentration of calcium rises (195). Elevation of the cytoplasmic concentration of calcium stimulates the sarcoplasmic reticulum and membrane Ca-ATPase to remove calcium from the cytoplasm, resulting in relaxation. The calcium is then transferred in some way from the intracellular pool to the extracellular space (229).

Vasogenic substances or agonists exert their effects via cAMP or cGMP (195). Kukovetz et al. (235) reviewed the concept of a causal relati[redacted]tween cyclic-AMP increases produced by beta-adrenergic [redacted] relaxing effect on smooth muscle. Drugs such as amino-[redacted]erine lowered smooth-muscle tone by inhibiting cAMP

phosphodiesterase; and, in some arteries, isoproterenol and adenosine-stimulated adenylate cyclase raised cAMP levels when relaxation occurred. These actions are potentiated by phosphodiesterase inhibitors and can be mimicked by the dibutyryl cAMP (247,248,300). Sodium nitroprusside and nitroglycerin have dose-dependent effects on relaxation of vascular smooth muscle and increases in GMP levels. The relaxant effect of these guanylate-cyclase activators, which is mediated by cAMP, probably terminates in phosphorylation of membrane proteins that participate in transporting intracellular calcium into the sarcoplasmic reticulum or extruding it through the cell membrane. Cyclic AMP functions as a secondary messenger for hormonally induced relaxation, whereas cGMP is a regulator that can reverse transmitter-induced contractions.

The sarcoplasmic reticulum is probably the main intracellular storage site of calcium for regulating the normal cycle of concentration/relaxation. The mitochondria also can accumulate intracellular calcium and thus may serve as a large calcium sink during excessive overload (301). Some workers, however, have postulated that the major cellular sink of intracellular calcium is either the plasma membrane or the sarcoplasmic reticulum and that mitochondria are not a physiologically significant site (302).

Contractile proteins disengage when the amount of free calcium within the cell falls as a result of decreased rate of entry or extrusion. The rate of extrusion of calcium from the cell increases when protein kinases are activated, as when activation of beta-receptors increases the intracellular concentration of cAMP. Actin-myosin interactions decrease when myosin's P-light chain is dephosphorylated by its light-chain's phosphate.

Prevention and Treatment of Cerebral Arterial Spasm

Vasospasm is most likely due to the action of two or more vasogenic agents that attach to specific sites on the smooth-muscle membrane (receptors), increasing the concentration of calcium intracellularly. The simplest way to prevent or treat vasospasm would be removal of the agent(s) from the vicinity of the receptors (303-305). Taneda (305) studied the effects of removing blood clots on the prevention of chronic VSP in 239 patients operated on within 48 hours of SAH or later. Permanent disability from VSP occurred in 25% (11/44) of patients in whom surgery was delayed 10 days or longer and in 27.7% (26/94) of those subjected to surgery within 48 hours but undergoing minimal removal of blood clots from the basal cisterns, whereas cerebral ischemia from vasospasm occurred in only 10.9% (11/101) of patients in whom extensive removal of blood clots was performed within 48 hours. Mizukami et al. (304) reported a similar experience with 64 patients operated on within 4 days: CT scan before and after surgery, to evaluate the completeness of blood clot removal, showed that SAH could be removed except in the region of the frontal interhemispheric fissure, on the ipsilateral side of the insular cistern (surgical approach), and the contralateral insular cistern. Vasospasm did not develop or was significantly milder after successful removal of the clots, whereas neurological deterioration from VSP occurred in those cases in which hematomas remained in the cisterns. Thus it seems that the incidence of symptomatic VSP can be reduced by early, extensive removal of blood clots from the subarachnoid space; however, this procedure is technically very difficult and, even in experienced hands, complete removal of hematomas has not been possible (304). Furthermore, some other surgeons have questioned the

usefulness of early surgery and removal of blood clots. In Ljunggren et al.'s series of 81 good-risk patients operated on within 48 to 60 hours post-SAH, 17 (21%) suffered delayed ischemia from VSP and 8 of them died (306). These authors concluded that early removal of hematomas and rinsing of the basal cisterns does not eliminate the risk of cerebral ischemia from vasospasm.

Regardless of which agent(s) operate in the genesis of vasospasm, it may be possible to interfere with the final common pathway of vasoconstriction (ingress of Ca into the cerebral arterial smooth-muscle cells) but still not disrupting cardiac, systemic arterial, and neuronal function. Some possibilities of therapy for vasospasm have been summarized by Towart (131). Potential therapies include calmodulin antagonists, to prevent or reverse calcium-induced activation of the actin-myosin complex; phosphodiesterase inhibitors, to increase cAMP and the intracellular sequestration of calcium; prostacyclin analogs, to increase cAMP; and calcium antagonists, to inhibit calcium ingress into the vascular smooth-muscle cell. Silver and Stull (232) suggested that calmodulin antagonists directly inhibit the activation of myosin light-chain kinase and thus the development of tension; these substances may also inhibit calmodulin-mediated effects and thus influence cytoplasmic calcium levels. Beta-adrenergic stimulation and consequent activation of cAMP-dependent protein kinase may cause relaxation by decreasing cytoplasmic calcium levels or attenuating the activation of myosin light-chain kinase. Alternatively, relaxation may result from the phosphorylation of myosin light-chain kinase by cAMP-dependent protein kinase, whose affinity for the calcium-calmodulin complex is thereby decreased, in this case, direct antagonism of calmodulin or beta-

adrenergic stimulation of adenylate cyclase would reduce the amount of calcium-calmodulin bound to myosin light-chain kinase and thus the phosphorylation of myosin's P-light chain.

Mechanisms of Action of Calcium-entry Blockers

Calcium action in vascular smooth muscle is more complex than in either skeletal or cardiac muscle, calcium ions in the vessel wall serving not only as mediators of excitation/contraction coupling but also as carriers of transmembrane charges in their spontaneous production and propagation of action potentials (230). Fleckenstein et al. (307) found that K^+-induced contraction of coronary-artery strips was inhibited when the bathing medium was replaced with a calcium-free solution or when its pH and/or oxygen content was reduced. Calcium antagonists reduced tension slowly in such K^+-depolarized coronary strips, in contrast to the extremely rapid relaxation induced by nitroglycerin, and nitrates induced only partial relaxation and did not prevent spontaneous recovery. These investigators concluded that both the organic calcium antagonists and nitrates prevent calcium influx, basing this on the lack of inhibition when smooth-muscle fibers were directly activated by the addition of ATP and calcium. Fleckenstein et al. further reported (308) that the contractility of depolarized vascular smooth muscle is proportional to the extracellular calcium, but that H^+ ions are highly potent antagonists of calcium; the vasoconstricting effect of increasing extracellular calcium disappears in acidosis or hypoxia. H^+ ions may compete with calcium for the same sites in the membrane's transport systems and with myofibrillary ATPase.

Calcium blockers act on voltage-sensitive channels to inhibit the uptake of calcium that accompanies depolarization. All agents of this type block K^+-induced tension, and the associated increased uptake of ^{45}Ca from the extracellular space, at lower concentrations than are required to block similar contractile responses to norepinephrine (309). The calcium antagonists, as a group, block the uptake of only the extracellular calcium associated with POCs; they do not affect calcium-binding, equilibration of resting calcium, or the distribution of resting free intracellular calcium, and probably do not act directly to decrease sensitivity to norepinephrine or the affinity of calcium-binding pools.

Cerebral arterial smooth-muscle cells depend solely on extracellular calcium for stimulating alpha-adrenergic, 5-HT, and prostaglandin-F_2 receptors. The extent of their dependence on extrinsic sources of calcium perhaps differentiates them from other classes of smooth-muscle cells (310). In studies of strips of rabbit aorta (311), nimodipine, a calcium antagonist, blocked KCl-induced but not norepinephrine-induced contractions, whereas phentolamine, an alpha-agonist blocker, had the reverse effect. Norepinephrine probably contracts this vascular tissue by releasing calcium stored intracellularly.

The variation in responsiveness of vascular smooth-muscle populations to calcium antagonists was illustrated by Vanhoutte (312), who showed that contraction of the basilar artery is inhibited by much lower concentrations of the calcium antagonist phenarizine than are the gastrosplenic, coronary, or tibial arteries. Pearce and Bevan (313), in their preliminary report on the cerebrovascular actions of diltiazem, stated that infusion rates of 30 $\mu g/kg^{-1}/min^{-1}$ caused cerebral vaso-

dilation without reducing peripheral resistance, cardiac output, cerebral metabolic rate or mean arterial pressure. They interpreted the vasodilation without significant change in cerebral metabolic rate as evidence of greater dependence on extracellular calcium by cerebral than by most other vascular smooth-muscle cells. Others, also, have provided evidence that cerebral smooth-muscle cells contain relatively small calcium pools and depend mainly on extracellular calcium for their contractions (314,315).

Comparing the responses of segments of rabbit basilar and ear arteries to various agonists, McCalden and Bevan (314) suggested that steady-state norepinephrine contractions in the former arise almost entirely from tightly bound extracellular calcium, and that the difference in responses by the ear artery relates to the additional tightly bound intracellular calcium pool - a source that in the basilar artery contributes to only a brief phasic component insensitive to verapamil (230). In basilar artery, the contractile response to K^+ and to 5-HT was reduced by incubation in Ca-free solution (314). The initial rates of decline were similar for both and probably represented depletion of an extracellular source, but the response to 5-HT declined more slowly, possibly reflecting a second clearance compartment: McCalden and Bevan thought this might be intracellular. The norepinephrine curve did not differ from the K^+ curve, perhaps indicating that steady-state contractions use only extracellular calcium. Although these investigators concluded that the basilar artery mobilizes primarily extracellular calcium in response to norepinephrine, they pointed out that the phasic (initially brief) responses to norepinephrine and 5-HT could occur independently of calcium ingress from the extracellular space but that

these phasic responses play little or no part in the basilar artery's sustained contraction in response to norepinephrine.

Kazda and Towart (49), in studies of strips of rabbit aorta showed a dose-dependent inhibitory effect of nimodipine on K^+-induced contractions, but no inhibition of norepinephrine-induced contractions - even with higher concentrations. By contrast, nimodipine strongly inhibited responses to 5-HT in rabbit basilar artery (but not systemic artery). Verapamil, another calcium antagonist, inhibits POCs in all blood vessels as well as ROCs in cerebral and coronary arteries (317).

Towart (132) compared nimodipine's effect on responses by rabbit arteries in vitro. In basilar artery, the sustained tonic phase of contraction in response to 5-HT was strongly inhibited by nimodipine but the initial, brief, phasic portion of the contraction was relatively unaffected; this was thought to indicate that nimodipine's antagonism did not occur at the 5-HT receptors but probably in the ROCs. In saphenous artery, nimodipine in doses of up to 2.4×10^{-5}M did not affect initial phasic or tonic contractions in response to 5-HT.

Calcium-entry blockers block POCs to inhibit the increased uptake of extracellular calcium that normally accompanies depolarization. All calcium antagonists block K^+-induced tension responses and associated increases in ^{45}Ca uptake at much lower concentrations than required to block similar responses to norepinephrine (309), but it is not known whether calcium antagonists have additional mechanisms of action when used in therapeutic concentrations; only POCs and ROCs are affected at calcium-antagonist concentrations below 10^{-5}M (229). Calcium-entry blockers do not inhibit tension developing from leakage of calcium or its release from superficial calcium pools. Some cerebral blood vessels

may use calcium channels insensitive to calcium-entry blockers to maintain vessel tone, the magnitude of the releasable calcium pool varying according to the source of the smooth muscle. In cerebral arteries, antagonists are more efficacious if the ingress of calcium exceeds the amount of releasable calcium. In systemic arteries, norepinephrine-induced contractions ranged from 80% of control proximally to zero in the most distal branches. Thus, the more peripheral an artery, the more likely it may be to depend completely on calcium ingress to contract in response to norepinephrine.

Because calcium antagonists may have specific blocking actions, and the origin of vasospasm is likely to be multifactorial, White et al. (237) studied the effects of nimodipine on contractions induced by 5-HT, prostaglandin-$F_{2\alpha}$, thrombin, and whole blood, in isolated canine basilar arteries. Nimodipine significantly inhibited contractile responses induced by these diverse agonists whether given before or after them, leading these workers to postulate a common calcium-entry channel and elicitation of the response by varied receptor mechanisms. This is the rationale for the use of nimodipine in the treatment of cerebral ischemia from SAH-induced VSP.

From a literature review, Flaim (317) concluded that nifedipine caused 50% inhibition of contractile activity at a concentration (IC_{50}) of 10^{-9}M against KCl, 3×10^{-7} against norepinephrine and 5-HT, and 10×10^{-8} against prostaglandin-$F_{2\alpha}$. Nifedipine was a more potent inhibitor of contraction to all agents than verapamil. Like verapamil, it inhibited contraction more strongly in cerebral arteries than in peripheral vessels. Studies of the effects of seven calcium blockers on isolated cerebral arteries gave a mean IC_{50} of 5×10^{-8}M for verapamil and 6 x

10^{-9}M for nifedipine, and lowest of all (3×10^{-9}M) for nimodipine.

Brandt et al. (219) studied contractions induced in human pial and mesenteric arteries by K^+, norepinephrine, 5-HT and prostaglandin-$F_{2\alpha}$. In the pial vessels, nifedipine and nimodipine almost abolished contractions induced by K^+ and partly inhibited those induced by $F_{2\alpha}$. Nifedipine, but not nimodipine, more potently relaxed cerebral than mesenteric arteries contracted by K^+, whereas both of these calcium antagonists inhibited contractions induced by calcium in pial arteries. These results indicate that K^+, amines, and prostaglandin-$F_{2\alpha}$ activate the two types of vessels by different calcium-dependent mechanisms, and confirm the potent relaxing effects of these calcium antagonists. In studies of dogs, significant relief of spasm was achieved with a small dose (0.28 mg/kg) of nimodipine but not with the same dose of nifedipine (51). In cats in which VSP was induced by the injection of 0.2 - 0.3 ml fresh autologous blood into the cisterna magna and the pial vessels were monitored with a television camera, nimodipine (0.1 mg/kg) injected iv, 20-30 minutes after SAH, abolished the spasm; vasodilation was greatest in arteries under 100 μm in diameter (321).

Auer et al. (250) applied a solution of nimodipine, 2.4×10^{-5}M, to exposed cerebral vessels in 17 patients while the aneurysm was being clipped, 42-72 hr after SAH, and inserted a cannula for topical administration postoperatively in 13 of them. CT scan had shown blood in the basal cisterns in all 17. Vasodilation in response to the nimodipine, relatively greater in the smaller vessels, was observed in all instances. A neurological deficit combined with radiographically verified VSP was present postoperatively in two patients, but severe vasospasm was absent in all of the angiograms taken on or about Day 7. It was

concluded that the topical intracisternal administration of nimodipine intra-operatively reversed the vascular spasm and decreased the probability of symptomatic VSP after early surgery. More recently, in a prospective, multi-institutional, randomized, double-blind placebo-controlled trial of nimodipine in 125 neurologically normal patients who had suffered a ruptured aneurysm, a cerebral angiogram and CT scan were obtained and treatment was started within 96 hours of the SAH (50). Nimodipine was given orally, 0.7 mg/kg as a loading dose and then 0.35 mg/kg q4h for 21 days; no side effects were seen. A deficit from vasospasm that persisted and was severe, or caused death before the end of the treatment, developed in 8 of 60 patients given placebo and 1 of 56 given nimodipine, a difference that was significant ($p < 0.03$). As angiography was repeated only if a neurological deficit developed, no firm conclusion could be drawn whether nimodipine reduced the frequency and/or severity of VSP. However, the results provide strong evidence that this drug reduced the risk of both neurological deterioration and death from VSP.

CHAPTER TWO

THE PRESENT STUDIES

I. OBJECTIVES AND EXPERIMENTAL DESIGN

The present work consisted of three studies (A, B, and C).

STUDY A: DEVELOPING A MODEL OF CHRONIC VASOSPASM: CREATING A LARGE SUBARACHNOID HEMORRHAGE IN MONKEYS

Objectives

1. Create an animal model of chronic VSP following SAH, similar to that in humans.
2. Test the hypothesis that the size of the SAH as seen on CT scanning is critical to the development of VSP, as in man.
3. Study the effects of SAH on vessel caliber.
4. Examine the association between VSP and hemispheric cerebral blood flow.
5. Document the effect of VSP on neurological status.

Design

Twenty-five monkeys were entered into this observational investigation. Seven died within hours after the SAH and therefore were rejected from the study. The remaining 18 were studied for 48 hr or longer. Variables measured or observed included the cerebral-vessel caliber on angiograms, cerebral blood flow (CBF), mean arterial blood pressure (MABP), size of the SAH on computed tomography (CT) scans, and neurological status. Baseline values were established, and neurological

assessment and CT scanning were performed during the control period. An SAH was created on Day 0; neurological assessment was repeated thereafter, and the other variables were tested five times (between 30 min and 5 hr after the hemorrhage, and on Days 2, 7, 14 and 21). Animals that survived for more than 7 days were killed (by exsanguination under general anesthesia) on Day 14 or 21. The brain was removed within a few hours of death, for gross pathology.

STUDY B: (PILOT STUDY) ESTABLISHING PREFERRED METHODS FOR CREATING SAH-INDUCED VSP AND FOR CEREBRAL ANGIOGRAPHY

Objectives

1. Induce a large SAH within an isolated area of the subarachnoid space.
2. Develop an experimental angiographic technique to study the cerebral arterial circulation bilaterally.
3. Establish treatment and experimental procedures for Study C.

Design

Ten monkeys divided into two groups were entered into the study. Baseline measurements were established during the control period. In all 10 monkeys, an SAH was created through a frontolateral craniectomy (Day 0). In Group 1, in eight monkeys, the SAH was created by direct injection of blood into the subarachnoid space, into a cistern (right sylvian, 3; interpeduncular, 2; and chiasmatic, 3). In Group 2, in two monkeys (once in 1 and twice in 1) the arachnoid membrane was opened widely at the regions of the supraclinoid internal carotid artery, sphenoidal middle cerebral artery, and anterior cerebral artery, and a

hematoma of 1.5 ml (n = 1) or 5 ml (n = 2) was placed very carefully against these arteries. The variables, measured on Days 7 and 14, were as in Study A.

STUDY C: A MODEL OF CHRONIC VSP OF THE MIDDLE CEREBRAL ARTERY AFTER LARGE SAH IN MONKEYS: A RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL OF NIMODIPINE

i) Clinical and Radiological Findings

ii) Pathological Findings

Objectives

1. Create an animal model of severe spasm of the middle cerebral artery, by inducing a large localized SAH.
2. Study the effects of the SAH on vessel caliber.
3. Examine the association between chronic VSP and hemispheric CBF.
4. Document the effects of chronic VSP on neurological status.
5. Investigate the efficacy of one pharmacologic agent (nimodipine) in preventing or reducing chronic VSP and/or delayed ischemic neurological deficits.
6. Determine the extent of structural and/or morphological changes in cerebral arteries that have been in spasm.

Design

This project was designed as a randomized, double-blind, placebo-controlled trial.

Thirty-three monkeys were entered into study; three died shortly after the SAH was created and were therefore excluded. The other 30 were studied for 7 to 14 days post-SAH. They were allocated into two groups of 15 by simple blind randomization of drug therapy; the investigator and statistician were unaware of group allocation until after the completion of data analysis.

In all 30 monkeys, the operative procedure was the same (right frontolateral craniectomy in 28 and left in 2, exposure of the circle of Willis, and placement of a hematoma obtained from 5 to 9 ml of blood) and a solution of polyethylene glycol with water was administered via a nasogastric tube inserted tid for 14 days post-SAH; for the experimental group, the solution contained nimodipine. Variables measured or observed were as for study A, together with determination of structural and/or morphological changes in cerebral arteries. Two of the monkeys died, one on Day 8 from surgical complications and the other on Day 13 from accidental self-suffocation. After completion of the last angiogram, on Day 14, while still under general anesthesia the remaining 28 monkeys were killed by intra-arterial fixation of the brain. The cerebral vessels were carefully dissected; they were examined with light microscopy and both transmission and scanning electron microscopy.

II. MATERIALS AND METHODS

Preparation of Animals

There were some differences in preparation in the three studies; these are noted where appropriate. Otherwise, the techniques described pertain to all three studies.

Juvenile and adult female cynomolgus monkeys (Macaca fascicularis) weighing 2.3 to 4.8 kg were studied.

Anesthesia was induced with sodium pentobarbital injected iv, 26 to 32 mg/kg b.wt, for all procedures in Study A and for craniectomy only in Studies B and C, and with ketamine HCl injected im, 6 to 10 mg/kg b.wt, for other procedures in B and C. The anesthetic agent was supplemented with a 2:1 mixture of N_2O:O_2 administered with a variable-phase respirator (Harvard Apparatus Inc., Dover Road, Millis, MA) via an endotracheal tube. In Studies B and C, a cephalic or small saphenous vein was cannulated with a no. 22 catheter for administration of drugs and fluids. Paralysis was induced and maintained with gallamine, 2 mg/kg, every 45 min; it was injected intra-arterially in Study A and into the cannulated vein in B and C. Procaine penicillin, 100,000 U/kg b.wt, was injected im after the induction of anesthesia. Body temperature was monitored peroperatively with a rectal thermometer (Yellow Springs Instrument Co., Yellow Springs, OH) and was maintained at 37°C by means of a thermostat attached to the thermometer and a heating pad beneath the monkey. A wide operative area was shaved and the skin was prepared with betadine (scrub and solution). An operating microscope was used during the intracranial surgical procedures.

In Study A, the left common carotid artery was dissected and a no. 20 catheter was inserted into it through an 8-0 silk purse-string suture placed approximately 3 cm proximal to the bifurcation. In Studies B and C, the femoral artery on either side was dissected and a no. 5.0 French radiopaque polyethylene catheter with a sigmoid tip (Fig. 7) was inserted into it through a transverse arteriotomy between two ligatures. The ligatures were tied at the end of each experiment. Xylocaine (2% simple soln) was injected to prevent femoral constriction, 1 ml sc and, after the skin had been opened, 1 ml in the femoral sheath. Under fluoroscopic control, the catheter tip was advanced into the innominate artery; 0.5 to 1.0 ml of contrast medium was injected, and correct positioning of the catheter was confirmed by filling of both common carotid arteries. Arterial blood samples were obtained periodically throughout, via the catheter, for blood-gas analysis, determination of pH and hematocrit, and calculations of CBF; the catheter was flushed intermittently with 1.0 ml of 0.9% saline containing heparin (10 U/ml) to prevent clotting. The respirator was adjusted as required to keep arterial blood gases within physiological range, particular attention being paid to the $PaCO_2$ level; this was kept as close as possible to 38 mm Hg during measurement of CBF and cerebral angiography, and between 30 and 35 mm Hg during induction of SAH. The catheter(s) in the carotid (Study A) and femoral arteries (Studies B and C) were connected to a no. 16 cathether that led via a three-way stopcock to an injector for the administration of xenon-133 (^{133}Xe), a Cordis injector (Cordis Corp., Miami, FL) for applying the contrast medium meglumine iothalamate, and a pressure transducer (model 23 dB; Statham Instrument Co., Oxnord, CA) for monitoring arterial blood pressure. The Beckman

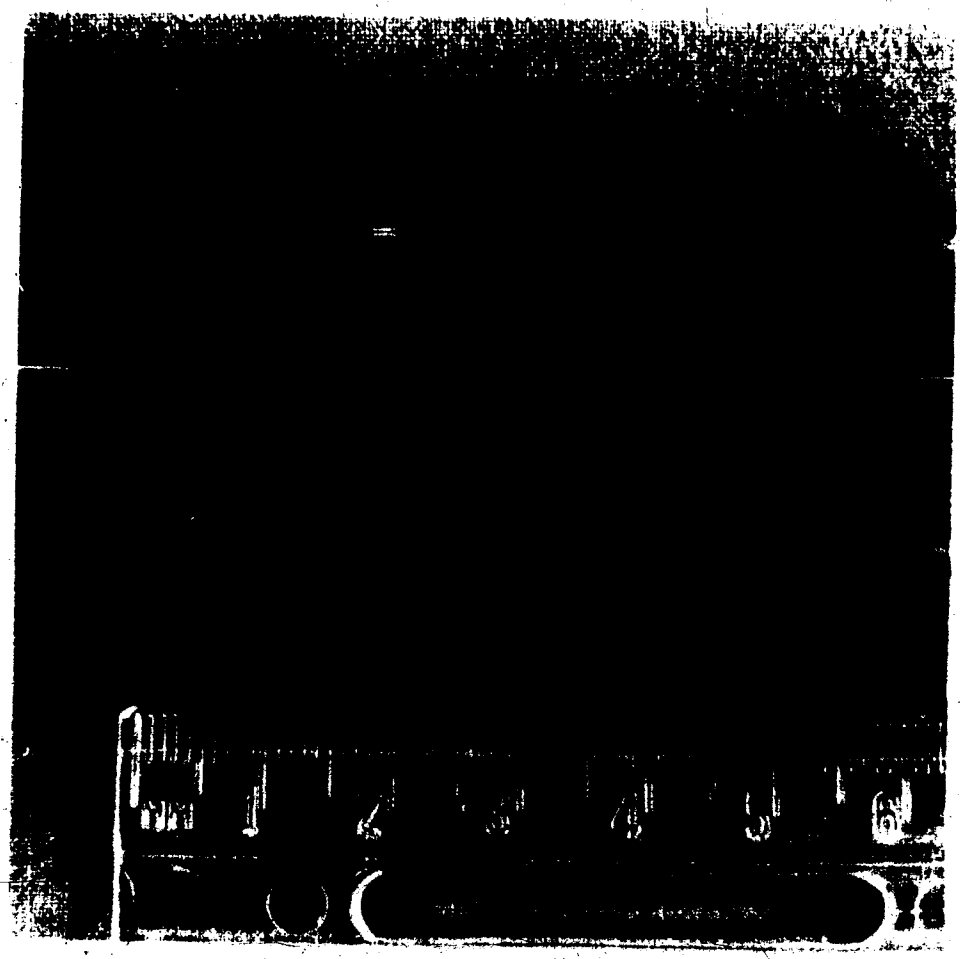


FIGURE 7

Polyethylene catheter with a sigmoid tip, used in studies B and C. Note the side holes, and size of the primary curvature, needed to fit the junction of the aortic arch and innominate artery in the monkey.

dynagraph used to record blood pressure was calibrated against a mercury manometer immediately before each experiment; when drift occurred during the experiment, the baseline was adjusted to zero.

Induction of Subarachnoid Hemorrhage

Surgical procedures were performed under aseptic conditions as for humans.

In Study A, under fluoroscopic monitoring a circumferentially bevelled no. 20 spinal needle was advanced through a midline frontal-twist drillhole, along the floor of the anterior fossa to 0.5 to 1.0 mm beyond the anterior clinoid process. With its tip in the chiasmatic cistern, the needle was secured to the skull by a screw device. In most cases there was drainage of 1.0 to 1.5 ml of CSF. Autologous arterial blood (mean $\pm$ SD = 1.48 $\pm$ 0.19 ml/kg b.wt) was injected manually in three equal volumes, each over 5 to 7 min; the injection was arrested immediately if signs of intracranial hypertension (pupillary dilation and/or Cushing response) became apparent.

In Study B, two new methods for creating an SAH were investigated; both were carried out through a right frontolateral craniectomy. With the animal's head fixed in a head holder and the arterial $PaCO_2$ stabilized at 30-35 mm Hg, a right frontotemporal skin flap centered at the pterion was created with an electrosurgical unit (Valley Lab, PO Box 9015, Boulder, CO). A sigmoid incision was made, beginning at the zygoma, crossing the temporal region toward the frontoparietal region, and terminating frontally parallel to the sagittal suture (Fig. 8A). Then the temporalis muscle was cut and cauterized, creating a U-shaped muscular flap with its concavity toward the external auditory canal

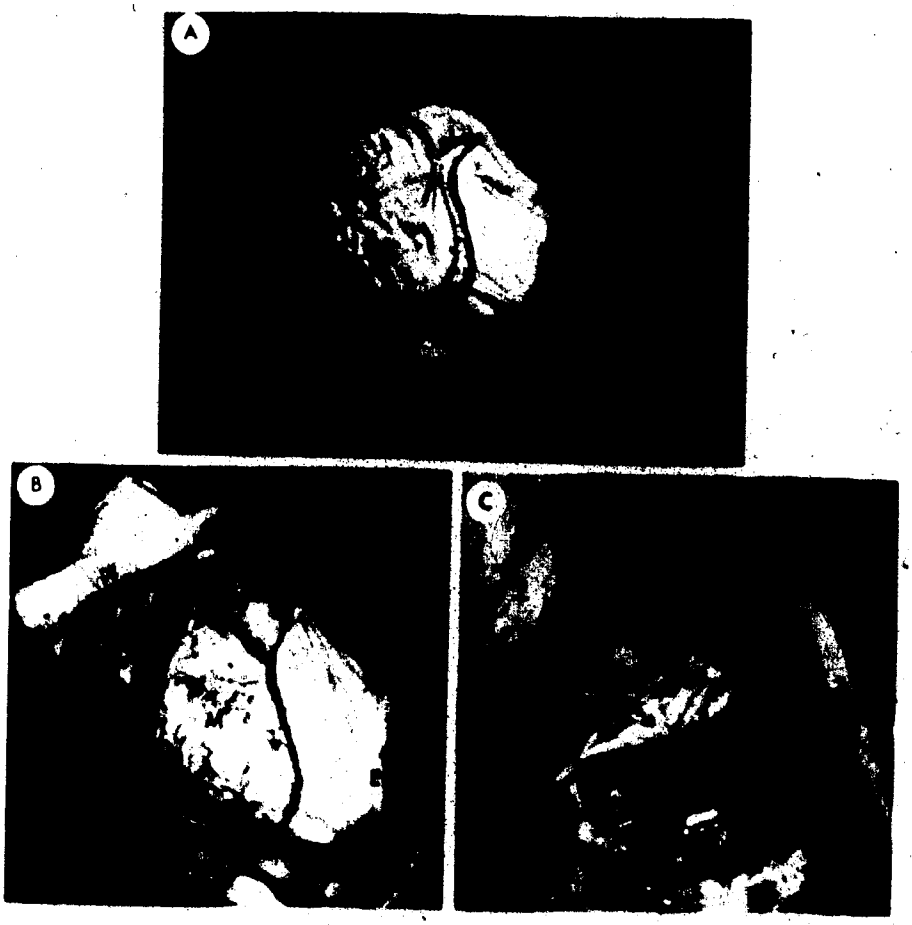


FIGURE 8

A: Skin opened with a sigmoid incision, starting at the zygoma and with its major concavity pointing toward the eye (arrow).

B: Temporalis muscle (M) incised in a U-shape, with its concavity pointing toward the ear (E).

C: Renal-shaped craniectomy, and dura mater (D) opened in a semicircle; frontal (F) and temporal (T) lobes partly exposed.

(Fig. 8B). A trephine and a Cloward clamp were used to create a small kidney-shaped craniectomy (base centered at the pterion) and bone wax was applied to arrest bleeding from the diploic veins. The middle meningeal vessels were cauterized and cut while the dura mater was being opened with a semicircular incision (concavity toward the sphenoid ridge) (Fig. 8C). The temporal lobe was gently retracted with a self-retaining retractor, the arachnoid membrane was incised at the origin of the sylvian cistern, and 3 to 4 ml of CSF was drained.

In SAH method 1 (n = 8), 5 to 6 ml of blood was manually injected over 10 to 15 min into the subarachnoid space in a cistern (right sylvian, 3; interpeduncular, 2; and chiasmatic, 3). In method 2 (n = 3), the arachnoid membrane was opened widely at the regions of the supraclinoid segment of the internal carotid artery, posterior communicating artery, anterior cerebral artery, and sphenoidal segment of the middle cerebral artery, and a hematoma obtained from fresh autologous arterial blood (1.5 ml and 5.0 ml on either side in one monkey and 5.0 ml in the other one) was placed very carefully against all four arteries.

In Study C, in 33 monkeys an SAH was created with the latter method (placement of a clot) but using a larger hematoma obtained from 5 to 9 ml of autologous arterial blood (Fig. 9).

After creation of the SAH in Study A, the spinal needle was removed, the cranial defect was sealed with bone wax, and the skin was sutured with 3-0 nylon. In studies B and C, when the injection had been completed or hematomas had been placed, the dura mater was closed in watertight fashion with 7-0 silk, the muscular flap was returned to its original position, the galea was closed with 2-0 silk, and the skin was

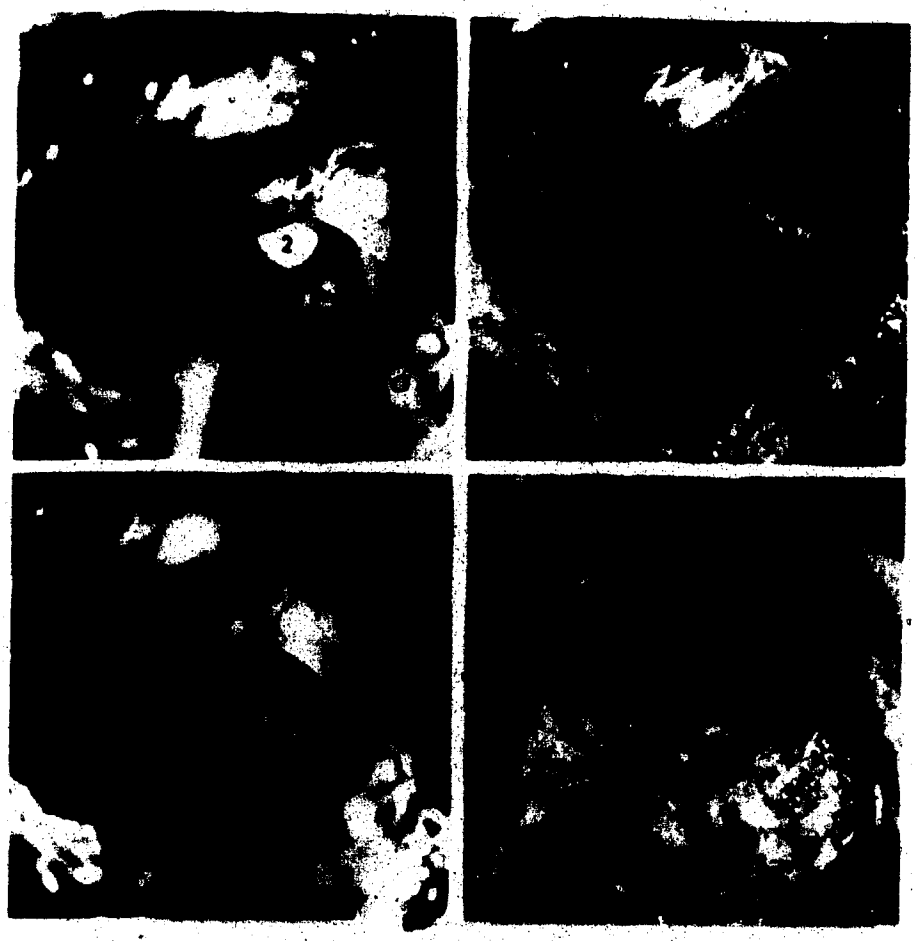


FIGURE 9

Photomicrographs taken peroperatively. A: The arachnoid membrane has been opened, revealing normal intracranial structures, including the optic nerve (2), internal carotid artery (c), and the middle (m) and anterior (a) cerebral arteries. No vasospasm or bleeding from surgery is present. B: A small fragment of blood clot has been placed against the arteries. Acute VSP of the middle cerebral artery is apparent, after placement of hematoma. C: A clot from approximately 7 ml of blood fills the middle fossa. D: The dura mater has been closed after clot placement. F = frontal lobe; R = retractor.

sutured with 3-0 nylon. Finally, arterial PCO_2 was returned to about 38 mm Hg, the paralysis was reversed with prostigmine (0.07 mg/kg) and atropine (0.02 mg/kg) injected iv, and the catheters were removed. After extubation, the monkeys were given routine postoperative care for 3 to 5 hr and then were returned to their cages.

Cranial Computed Tomography

CT scans were performed under general anesthesia with intravenously administered sodium pentobarbital, 1 to 4 wk before the SAH (control) and on Days 0, 2 (Study A only), 7, and 14 (Study A only) after its creation. On each occasion, eight horizontal sections 5 mm thick were obtained from base to top of the cranium. The scans were obtained with a scout-view body-scanner (GE model CT/T 8800), with a 320^2 matrix, 1.5 mm^2 pixel, and a scanning time of 9.6 sec (Fig. 10).

The observer grading the SAH on CT scans was unaware of the monkey's angiographic status. The edges of intracranial hematomas or layers of blood were delineated on selected frames, and the observer used a previously calibrated (1:1) optical measuring system to grade the SAH. Grading, which was established in Study A, was as follows:

Grade 1: small SAH; not apparent on CT

Grade 2: medium SAH; layers less than 2.0 mm thick

Grade 3: large SAH, big hematoma or layers 2.0 mm thick or more.

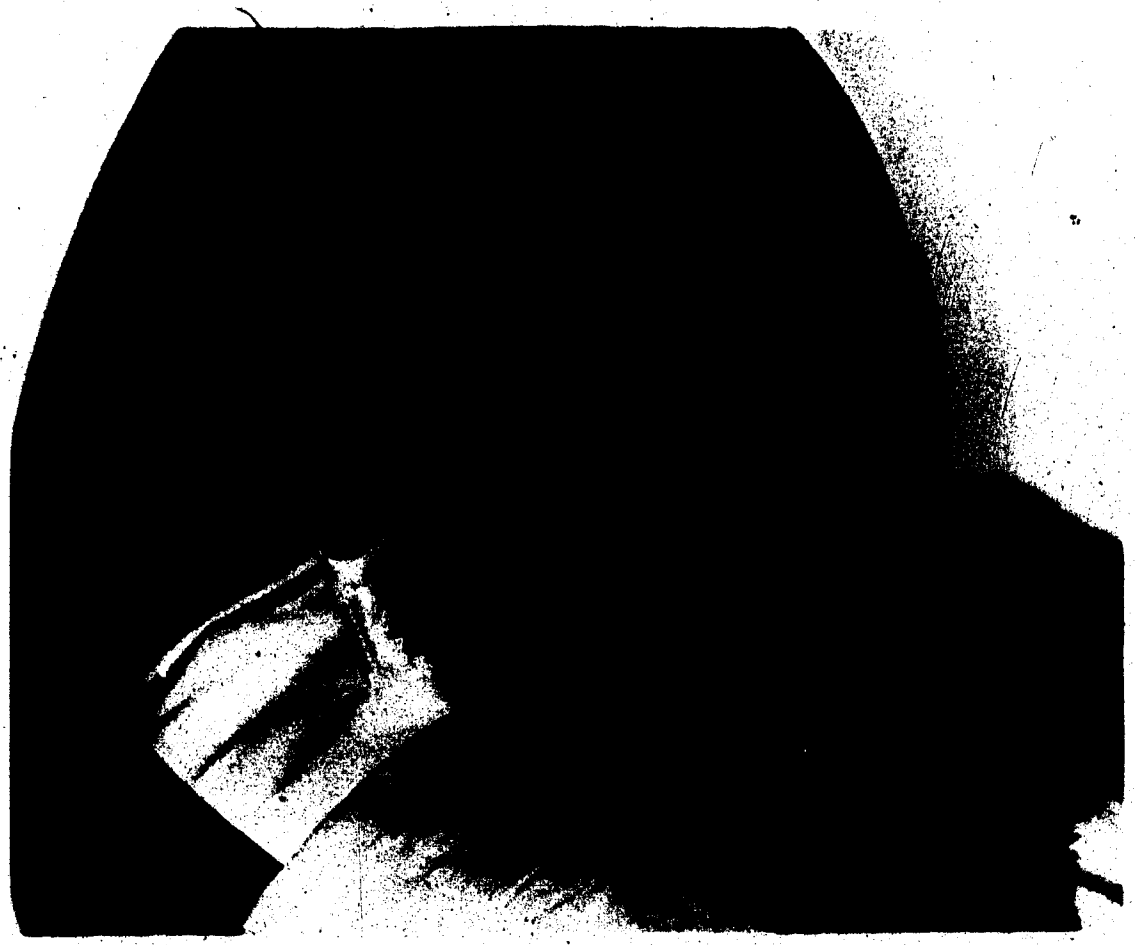


FIGURE 10

Monkey subjected to computed tomography of the head (under general anesthesia).

[With the magnification used, mean cranial CT diameters (± SD) were 38.5 ± 3 mm on fronto-occipital scans and 32.3 ± 3 mm on temporo-temporal scans.]

Cerebral Angiography

Cerebral angiography was always performed after CBF studies. The radiography equipment was of the same specifications as in previous investigations in this laboratory (106).

Study A

Lateral cerebral angiograms and in some cases oblique views were obtained twice on Day 0 (immediately before and within 1 hr afterward) and once on each occasion on Days 2, 7, 14 and 21; one film was obtained during the arterial phase. Cerebral vessels were measured at four points: the internal carotid artery (ICA) just above the posterior communicating artery, the proximal middle cerebral artery (MCA), the proximal pericallosal artery (PPA), and the distal pericallosal artery (DPA).

Studies B and C

Retrograde (femorocerebral) angiography was performed, using a no. 5 French radiopaque polyethylene catheter with a sigmoid tip (designed and shaped by the author) and two side-holes (Fig. 7). An AP-view film was obtained during the arterial phase 1 wk before the SAH (Day -7) and on Days 7 and 14 post-SAH. The X-ray beam was directed parallel to Reid's baseline and centered at the nasion. Cerebral vessels were measured bilaterally (except for the PPA and DPA) at 10 points: the segments of the extradural internal carotid (EICA) just before entrance into the cavernous sinus, the segments of the ICA between the posterior communicating and ophthalmic arteries, and the sphenoidal segments of the MCA, the anterior cerebral arteries (ACA), and the PPA and DPA. The Cordis injector was used to inject contrast medium, at approximately 10 ml/sec.

In Study A, 3 ml of Reno-M-60 was injected into the left carotid artery at a pressure of 10 PSI. In Studies B and C, 10 ml of Conray 60 was injected into the innominate artery and the common carotid arteries bilaterally, at a pressure varying from 100 to 500 PSI in Study B and steady at 300 PSI in Study C.

Magnification factors were kept constant and a radiopaque control device was used to correct for variation in film, exposure, and development (106). The observer grading variation in vessel caliber was unaware of the animal's identity. Caliber at each of the arterial sites was measured six times, with the same optical system as for CT measurements, and the average for each site was calculated. Serial measurements were plotted as percentage change in vessel caliber, and this was graded as follows: $\geq$ + 1% to -10% = no spasm; -11% to -30% = mild vasospasm; -31% to -50% = moderate vasospasm; and $\geq$ 51% reduction = severe vasospasm. At the end of Studies A and C, for each monkey the overall incidence of VSP was determined, the time course of the VSP was plotted, and reduction in vessel caliber (based on measurements of the maximally constricted vessel) was analyzed. In addition, in Study C the angiographic appearances of collateral circulation on Days 7 and 14 were graded: 0 = collateral circulation not seen; + = appearance of a small new channel, or mild dilation of collateral circulation apparent on the initial (control) angiogram; ++ = one or more medium-sized new channels; and +++ = one or more large new channels.

Measurement of Cerebral Blood Flow

CBF was measured twice before cerebral angiography on each occasion. In studies B and C it was done 15 and 60 min after the injection

of 0.5-1.0 ml of contrast medium (to confirm correct positioning of the catheter tip in the innominate artery). In Study C, CBF was measured 2-3 hr or 6-7 hr after administration of the drug (nimodipine or placebo). Hemispheric CBF bilaterally (Studies B and C) or on the left (Study A) was determined by intra-arterial ^{133}Xe clearance, using a single collimated scintillation detector (1 inch diam; activated with NaI-thallium) to record radioactivity from the dorsolateral fronto-parietal brain regions (Fig. 11). CBF values were calculated from the initial-slope index and were corrected to a $PaCO_2$ of 40 mm Hg.

Methods of handling, dispensing, administering and detecting ^{133}Xe, and for calculating CBF, were very similar to those used in this laboratory since 1972; they have been reported in detail (107-109).

Preparation and Administration of Drugs

The drugs (including placebo) were prepared by Dr J.A. Rogers, Associate Professor of Pharmacy at the University of Alberta. As nimodipine is very sensitive to light of ordinary wavelength, especially in solution (51), all preparations used in Study C were dispensed and given under gold light (F-15T8-60 gold, Westinghouse Electric, Pittsburgh, PA) and were stored in amber-colored glass bottles.

Two methods of administration were tried in Study B. Method 1. A total of 25 gelatin capsules containing 0.5 ml of the vehicle (polyethylene glycol 400), inserted into pieces of banana or sections of orange, was given to 14 monkeys: it was hoped the animals would swallow both fruit and capsule, but four of the animals discovered the capsule and threw it away (but ate the fruit!). Method 2. Ten monkeys were lightly sedated with ketamine (6-10 mg/kg) injected im and the vehicle

FIGURE 11

Measuring cerebral blood flow in a monkey. Note the position of the detectors, away from the temporalis muscle.

was administered via a nasogastric tube: this method was successful in all cases and therefore was used in Study C.

Study C. For each of the 30 monkeys studied, 50 ml of the glycol solution, containing nimodipine 3 mg/ml (n = 15) or placebo (n = 15), was dispensed into a bottle labeled N/P followed by a number from 1 to 30. The average weight of the monkeys was about 3 kg and the solution was given in 1-ml aliquots with 1 ml of water tid, at 0730, 1530, and 2330 hours (i.e., the dosage was 1 mg/kg b.wt, tid), from Day 1 post-SAH to Day 13, and at 0730 hours on Day 14. The animal-care technician gave the first two doses each day and the author gave the last one, except at weekends, when the author gave all doses.

Gold light was used when giving the drugs. In addition to protecting the nimodipine-containing solutions from normal light, this maintained 'blinding' of medications: nimodipine is yellow, and under gold light the solutions appeared identical. Only Dr. Rogers had access to the coding system for medications. After completion of the study the code was broken in two stages: when all the data had been collected, to identify animals as belonging to group A or B; and when the data had been analyzed, to identify which group had received nimodipine and which had had placebo.

Neurological Assessment and Termination of Experiments

Each monkey underwent neurological examination before and 5 hr after SAH (allowing recovery from anesthesia) and daily thereafter until it died or was killed (under general anesthesia, by exsanguination on Day 14 or 21 in Study A or by cerebral fixation by intra-arterial per-

fusion on Day 14 in Study C). A five-division neurological grading system was used for evaluation:

Grade 1: active, vocal, normal
Grade 2: lethargic, upright but unsteady, and not as active or vocal, but no significant neurological deficit (paresis, paralysis)
Grade 3: no spontaneous attempt to stand upright but appropriate responses to stimulation (touch, sound); moderate neurological deficit (monoparesis, hemiparesis)
Grade 4: severely obtunded, apathetic, with little response to stimulation; severe neurological deficit (monoplegia, hemiplegia, quadriplegia)
Grade 5: moribund, failing vital signs, no response to stimulation.

Pathology

The primary fixation solution consisted of 2% glutaraldehyde and 2% formaldehyde in Millonig's buffer, 0.12 M; pH 7.4, at 4°C.

Gross Pathology

The brain was removed within a few hours of death in most cases. Gross pathology was noted in detail and the brain was photographed. It was then placed in 10% formaldehyde for 2 to 4 wk (Studies A and B) or in primary fixation solution for 24 hr to 4 wk (Study C). Gross coronal or horizontal sections were cut and photographed.

Microscopic Pathology

This section pertains only to Study C.

Cerebral fixation by intra-arterial perfusion

Except in the case of monkeys who died before Day 14, the brain was fixed by intra-arterial perfusion after the last angiogram on Day 14. Under continuing general anesthesia, paralysis, and mechanical ventilation with 66% O_2 in N_2O, a wide thoracotomy was performed by cutting through the fifth intercostal space bilaterally. The ascending aorta was cannulated via the left ventricle, the right atrium was widely

opened, and the descending aorta ligated. Heparin (3000 U) was injected iv 5 min before the circulating blood was washed out (with 150 ml of 0.9% saline, at 20°C, infused over 30 sec at a pressure of 110 mm Hg), and this was followed immediately by perfusion with 1 L of the primary fixation solution for 5 to 10 min at 110 mm Hg. The brain was removed within 15 to 30 min, and was placed whole in primary fixation solution for a minimum of 24 hr and a maximum of 4 wk at 4°C. The cerebral vessels (basilar and middle cerebral arteries, bilaterally) were dissected out very carefully with the aid of an operating microscope; each was cut into three pieces, which were placed in separate small vials of the same fixation solution. In most cases, the proximal segment (in which a window 0.5 mm^2 had been opened to allow visualization of the luminal surface) was studied with scanning electron microscopy (SEM), the mid segment with transmission electron microscopy (TEM), and the distal one with light microscopy (LM).

Light Microscopy

After fixation for at least 24 hr, the tissues were dehydrated through a graded ethyl-alcohol series and cleared in xylene (dehydrating and clearing series: 70% ETOH X 2 for 15 min each; 85% ETOH for 15 min; 95% ETOH for 15 min; absolute ETOH X 2 for 30 min each; and xylene X 2 for 30 min each.) After transfer to an incub[redacted]8°C), the samples were moved through two changes of paraffin (TissuePrep embedding pellets; Fisher Scientific) at 56.5°C for 30 min each. The blocks were sectioned at 8 μm on a steel knife in a rotary microtome, floated on albuminized slides, and allowed to dry overnight at 40°C. The slides were stained with Harris's hematoxylin and counterstained with alcoholic eosin Y, and were examined and photographed in a Zeiss microscope

(Standard 14 laboratory light microscope, model D-7082; Zeiss, Oberkochen, W. Germany).

Electron Microscopy (SEM and TEM)

After primary fixation as above, the tissues were washed for 45 min (3 changes) with Millonig's buffer, 0.13 M, and re-fixed for 1 hr in 1% osmium tetroxide in Millonig's buffer, 0.07 M. They were then washed for 30 min (3 changes) with double-distilled water, and dehydrated through the graded series of ethanol solutions.

Samples for SEM were transferred in absolute ethanol to a CO_2 critical-point dryer (Seevac Inc., Pittsburgh, PA) and when dried were mounted on aluminum stubs, sputter-coated with gold (model S150B; Edwards, Crawley, West Sussex, England) and examined in a scanning electron microscope at 25 kV (Phillips model 505).

For TEM, samples in absolute ethanol were transferred to propylene oxide for 30 min (3 changes), fixed in 1:1 propylene oxide:araldite (CY212) epoxy resin for 3 to 4 hr, and put into pure resin in embedding blocks. They were left overnight at room temperature, then polymerized for 48 hr at 60°C. Thin sections cut on an ultramicrotome (Reichert-Jung Ultracut) were mounted on 300-mesh copper grids, counterstained with uranyl acetate and lead citrate, and examined in a transmission electron microscope at 80 kV (Phillips model 410; N.V. Phillips' Gloeilampenfabrieken, Eindhoven, The Netherlands).

Analysis of Data

Study A

Descriptive statistics were computed for each variable. Comparisons within groups were made with Student's *t* tests, and relationships

between variables were determined with Pearson's correlation coefficient. Intergroup comparisons were made with tests of proportion and t test. Linear regression lines were fitted and goodness-of-fit measures were applied. Significance was assessed as $p < 0.05$ except where otherwise stated. Results are expressed as mean ± SE, except in assessing the Cushing response (mean ± SD).

Study B

No statistical analysis was made.

Study C

The data were coded, entered into a computer, and edited. For each monkey, descriptive statistics and frequencies were determined for each variable at each time of testing, and relationships between VSP and CBF with time were determined by Pearson's correlation coefficient. When the pharmacist had identified the nimodipine and placebo groups, intra- and intergroup differences were determined. Changes in CBF in the two groups were compared with two sample t tests, and the intragroup incidence of VSP and collateral circulation were analyzed with the Fisher-Irwin test. Intergroup comparison of the degree of VSP with time was by analysis of variance, and of changes in MABP with time by analysis of covariance with the initial MABP value as covariate.

The level of significance for all tests of comparison or association was $p < 0.05$ except where otherwise stated. Results are expressed as mean ± SE.

III. RESULTS AND COMMENTS

STUDY A: CREATING A LARGE SUBARACHNOID HEMORRHAGE IN MONKEYS

Summary of Subjects

Seventy-five sets of measurements were completed on the 25 monkeys after creation of the SAH. Seven monkeys died within a few hours; of the remaining 18, 8 were studied on Day 2, 17 on Day 7, 15 on Day 14, and 10 on Day 21.

The hemorrhage as seen on the first CT scan after creation of the SAH was graded as small in 3, medium in 7, and large in the other 8. In the 10 monkeys whose CT was graded as small or medium, the cerebral angiograms revealed similar degree, time of onset, and duration of VSP. Therefore (and because so few animals had a small hemorrhage), these two grades were considered together as the small/medium-SAH group. The other 8 monkeys were considered as the large-SAH group.

Comparison of the two groups showed no significant differences in pre-SAH values for body weight, the measured physiologic indices, roentgenographic appearances, and neurologic assessment, or in the volume of blood injected intracranially (Table 1).

There were no significant post-SAH alterations in average values for MABP, $PaCO_2$ or PaO_2 at any experimental stage except an increase in MABP during induction of the SAH.

TABLE 1

STUDY A: Control Values (mean + SD) for Measured and Observed Indices

Variable	SAH on CT Scan	
	Small/medium	Large
No. of monkeys	10	8
Body weight, kg	3.2 ± 0.5	3.5 ± 0.7
Mean arterial BP, mm Hg	91 ± 14	93 ± 13
$PaCO_2$, mm Hg	40 ± 3	39 ± 4
Vessel caliber, mm:		
internal carotid	1.02 ± 0.19	1.04 ± 0.15
middle cerebral	0.90 ± 0.19	0.88 ± 0.13
proximal pericallosal	0.89 ± 0.15	0.92 ± 0.16
distal pericallosal	0.72 ± 0.12	0.72 ± 0.19
Cerebral blood flow, ml/100 g/min	45 ± 9	51 ± 13
Neurologic assessment	grade 1	grade 1
Blood injected:		
ml/kg body weight	1.52 ± 0.18	1.43 ± 0.20
total, ml	4.81 ± 0.87	4.94 ± 0.79

Incidence of Vasospasm

With VSP considered as > 10% reduction in vessel caliber from the control value, it was present in the small/medium-SAH group in 6 of 10 animals on Day 0, in 5 of 6 on Day 2, in 6 of 9 on Days 7 and 14, and in all 4 studied on Day 21. In the large-SAH group, it was seen in all 8 on Day 0, in 2 of 2 on Day 2, in 7 of 8 on Day 7, and in 5 of 6 on Days 14 and 21. The vessel most often (and severely) affected was the pericallosal artery, especially its proximal segment (50% of the time); this was the segment closest to the site of blood injection. The distal segment of the pericallosal artery was next in frequency (33%), followed by the MCA (11%) and the ICA (6%).

Calculation of the incidence of VSP at the four arterial sites in 68 angiograms of the 18 monkeys showed that VSP occurred significantly more frequently in the large-SAH group (Fig. 12) on Days 0 ($p < 0.001$), 7 ($p < 0.01$), and 14. Intragroup frequencies showed no significant differences in the small/medium-SAH group but a significantly higher incidence of VSP in the large-SAH group on Day 0 than on Day 21.

Degree and Time-course of VSP

Intergroup comparison showed greater reduction in vessel caliber in the large-SAH group, the difference being significant on Day 0, Day 7 ($p < 0.02$), and Day 14.

Small/Medium-SAH Group

VSP was maximal (mean vessel caliber = 71% of control) on Day 2 and lessened thereafter (Fig. 13). Figure 14 illustrates the cerebral angiograms of an animal graded as having a small SAH on CT.

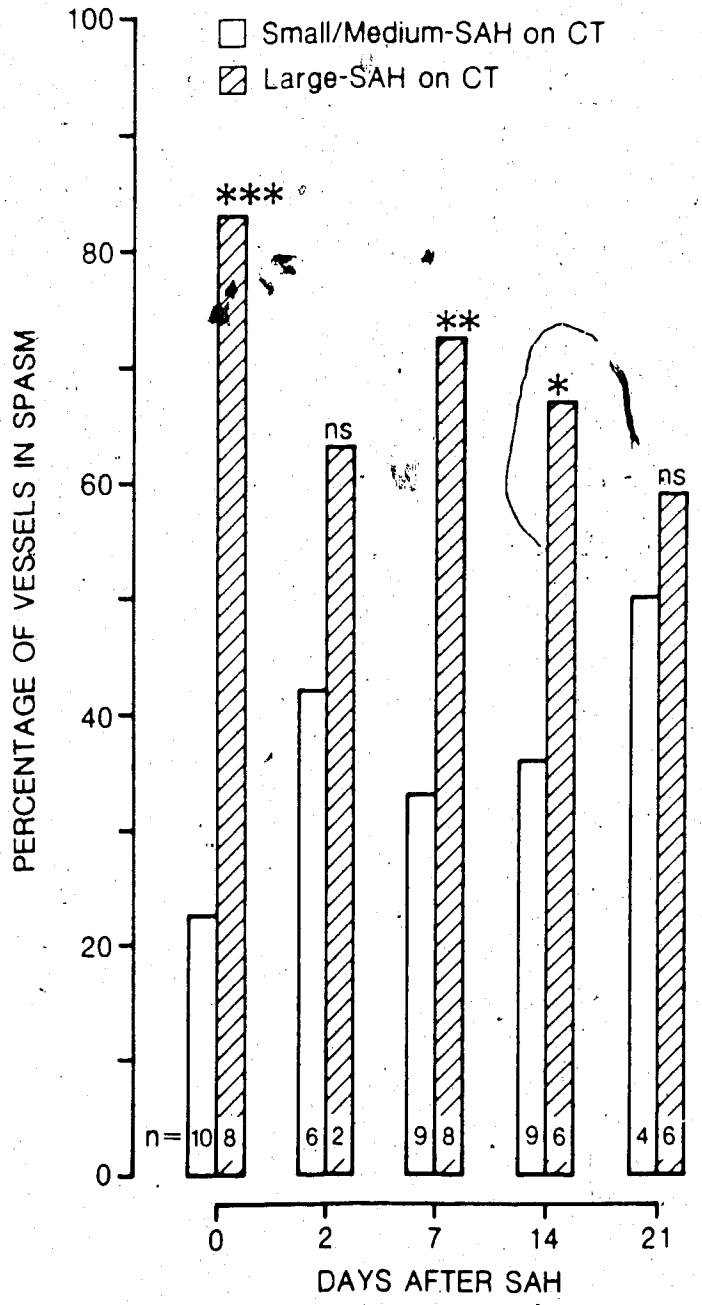


FIGURE 12

Incidence of VSP (>10% reduction in vessel caliber), based on data at the 4 arterial sites in 68 carotid angiograms of the 18 monkeys. VSP was significantly commoner on Days 0, 7, and 14 in the large-SAH group. Figures within columns are the numbers of animals studied at those times. Intergroup comparison: $P < 0.05$*; < 0.01**; < 0.001***; ns, not significant.

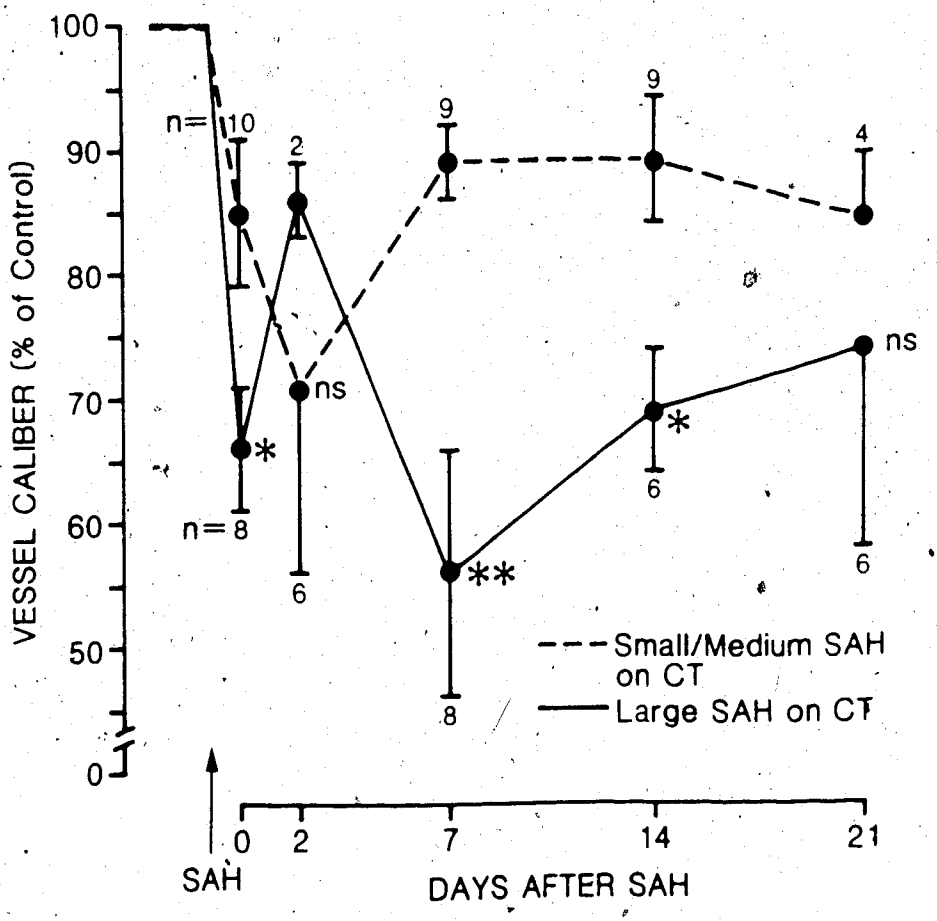


FIGURE 13

Time-course of VSP and mean (± SE) percentage change in vessel caliber, based on measurements of the vessel maximally constricted in each monkey. The large-SAH group had significantly greater VSP on Days 0, 7, and 14. Figures above and below SE bars indicate numbers of animals studied at those times. Intergroup comparison: * = $p < 0.05$; ** = $p < 0.02$; ns = not significant.

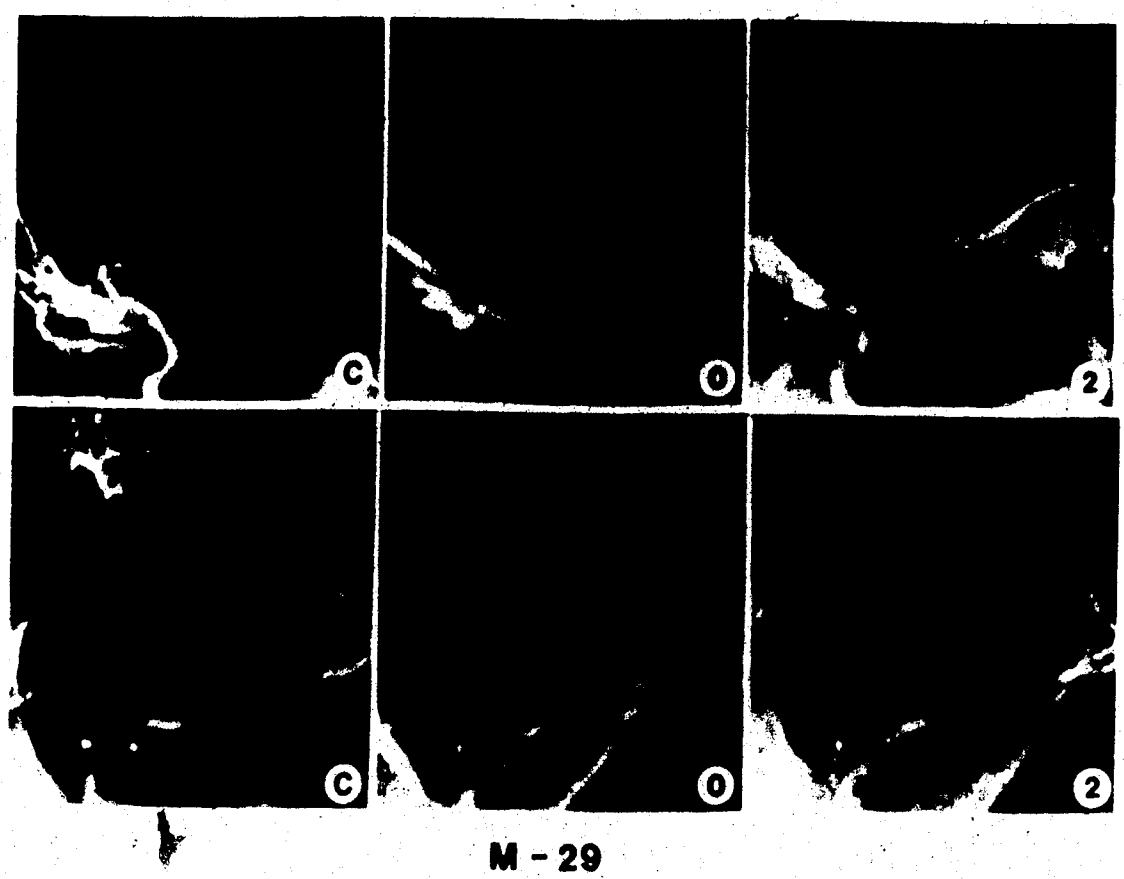


FIGURE 14

Lateral (upper) and oblique (lower) cerebral angiograms of monkey 29, which was graded as having a small SAH on CT scan: C, control; diffuse dilation on Day 0; severe VSP on day 2. This animal died (from surgical complications) on Day 3.

Large-SAH Group

VSP was maximal overall (mean vessel caliber = 56% of control) on Day 7, regardless of the presence or absence of VSP on Day 0 (Fig. 13 and Table 2). In three monkeys (No. 11, 25, and 26) the degree of VSP was biphasic. Figures 15 to 18 depict the CT scans and angiograms of two representative animals of the large-SAH group. Table 3 summarizes the relationship of VSP to the amount of blood seen in the subarachnoid space on the first post-SAH CT scan.

Cerebral Blood Flow

CBF was generaly decreased but not significantly. Values did not appear to correlate with the average percentage reduction in vessel caliber, in individual animals or in the groups overall.

Neurological Assessment

Neurological deterioration (right hemiparesis) developed on Day 0 in 7 monkeys, 4 of which were graded as having a small/medium SAH and 3 a large SAH. In 6 animals it was mild and disappeared within 48 hours. In one monkey of the large-SAH group, the paresis was severe and accompanied by right homonymous hemianopsia; these signs disappeared gradually over 12 days.

Delayed neurological deficit developed in two of the large-SAH group. In one monkey, apathy was noted from Day 17 to Day 21, coincident with severe VSP of the pericallosal artery and 29% reduction in CBF. In the other, unsteadiness and drowsiness were noted on Days 4 and 5; on Day 7, when CBF was reduced to 45%, the angiogram showed moderate VSP of the pericallosal artery.

TABLE 2

STUDY A: Degree and Time-Course of VSP in the Eight Monkeys in the Large-SAH Group

Monkey no.	Degree of VSP				
	Day 0	Day 2	Day 7	Day 14	Day 21
9	Moderate		None*		
11	Moderate		Severe	Moderate	Severe
13	Mild		Mild*		
16	Moderate		Severe	Moderate	Mild
19	None		Severe	Mild	Mild
23	Moderate		Severe	Moderate	None
25	Moderate	Mild	Moderate	Mild	None
26	Moderate	Mild	Mild	Moderate	Mild

* Died later that day. Death was due to technical complications.

TABLE 3

STUDY A: Summary of Relationship of SAH Size on CT and the Development of Chronic VSP*

	SAH on CT		
	Small/Medium	P	Large
Monkeys	10		8
Vessels in spasm	52/152 (34%)	< 0.001	85/120 (71%)
Degree of spasm:			
mild	44 (84.5%)	n.s.	48 (56%)
moderate	5 (9.5%)	n.s.	27 (32%)
severe	3 (6%)	n.s.	10 (12%)
VSP maximal	Day 2		Day 7

* Reduction in vessel caliber > 10% of control value.

n.s. = not significant.

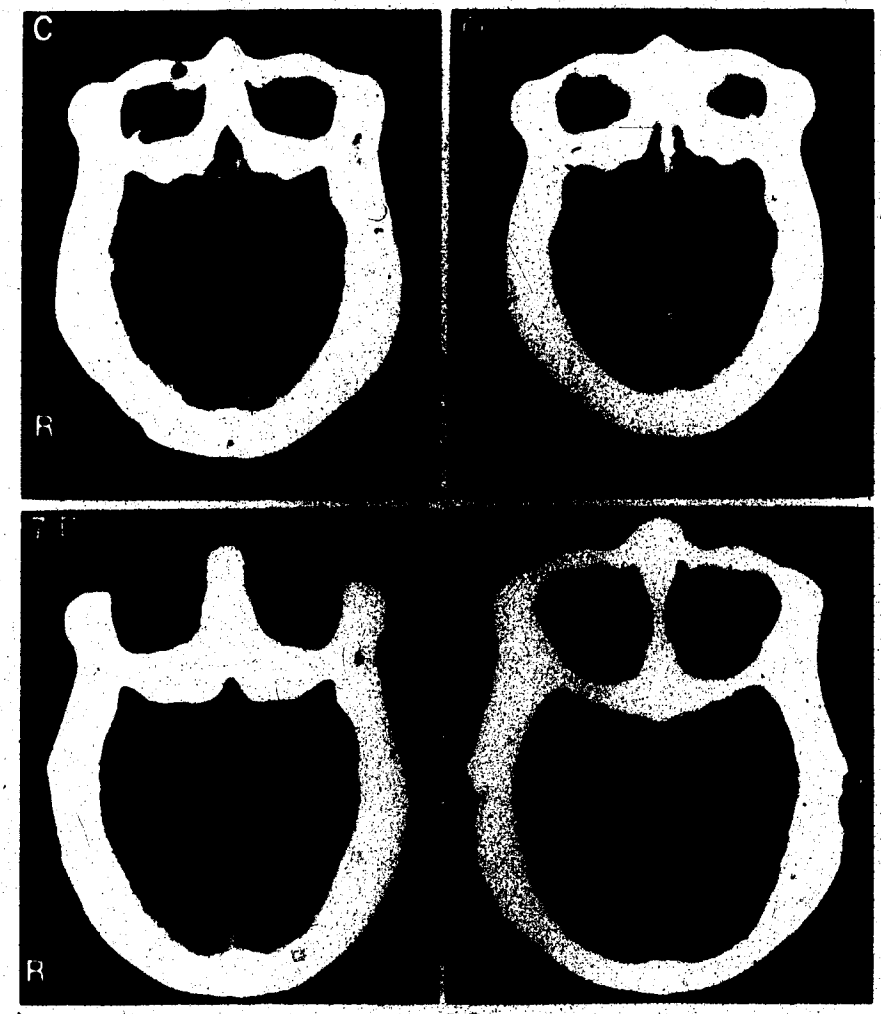


FIGURE 15

CT scans of monkey 23, showing large SAH. C, control. Thick layers of blood in the frontal and occipital interhemispheric fissures on Day 0; less blood on Day 7; almost complete reabsorption of blood by Day 14. This animal was not studied on Day 2.

FIGURE 16

Lateral angiograms of monkey 23. C, control. Diffuse moderate VSP on Day 0; severe proximal VSP on Day 7; moderate VSP on Day 14; no VSP apparent on Day 21.

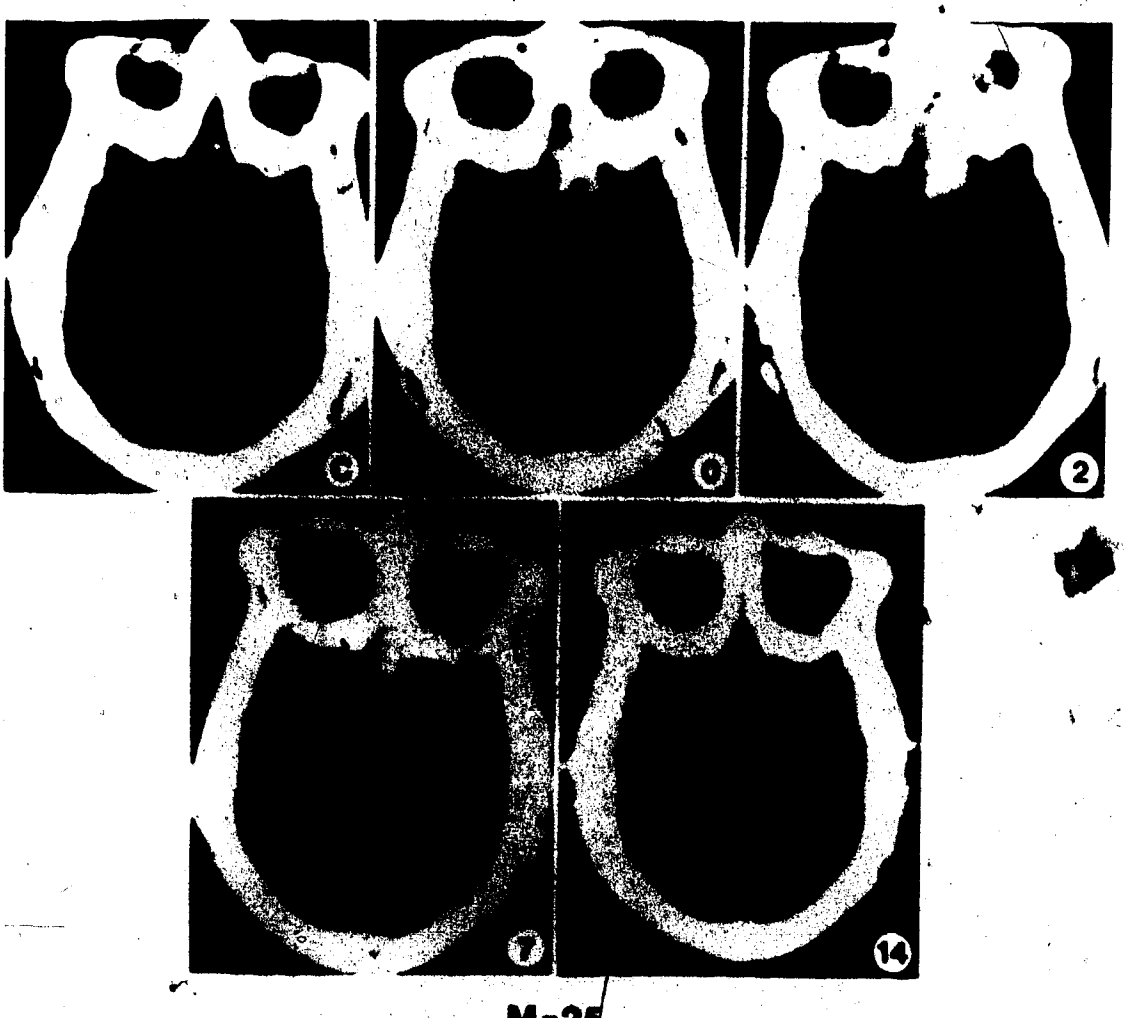


FIGURE 17

CT scans of monkey 25 (large-SAH group). C, control. Mixed (subarachnoid/intracerebral) frontal hematoma, and thick layers of blood, on Days 0 and 2; a low-density area on Day 7; almost complete reabsorption of blood and no low-density area apparent on Day 14.

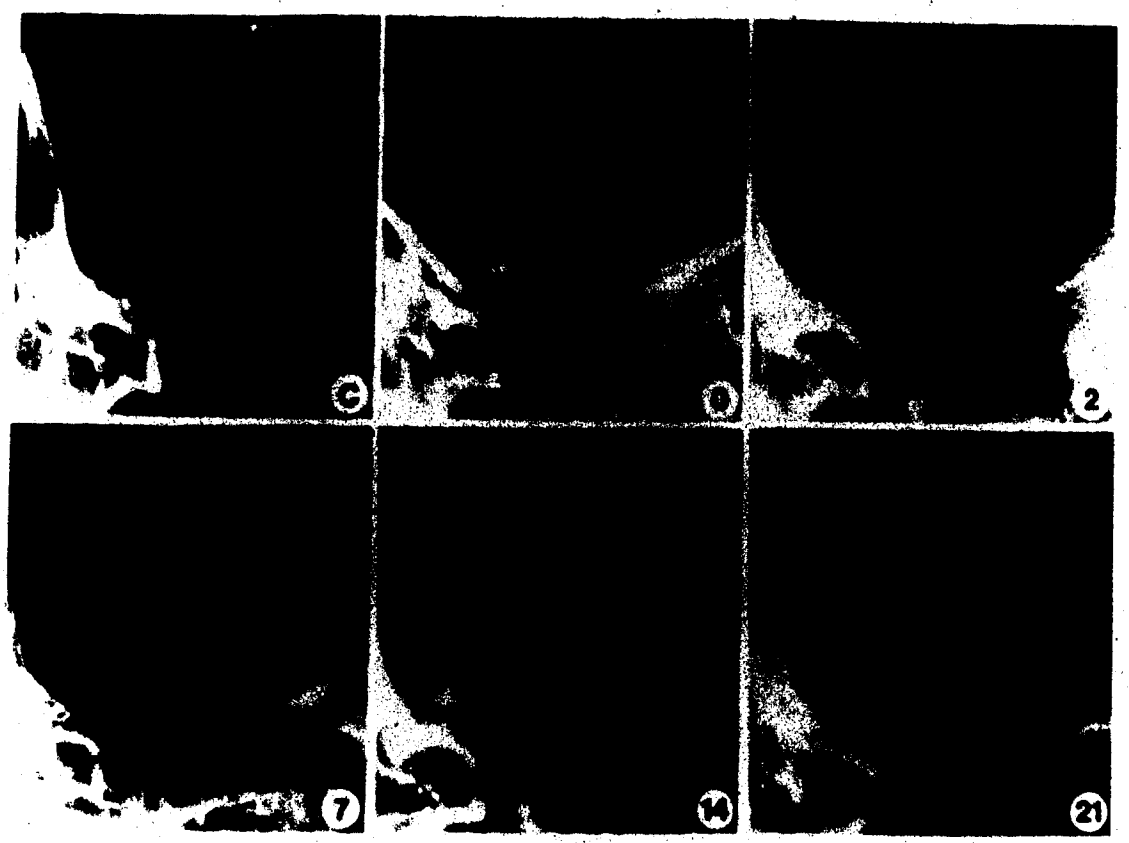


FIGURE 18

Lateral angiograms of monkey 25, showing a biphasic pattern of VSP. C, control. VSP moderate on Day 0 but only mild on Day 2; VSP again moderate on Day 7 and mild on Day 14; almost normal vessel caliber on Day 21.

In general, neurological condition tended to be poorer in the large-SAH than in the small/medium-SAH group; the average neurological grade was 1.1 and 1.4 respectively.

Cushing Response during Induction of SAH

Cushing response (25% increase in MABP from the pre-SAH value) developed in 7 of the 10 monkeys in the small/medium-SAH group and in 4 of the 8 in the large-SAH group. Mean ($\pm$ SD) increases in MABP during induction of the SAH were 49 $\pm$ 41% in the small/medium-SAH group and 50 $\pm$ 44% in the large-SAH group. Linear regression analysis showed no relationship between the percentage increase in MABP during creation of SAH and the development of VSP at any time post-SAH.

Pathology

Gross pathological examination revealed a major SAH component in monkeys graded as having a large SAH on CT scan. This was particularly noticeable in those which died within 7 days after the SAH. In monkeys killed on Day 14 or Day 21, the subarachnoid blood had almost completely reabsorbed. By contrast, pathological examination revealed a larger subdural than SAH component in monkeys graded as having a small/medium SAH on CT scan. Four monkeys in the large-SAH group and one in the small/medium-SAH group had a small intracerebral hemorrhage, in the right gyrus rectus in one and in the left in four.

Comment

Despite intensive effort to produce a model of VSP for the study of its pathophysiology and treatment, previous investigations have failed

to reproduce VSP as in humans (47,111). In most experiments, VSP has been maximal about 1 to 4 days after SAH and has lasted a few days, has been milder than in man, and has not given rise to delayed neurological deficit (112-118). Moreover, although some investigators have obtained fairly chronic VSP by inducing large SAH (119,120), this has not resulted in the prolonged arterial narrowing seen in humans. More recently, Frazee et al. (121) noted that when the SAH was induced with a large needle (which they presumed caused a large SAH), VSP occurred more frequently (in all of four cases) than when a smaller needle was used (in two of five). They also reported reflex asymmetry, but did not state whether this developed acutely and persisted up to Day 5 (the duration of their experiments) or had a delayed onset coincident with the observed 32% reduction in vessel caliber.

In the present study (A), a model in the monkey was developed in which the VSP resulting from a small/medium SAH resembled that reported in previous studies but the VSP following a large SAH closely resembled that which develops in man. In humans, reduction in vessel caliber is maximal on Day 7, lasts up to 2 wk, and in some cases gives rise to delayed neurological deficit. The success in mimicking VSP in man probably stems mainly from the volume of blood injected (more than most other investigators have used): this was calculated from the results of experiments in a similar strain of monkeys, in which 1.67 to 2.0 ml blood/kg body wt was lethal for 50% by 20 hr (122).

Fisher et al. (34) reported severe VSP in 23 of 24 patients whose SAH was apparent on CT scan as localized clots and/or thick layers of blood but in only 1 of 18 whose SAH was diffuse or not apparent on the CT scan. Davis et al. (36) also observed a direct correlation between

the extent of the bleeding and the severity of VSP. Although Mizukami et al. (123) did not measure the SAH, they found a relationship between CT density and cerebral VSP: 22 of 26 cases in which CT density was high and in none of 8 in which it was low. Suzuki et al. (124) reported a similar relationship between the amount of blood, CT density, clinical severity at the time of hemorrhage, and cerebral infarction due to VSP. The present results accord with these findings: significant VSP (severe in 4 and moderate in 2) developed in six of eight cases of large SAH but in only three (severe in 1 and moderate in 2) of the 10 with small or medium SAH.

The proportionality of the vasospastic reponse to the size of the SAH may explain why VSP is not invariably seen after SAH in man and could account for the variation in its time of appearance and duration. VSP may occur almost immediately after SAH in humans, as in the present large-SAH cases: cerebral angiography is usually not performed until hours or even days after the ictus, by which time the early phase of VSP probably is over.

The delay in development of maximal VSP may reflect the time required for erythrocytes to lyse. The contractile activity of incubated erythrocytes increases within the first 36 hr after SAH until day 3 and is maintained at that level for at least 14 days (125,126), and the vasoactivity of the hemolysate is dose-dependent on the concentration of oxyhemoglobin (126-128). Although the conditions for clot lysis and subarachnoid blood reabsorption in vivo should be different, it is highly likely that the larger the SAH the longer it will take to lyse and the greater the concentration of hemoglobin and/or other vasogenic substance(s) in the CSF.

The lack of correlation between SAH size and VSP in some of the monkeys (large SAH but no or mild VSP, and vice versa) accords with clinical experience. Such occurrences probably are exceptional; the findings indicate that the volume of a SAH is probably the most important determinant of severe VSP.

STUDY B: (PILOT STUDY) ESTABLISHING PREFERRED METHODS FOR CREATING SAH-INDUCED VASOSPASM AND FOR CEREBRAL ANGIOGRAPHY

A pilot study was carried out to establish the safest method for induction of SAH that would consistently induce chronic cerebral VSP. A new angiographic technique (retrograde femorocerebral) and a reliable route for administering medication to the monkeys were established, for later application in Study C.

Summary of Subjects

Ten animals were studied, in two groups. In Group 1, 8 monkeys were subjected to injection of blood into the subarachnoid space under direct vision. Five died acutely (within 20 hr): 2 from brain ischemia perhaps due to elevated intracranial pressure, as suggested by the Cushing response (25% increase above the pre-SAH MABP value) that developed during creation of the SAH, and 3 from hemorrhagic shock due to rupture of the external iliac artery during the complicated catherization. Three monkeys survived the acute period: 1 died on Day 7 and 1 on Day 14, both from angiographic complications (ruptured external iliac and coronary arteries respectively); the third (M-42), the only one subjected to a combined procedure (injection of 3 ml of blood and place-

ment of a 6-ml clot), was rendered quadriplegic, and died on Day 2 probably from brain ischemia due to elevated ICP and severe VSP.

In Group 2 (2 monkeys), in which the arachnoid membrane was opened at the region of the basal cistern and a blood clot (1.5 - 5.0 ml) was placed against the major anterior circulation arteries, both animals survived the acute period. Monkey 36 (5.0-ml clot) died on Day 9 during cerebral angiography; contrast medium was injected at 500 psi, causing cardiac tamponade from ruptured coronary arteries due to transmission of the high pressure of medium through the aortic arch. Monkey 37 survived for several weeks after the first procedure (1.5-ml clot), and was kept for another experiment (placement of a 5.0-ml clot over the left MCA); repeat angiography 20 hr after this second experiment was beset by technical complications and the monkey died later that day.

Incidence and Severity of VSP, and Neurological Assessment

In group 1, cerebral angiography showed mild to moderate diffuse cerebral VSP acutely in most animals (no measurements were made). Monkey 42, which had undergone the combined procedure (blood injection and clot) had left hemiplegia on Day 1, became quadriplegic and moribund on Day 2, and died later that day before angiograms could be performed. No other monkey in this group had a neurological deficit.

In Group 2, VSP was localized to the MCA on the clot side. It was mild in monkey 37 (first study) after placement of a 1.5-ml blood clot against the right MCA (Figs. 19 and 20), and severe in monkey 36, in which placement of a 5-ml clot against the right MCA resulted in delayed neurological deterioration (contralateral hemiparesis, developing on Day 9). When monkey 37 was restudied and a 5.0-ml blood clot was placed

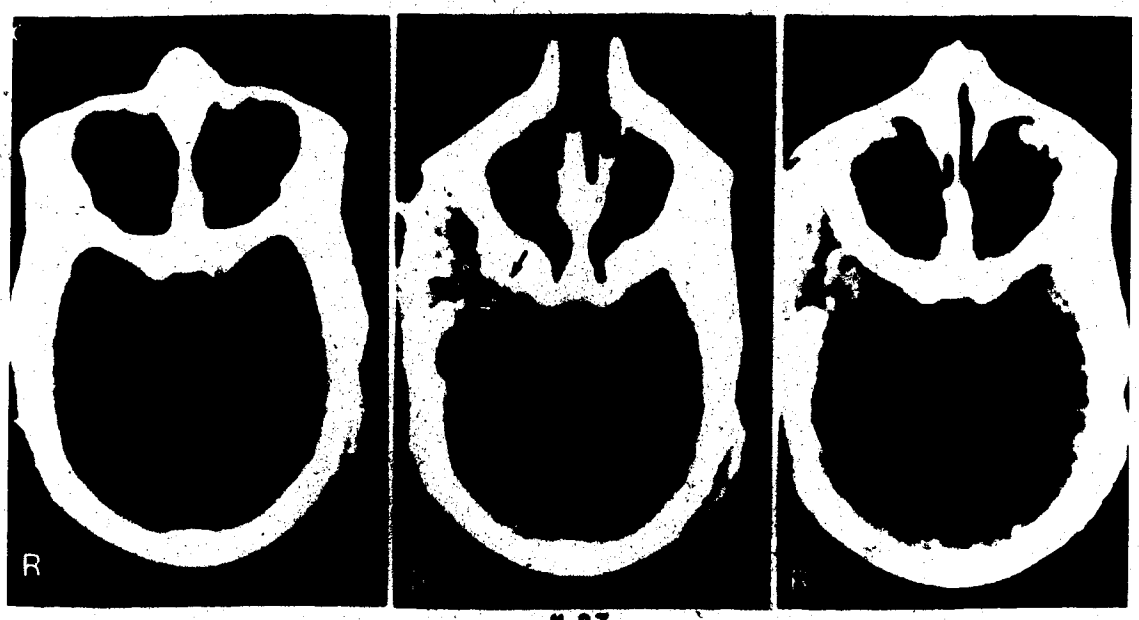


FIGURE 19

CT scans of monkey 37, at the time of control studies (C), just after creation of SAH (O) and 1 wk later. Day 0: A blood clot is apparent (arrow) in the region of the lesser wing of the sphenoid bone. Day 7: the hematoma has completely reabsorbed.

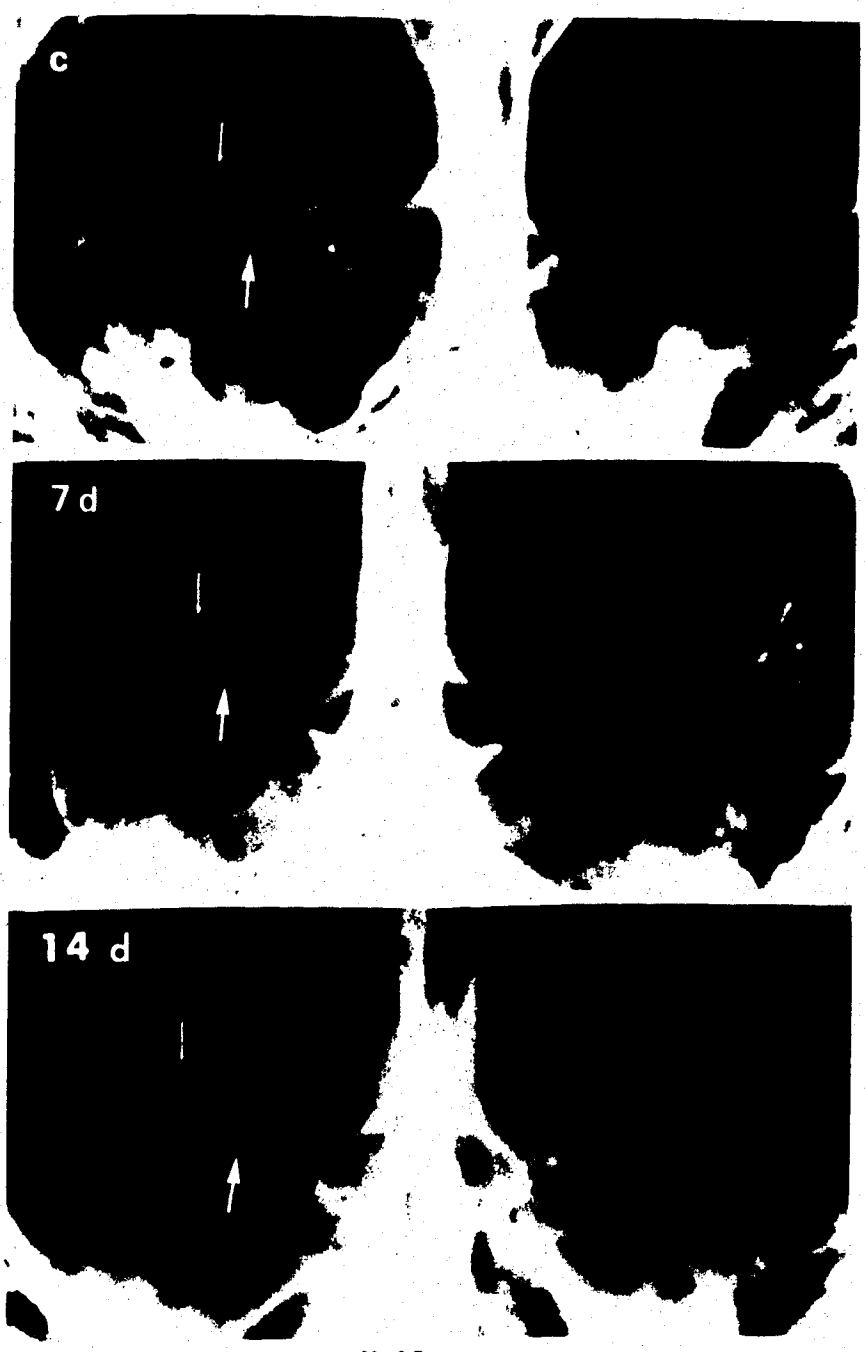


FIGURE 20

Anteroposterior cerebral angiograms of monkey 37, during control studies (C) and on Days 7 and 14 after induction of SAH. Mild VSP of the middle cerebral artery (arrow) and the opening of collateral circulation (arrowhead), apparent on Day 7, were absent on Day 14.

over the left MCA, right hemiplegia developed by 20 hr and angiograms showed severe VSP of the left MCA and ACA; the animal died on Day 1 from complications during the angiography.

Pathology

In Group 1, gross pathological examination revealed a major SAH component (blood diffusely distributed to the basal cisterns and surface of the brain) in six monkeys, and a major subdural component (> 90%) in two. There was a significant intraventricular (lateral and third ventricles) hemorrhagic component in two (nos. 41 and 42), a large retroperitoneal hematoma was found in four and cardiac tamponade from ruptured coronary arteries in one.

In Group 2, in both monkeys a well-formed blood clot was found lying against the MCA. In monkey 37 (second study), the entire area served by the left MCA was infarcted and the retroperitoneal space contained a large hematoma. Cardiac tamponade was found in monkey 36.

Cerebral Angiography (Retrograde Femorocerebral)

The use of pediatric catheters (#6 French; and HlH modified cerebral model, Cook Inc., Bloomington, IN) for cerebral angiography was attempted in several animals. This was unsuccessful, because of the catheters' large diameter in relation to the size of the femoral artery. Therefore, a #5 French straight catheter was shaped and adapted by the author (Fig. 7); the tip was curved into sigmoid shape, so it could fit into the monkey's aortic arch and remain steady at the origin of the innominate artery. Once the catheter had been introduced through either femoral artery it was advanced under fluoroscopic control to the innom-

inate artery, and contrast medium (Conray 60) was injected. Two conditions were tested: pressure (100, 200, 300, 400 and 500 psi) and volume of contrast medium (5, 7, 10, and 12 ml).

At 100 and 200 psi the injected material was diluted with blood, and therefore did not provide good opacification of the arterial system. At 500 and 400 psi, the coronary arteries ruptured; this was documented by fluoroscopy during the injection and confirmed on pathological examination. Injection of the contrast material at 300 psi was the safest and most efficient method; no animal died as a result of this pressure, and good opacification of the arteries throughout the anterior circulation on both sides was obtained.

In testing the volume of contrast medium, 5 and 7 ml were found insufficient to delineate the extra- and intracranial arteries, but 10 and 12 ml provided good opacification of the neck and cerebral vessels. As the maximal dosage of contrast medium was about 4 ml/kg body wt, and the monkeys averaged 3 kg, a total dose of 10 ml was preferred; this was used in all experiments in Study C.

Administration of Medication

Details of the methods tested are on pages 72 and 74 (insertion into pieces of fruit, and administration via a nasogastric tube under sedation with ketamine). The latter method was successful in all 10 animals in which it was tested and therefore was used in Study C.

Comment

There were two major disadvantages in Study A. 1. Creation of a mixed subdural hematoma and SAH, due to blind injection of blood via a

spinal needle placed intracranially under fluoroscopic control would have necessitated study of a large number of animals to achieve a good sample size with a major SAH component. 2. Blood was distributed throughout the basal cisterns and subarachnoid space, and especially in the frontal interhemispheric fissure. In most cases the VSP was localized to the pericallosal artery (the artery closest to the site of blood injection and the collection of blood visible on CT scans); therefore, no obvious neurologic deficits developed. Thus, as the aim of treatment of SAH-induced VSP is to prevent neurological deterioration, refinement of the model was required before treatments could be tested.

Isolation of the right side of the circle of Willis and placement of the hematoma provided the safest and most efficient method to induce SAH and VSP similar to that in man. Retrograde femorocerebral angiography enables one to study the intracranial circulation bilaterally, and isolation of one side of the circle of Willis permits use of the other side as control. In Study A, local injury to the common carotid or internal carotid arteries during catheterization for cerebral angiography resulted in a high morbidity and mortality rate. In Study B, once the technique for retrograde femorocerebral angiography was established it proved the best and safest method for studying the intracranial circulation bilaterally. Although many complications were encountered initially during development of this method, minimal or no complications occurred in Study C.

When the pilot study was concluded, no attention had been paid to the collateral circulation that developed on the side ipsilateral to the VSP. Later, in Study C, this proved to be a major factor in preventing

deterioration in neurological status from VSP - an important consideration when assessing models of ischemia from cerebral VSP.

STUDY C: A MODEL OF CHRONIC VSP OF THE MIDDLE CEREBRAL ARTERY AFTER LARGE SAH IN MONKEYS: A RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL OF NIMODIPINE

CLINICAL AND RADIOLOGICAL FINDINGS

Summary of Subjects

A total of 121 sets of measurements on the 30 monkeys was completed in 6 months. Two animals in the nimodipine group died, one from a lethal dose of contrast medium associated with a difficult catheterization on Day 7; the other, after being well throughout the study, suffocated itself with the cage's food container on Day 13.

The hemorrhage as seen on the first CT scan after its creation was graded as 'large' in all 30 monkeys, as illustrated in Figures 23 and 31 (see later). As shown in Table 4, there were no significant intergroup differences in pre-SAH values for body weight, measured physiologic indices, or cerebral vessel caliber, in the volume of hematoma placed intracranially, or in alterations in average values for body weight, $PaCO_2$, PaO_2 or MABP during the study. No effects of medication on blood pressure and pulse were detected in any of the animals. The only side-effect noted was diarrhea, which occurred in all 30 monkeys and most probably was due to the medication vehicle (polyethylene glycol 400).

TABLE 4

Study C: Mean Baseline Values for Measured and Observed Indices and Mean Volume of Hematoma

Variable	Placebo Group (n = 15)	Nimodipine Group (n = 15)
Body weight, kg	3.3 ± 0.4	3.2 ± 0.4
Mean arterial blood pressure, mm Hg	119 ± 9	126 ± 14
Heart rate, beats/min	172 ± 20	184 ± 20
$PaCO_2$, mm Hg	38 ± 2	38 ± 2
Vessel caliber, mm		
internal carotid artery:		
right extradural	1.6 ± 0.2	1.5 ± 0.3
left extradural	1.0 ± 0.2	1.6 ± 0.3
right supraclinoid	1.0 ± 0.1	1.1 ± 0.2
left supraclinoid	1.3 ± 0.2	1.1 ± 0.2
anterior cerebral artery:		
right	0.8 ± 0.2	0.8 ± 0.1
left	0.9 ± 0.1	0.8 ± 0.1
middle cerebral artery:		
right sphenoidal	0.9 ± 0.1	0.9 ± 0.1
left sphenoidal	0.9 ± 0.1	0.9 ± 0.1
pericallosal artery:		
proximal	0.9 ± 0.2	0.9 ± 0.1
distal	0.8 ± 0.1	0.9 ± 0.1
Hemispheric cerebral blood flow, ml/100 g/min		
right	44 ± 10	43 ± 10
left	43 ± 10	44 ± 14
Size of hematoma, ml	7.3 ± 1.1	7.1 ± 1.1

Neurological Assessment

Placebo Group

Delayed hemiparesis, contralateral to the side of clot placement, developed in one monkey during Day 4 (Fig. 21). The pupils remained equal and symmetrical. Function in the affected leg improved by Day 10, but the arm remained very weak until death by fixation perfusion on Day 14. The angiograms (Fig. 22) showed severe VSP of the right (clot-side) MCA on Days 4 and 7, and the CT scans (Fig. 23) showed a wedge-shaped low-density area in the distribution of the right MCA on Days 7 and 13.

Nimodipine Group

None of the monkeys suffered a neurological deficit.

Incidence of Vasospasm

Vasospasm (> 10% reduction from control vessel caliber) was seen in the placebo group in all 15 monkeys on Day 7 and in 12 of the 15 (80%) on Day 14, and in the nimodipine group in all 15 animals on Day 7 and in 12 of the 13 (92%) on Day 14. Assessment of spasm by degree showed moderate VSP (31 - 50% reduction in vessel caliber) more common, but not significantly, in the nimodipine group: it was seen in the placebo group in 12 of the 15 animals (80%) on Day 7 and in 4 of 15 (27%) on Day 14, and in the nimodipine group in 13 of the 15 monkeys (87%) on Day 7 and in 5 of 13 (38%) on Day 14. Severe VSP (≥ 51% reduction) was more common, but not significantly, in the placebo group: it was apparent in 5 of these 15 animals (33%) on Day 7 and in 1 of the 15 (7%) on Day 14, and in the nimodipine group in 2 of the 15 monkeys (13%) on Day 7 and in none of the 13 on Day 14.

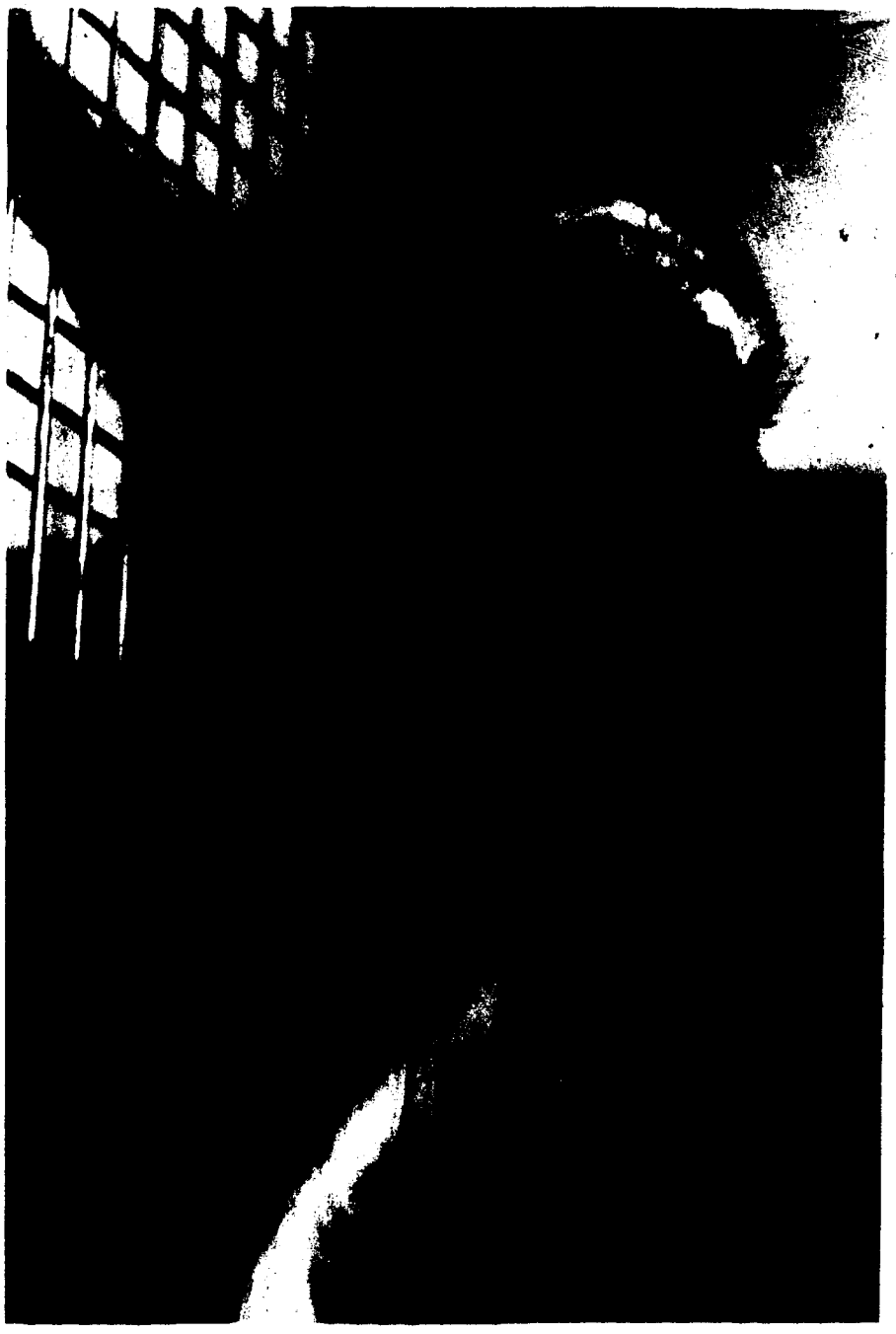
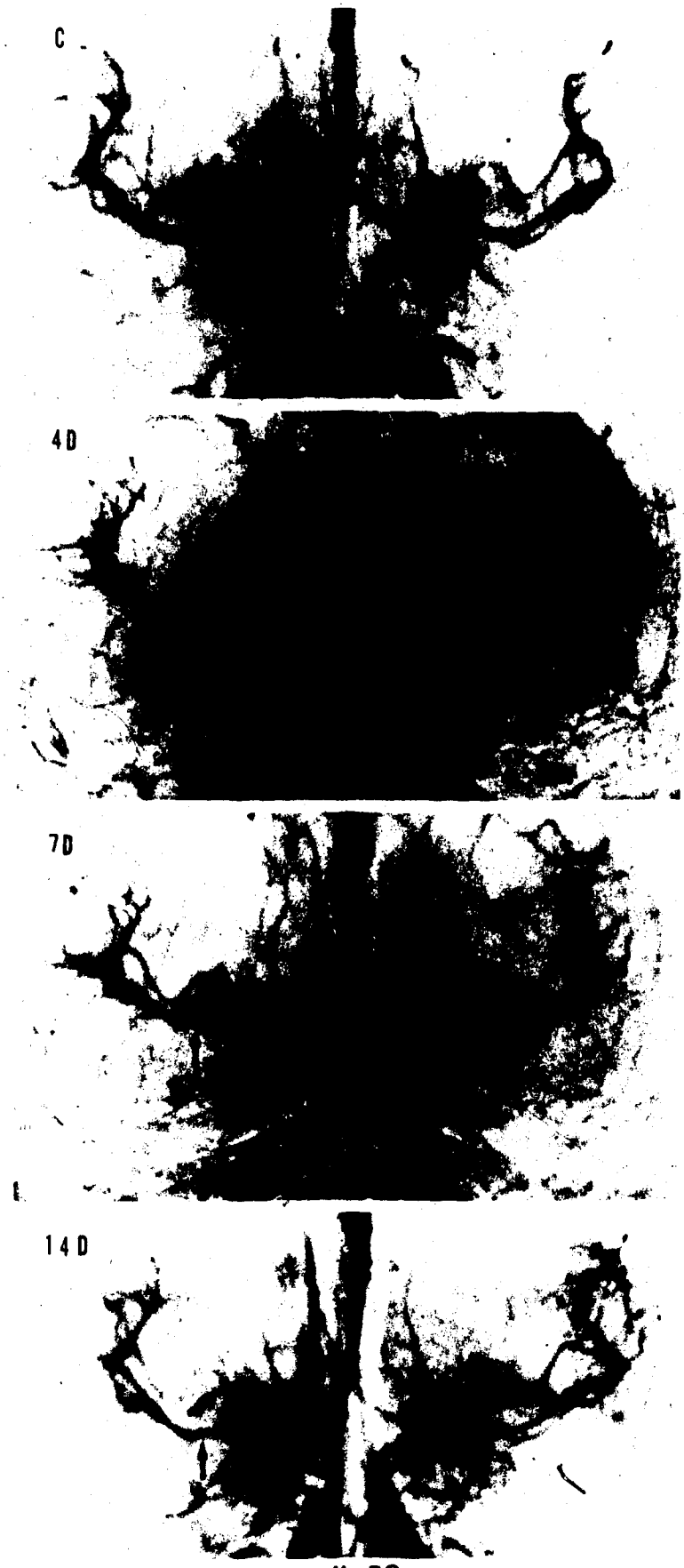


FIGURE 21

Monkey 58 (placebo group) on Day 7, 3 days after the onset of left hemiparesis. The craniectomy incision is visible on the right.

FIGURE 22

Monkey 58; placebo group. Anteroposterior cerebral angiograms at the time of control studies (top) and serially after induction of SAH. Days 4 & 7: Severe VSP has developed in the sphenoidal segment of the middle cerebral and supraclinoid internal carotid arteries (large arrows), and moderate VSP in the anterior cerebral artery (medium-sized arrow) and extradural internal carotid artery (small arrows). Day 14: The VSP is of mild degree.



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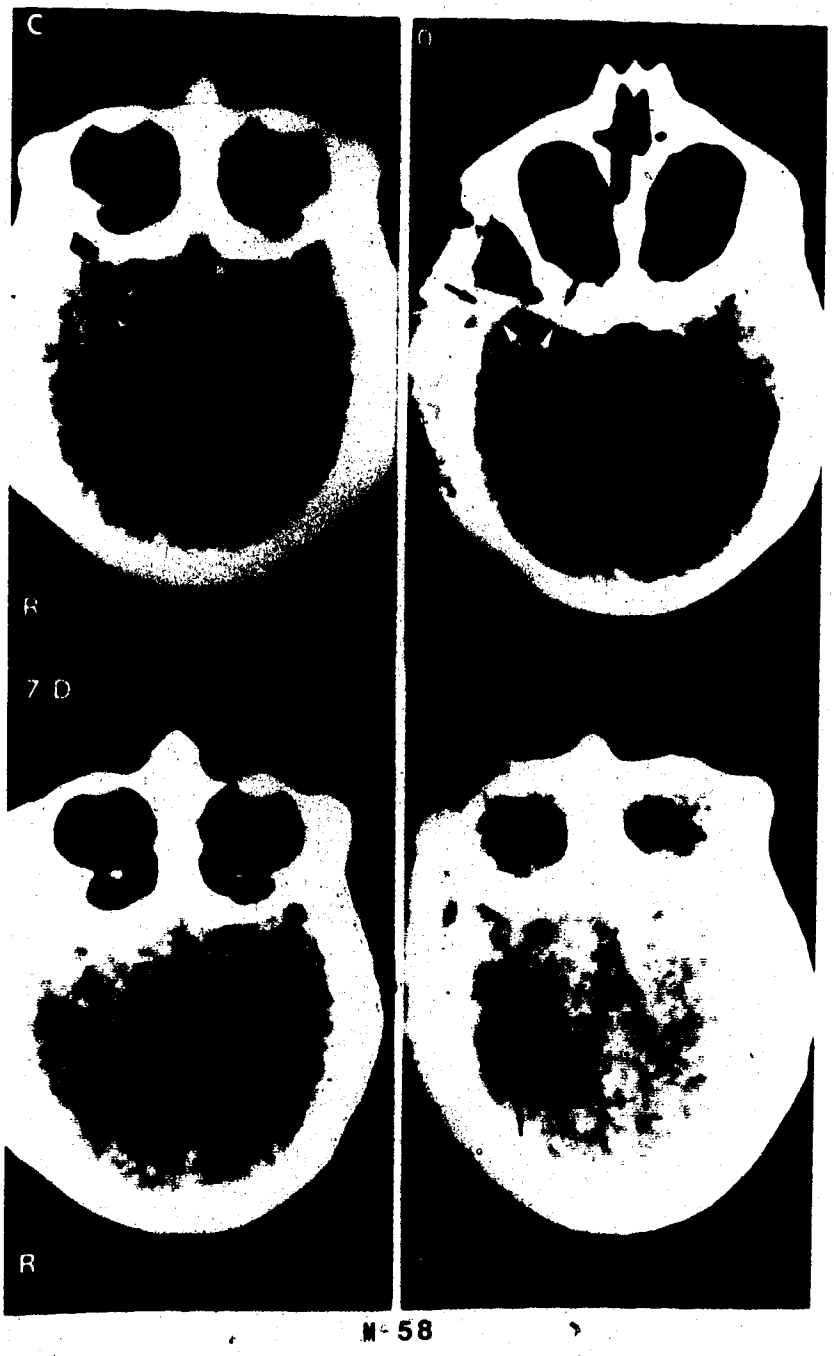


FIGURE 23

Monkey 58; placebo group. CT scans at the time of control studies (C), just after creation of the SAH (0), and 1 and 2 wk later.

Day 0: thick layers of blood are apparent (large arrows). Days 7 & 13: there is a wedge-shaped low-density area in the distribution of the right middle cerebral artery (small arrows), not affecting areas supplied by the anterior and posterior cerebral arteries.

Significant VSP, moderate or severe (31-100% reduction in vessel caliber), developed in 26 (87%) of the 30 animals, 13 in each group. VSP averaged 47% ± 5 in the placebo group and 43% ± 5 in the nimodipine group. In the placebo group, on Day 7 the vessels most commonly and most severely affected were the clot-side middle cerebral (47%) and intradural internal carotid and anterior cerebral arteries (20% each), and the least-often affected was the extradural internal carotid artery (13%). In the nimodipine group, on Day 7 this frequency was as follows: the clot-side middle cerebral and intradural internal carotid arteries (31% each) and anterior cerebral artery (25%), and the pericallosal artery (13%). Calculation of the incidence of VSP at the 10 arterial sites in 58 angiograms of the 30 monkeys showed spasm significantly commoner in the placebo group on both Day 7 and Day 14 ($p < 0.05$) (Fig. 24). Within the groups, VSP was significantly commoner on the clot side than non-clot side ($p < 0.0001$). Intergroup comparison of the incidence of VSP on the clot side showed no difference at any time, but on the non-clot side it was commoner ($p < 0.05$) overall in the placebo than in the nimodipine group (Fig. 25).

Time-course and Degree of Vasospasm

Overall, VSP on the clot side was more common ($p < 0.01$) and severe ($p < 0.001$) on Day 7 than on Day 14 (Figs. 25 & 26). There was no intergroup difference in the severity of VSP at any time (Fig. 26). In the placebo group, VSP was maximal (mean vessel caliber = 53% of control) on Day 7 and decreased thereafter (Figs. 22, 27, and 28). In the nimodipine group, also, VSP was maximal (mean vessel caliber = 57% of control) on Day 7 and decreased by Day 14 (Figs. 29, 30 and 31).

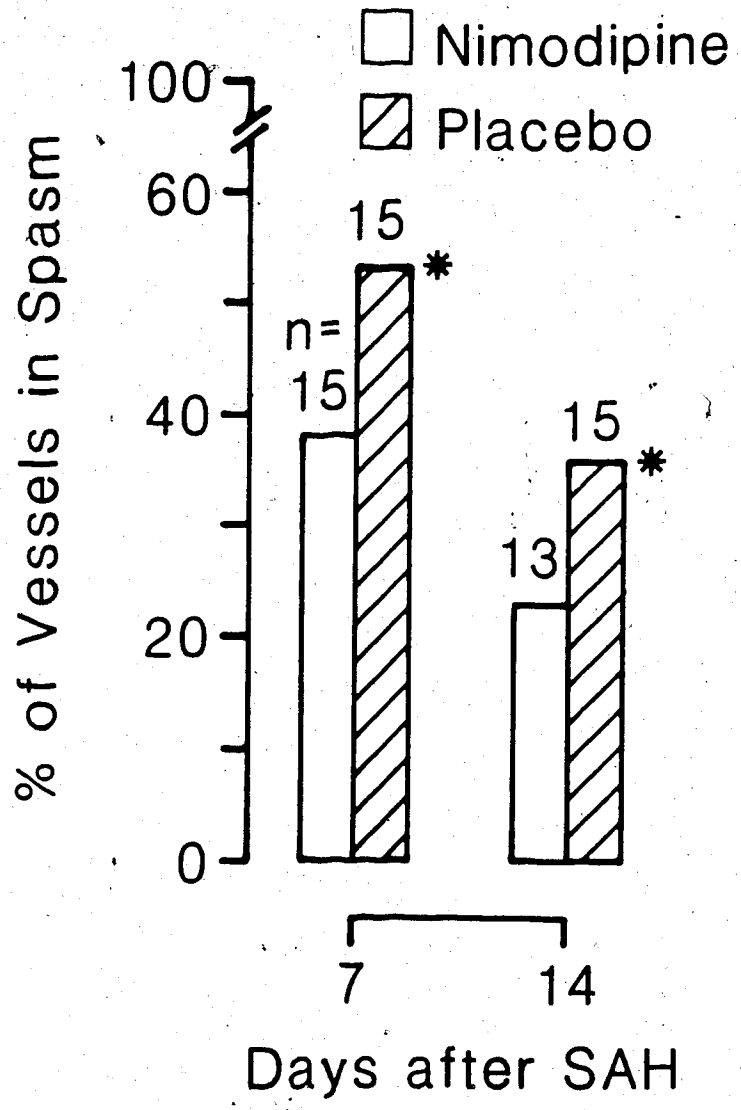


FIGURE 24

Incidence of vasospasm (> 10% reduction in vessel caliber), calculated from measurements at 10 arterial sites in 58 angiograms of the 30 monkeys. Asterisks indicate significant difference ($p < 0.05$) between the nimodipine (open bars) and placebo (hatched bars) groups.

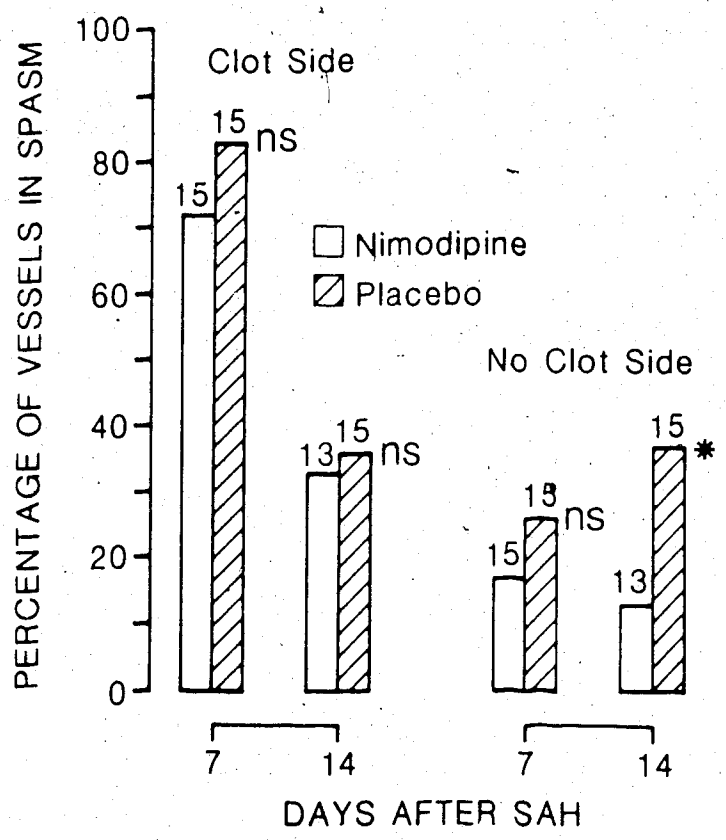


FIGURE 25

Incidence of vasospasm (> 10% reduction in vessel caliber), calculated from findings at the four arterial sites measured on each side in 58 angiograms of the 30 monkeys. Vasospasm was significantly commoner ($p < 0.05$; indicated by asterisk) on the non-clot side on Day 14 in the placebo group than in the nimodipine group (open bars). There was no significant difference (ns) on the clot side at any time.

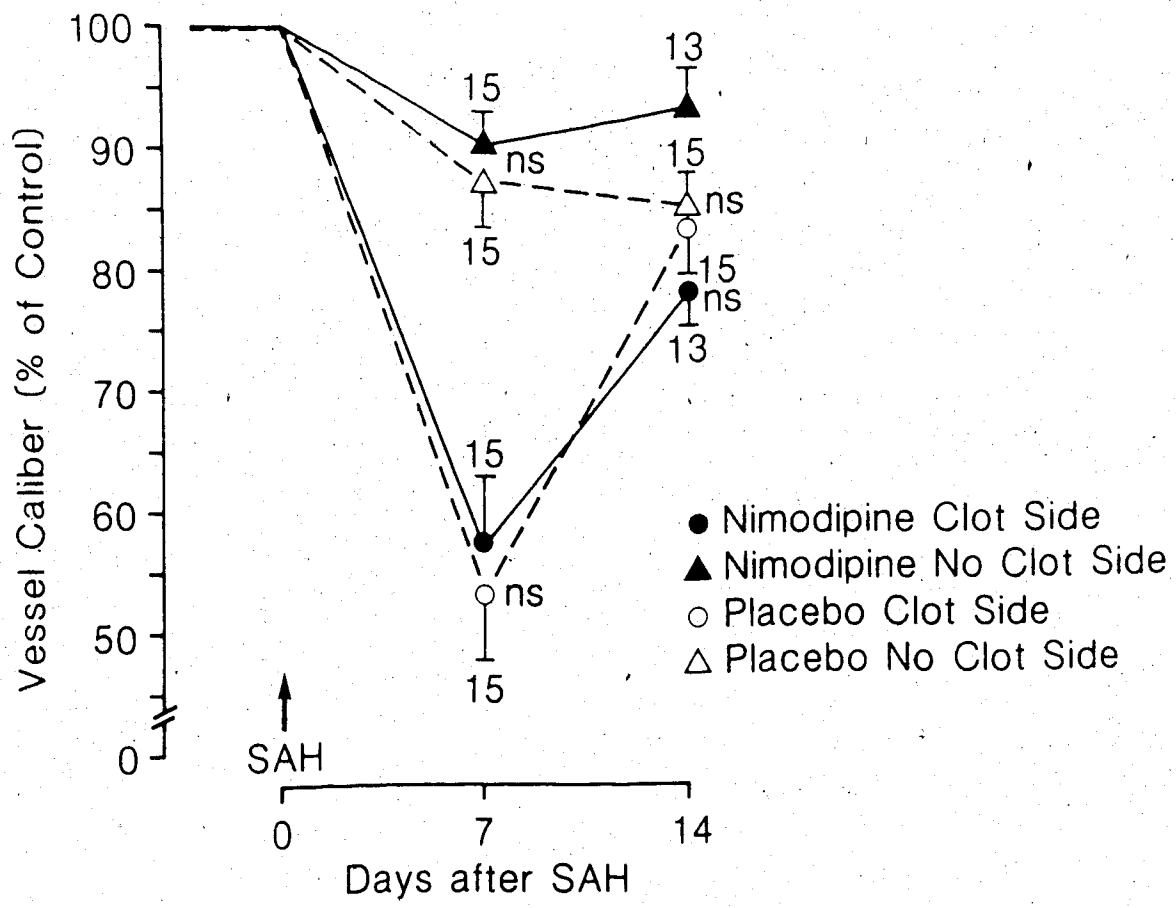


FIGURE 26

Time course of vasospasm and mean (± SEM) percentage change in vessel caliber, on the clot and non-clot sides, based on measurements of the most-constricted vessel in each monkey. In both groups, vasospasm was significantly greater ($p < 0.001$) on the clot side on Day 7. Intergroup differences in the degree of vasospasm were not significant (ns). Figures above and below SE bars indicate the numbers of animals studied at those times.

FIGURE 27

Anteroposterior cerebral angiograms of monkey 53 (placebo group). C, control angiogram. Severe vasospasm of the middle cerebral and supraclinoid internal carotid arteries (large arrows) and anterior cerebral artery (small arrows), apparent on Day 7, is absent on Day 14. The collateral circulation is very dilated (arrowhead) on Days 7 and 14.

FIGURE 28

Monkey 79; placebo group. Anteroposterior cerebral angiograms at the time of control studies (top) and on Days 7 and 14 after induction of SAH. Severe vasospasm of the middle cerebral and supraclinoid internal carotid arteries (large arrows) and anterior cerebral artery (small arrow), apparent on Day 7, is absent on Day 14. The collateral circulation is very dilated (arrowhead) on both occasions.

FIGURE 29

Anteroposterior cerebral angiograms of monkey 57 (nimodipine group). C, control angiogram. Day 7: Moderate vasospasm of the middle cerebral and internal carotid arteries (arrows) and enlargement of collateral circulation (arrowhead) are apparent. Day 14: Vasospasm is minimal and collateral circulation absent.

FIGURE 30

Anteroposterior cerebral angiograms of monkey 68 (nimodipine group). C, control. VSP of the middle cerebral and internal carotid arteries (large arrows) and anterior cerebral artery (small arrow), severe on Day 7, is absent on Day 14. The collateral circulation (arrowhead) is moderately dilated on Day 7, when spasm is maximal and the insular segment of the middle cerebral artery is dilated (W).

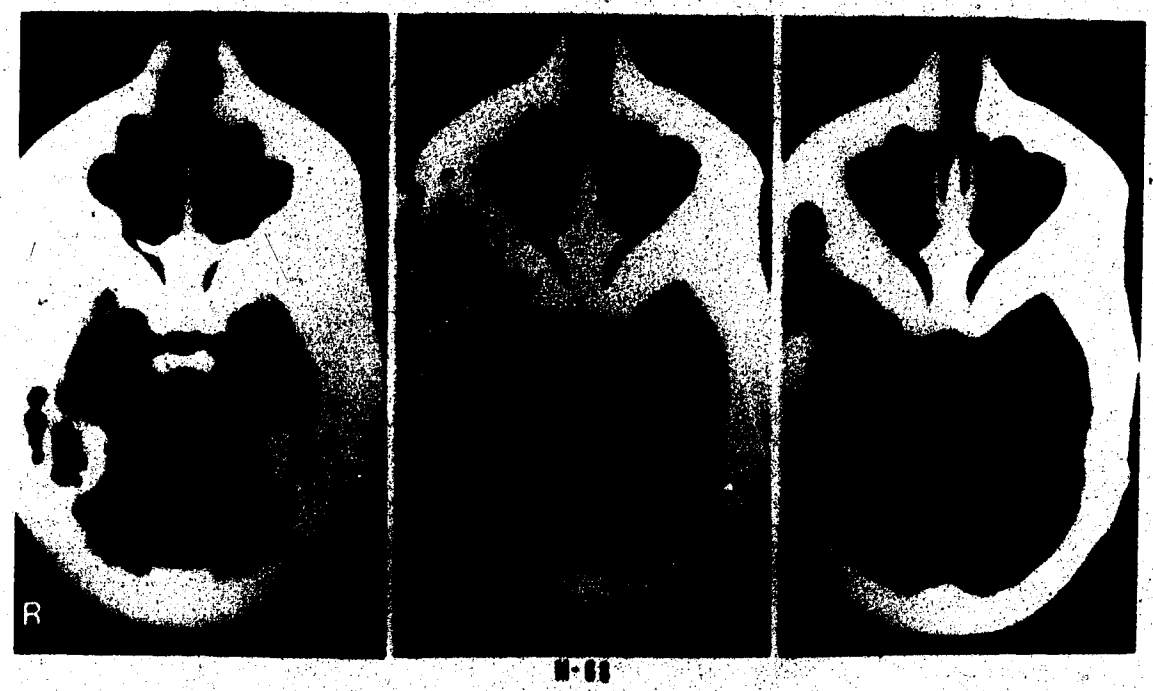


FIGURE 31

Monkey 68; nimodipine group. CT scans at the time of control studies (C), just after SAH (0), and 1 wk later. Day 0: Thick layers of blood are apparent (arrows) in the region of the sylvian cistern on the right. Day 7: The hematoma has almost completely resorbed.

Collateral Circulation

Collateral circulation seen angiographically, an incidental finding, was maximal on Day 7 and in most cases reverted to the control appearance by Day 14. Figures 27 to 30, depicting moderate or severe VSP and increased collateral circulation on Day 7, illustrate representative cases from the placebo and nimodipine groups. Intergroup comparison of the incidence of collateral circulation showed no significant difference (Table 5).

Cerebral Blood Flow

Hemispheric CBF was not significantly different in relation to clot and non-clot sides, days post-SAH, or the treatment groups. CBF was not increased 2 and 6 hr after the administration of nimodipine on Days 7 and 14.

Comment

The high reproducibility of cerebral VSP after SAH in this primate model makes it a reliable one for assessing therapies to prevent and treat VSP in man. The time-course, the association with a large collection of blood in the subarachnoid space, and VSP followed by delayed-onset obvious neurological deficits, are all features of the syndrome in humans (34,124,129). However, assessment of treatment for neurological deficits from VSP must take into account species-related anatomical and physiological variations in the collateral circulation.

The failure to demonstrate reduction in hemispheric CBF was probably due to the compensation by the collateral circulation for the VSP;

TABLE 5

Study C: Incidence of Collateral Circulation Seen Angiographically

	Placebo group				Nimodipine group			
	Day 7 (n=15)		Day 14 (n=15)		Day 7 (n=15)		Day 14 (n=13)**	
Collateral circulation*	Clot side	Non-clot side	Clot side	Non-clot side	Clot side	Non-clot side	Clot side	Non-clot side
0	2	11	2	11	3	12	4	10
+	8	4	8	4	4	3	7	3
++	2	0	3	0	6	0	2	0
+++	3	0	2	0	2	0	0	0

* 0, Not seen; +, small new vessel, not seen previously;

++, medium-sized new channel; +++, large new channel.

** Two animals had died.

in addition, the size of the detectors did not permit selective assessment of CBF supply to the territory of the middle cerebral artery. Harper et al. (130) reported increases in CBF during the administration of nimodipine through an intravenous or intra-arterial catheter and for up to 20 min afterward, but in the present study no significant increase in CBF occurred by 2 and 6 hr after giving nimodipine (1 mg/kg) orally on Days 7 and 14.

Cerebral arterial spasm probably originates from the interaction of multiple factors that ultimately induce contraction by increasing the concentration of free intracellular calcium (131,132). Therefore, it might be expected that blocking the ingress of calcium to smooth-muscle cells would prevent or lessen cerebral VSP despite its manifold origin. Studies of canine basilar and femoral arteries in vitro demonstrated that nifedipine and nimodipine inhibit contractions induced by serotonin, phenylephrine, and KCl (51). Nimodipine, which was the stronger inhibitor, had a more potent effect on basilar artery, a preferential effect that has been confirmed by others (49,132). Studies in vivo (51,133) also have shown a beneficial effect of both of these drugs on post-SAH cerebral VSP in dogs: nifedipine, 1 mg/kg, reversed both the acute state and early chronic phase (2 days) of VSP; nimodipine, 0.28 mg/kg, was more effective than nifedipine in reversing acute VSP (it was not tested after the acute phase).

Results in the present study do not entirely agree with earlier reports. Compared with placebo, nimodipine (1 mg/kg, orally, q8h) reduced the number of vessels in spasm on Days 7 and 14. However, assessment of VSP by side showed a beneficial effect of nimodipine on only the non-clot side - it did not lessen either the incidence or severity of

chronic VSP on the clot side. This suggests that, at the given doses, it prevented acute and chronic spasm of vessels exposed to low concentrations of vasoactive substances derived from fresh blood; i.e., on the non-clot side. This postulate may relate to Cohen and Allen's experiments (51) in which they created an SAH with 2.5 ml of autogenous blood in dogs and assessed the efficacy of nimodipine in reversing acute VSP (80 min post-SAH) rather than chronic (7 - 14 days). Factors in the genesis of acute and chronic VSP may be different; it is chronic VSP that causes ischemia of delayed onset (34,124).

In a recent clinical trial of nimodipine (50), VSP was considered the sole cause of severe, persistent, delayed neurological deficit or death in 9 of 125 patients (7.2%) treated with nimodipine or placebo. Eight of these patients had been given placebo and the other had been treated with nimodipine (0.35 mg/kg q4h), leading the authors to conclude that nimodipine significantly improved the neurological outcome for patients in whom VSP caused a deficit. In the present study, delayed ischemic neurologic deficit occurred in only 1 of 15 monkeys (3.3%) thus treated. Presumably, one factor that prevented VSP-induced deterioration in neurological status in these young female monkeys was the development of their collateral circulation when VSP was maximal.

Although the dose of nimodipine was larger in this study (1 mg/kg q8h) than in the clinical trial by Allen et al. (50) (0.35 mg/kg q4h), neither incidence nor severity of chronic VSP on the clot side was less than with placebo, perhaps indicating greater efficacy of nimodipine when its concentration in the blood is maintained by more frequent administration. As no deleterious side-effects were observed with

1 mg/kg every 8 hr, trials with increased doses of this calcium-entry blocker are warranted.

PATHOLOGICAL FINDINGS

Summary of Subjects

In all of the animals, gross inspection revealed a hematoma surrounding narrowed arteries on the side of the craniectomy and no abnormality of the contralateral vessels or basilar arteries. One brain, from the monkey in which delayed ischemic deficit had resulted from severe VSP, contained a wedge-shaped infarction in the territory of the MCA (Fig. 32); this correlated with the clinical and radiological features.

Control vessels were the MCA from the non-clot side and the basilar arteries.

Light Microscopy

In all of the control vessels, the endothelium was single-layered and flat, the internal elastic lamina was smooth and apparently intact, and the media and adventitia appeared normal (Fig. 33A).

Few changes were apparent in the vessels that had been in spasm. Compared with their controls, in general the adventitia looked thicker and had more abundant collagen and the media appeared thickened. The internal elastic lamina and intima were corrugated, and in some cases the endothelial cells at the crests of these ridges appeared rounded (Fig. 33B, 33C).

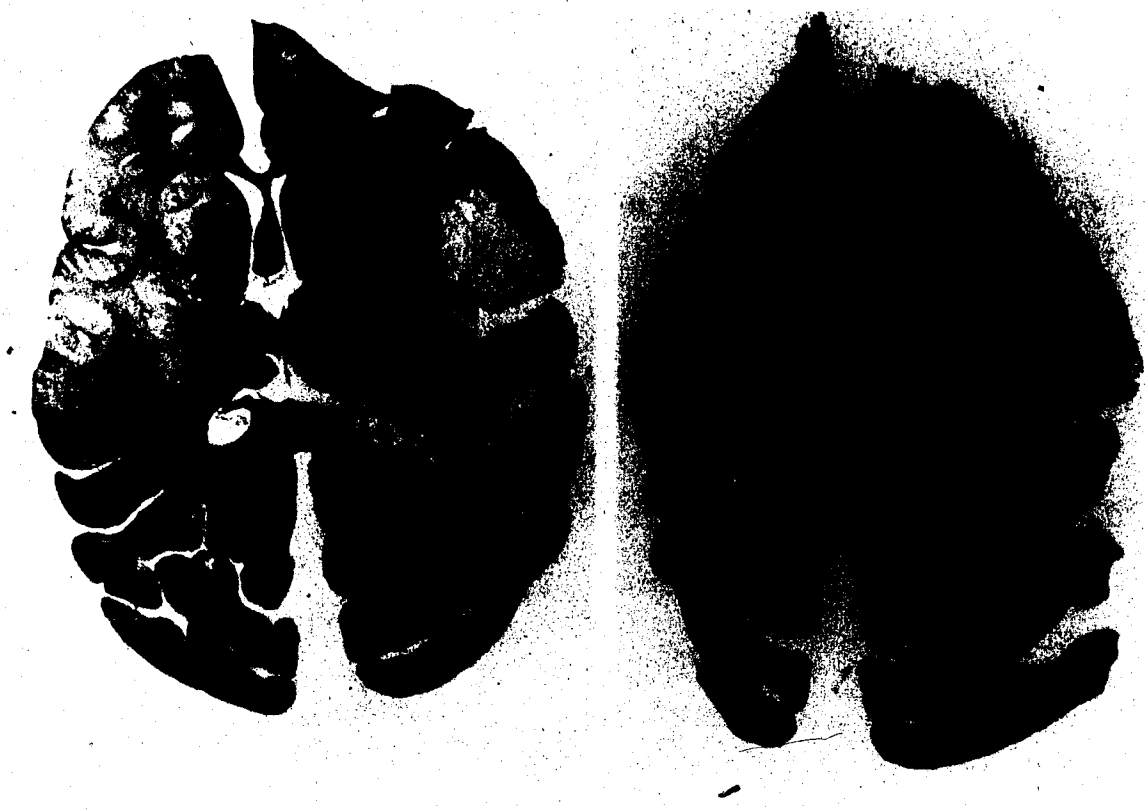


FIGURE 32

Light micrograph of the brain from the monkey that had a delayed ischemic deficit from severe vasospasm, showing a right-sided pale infarction of the territory of the middle cerebral artery.

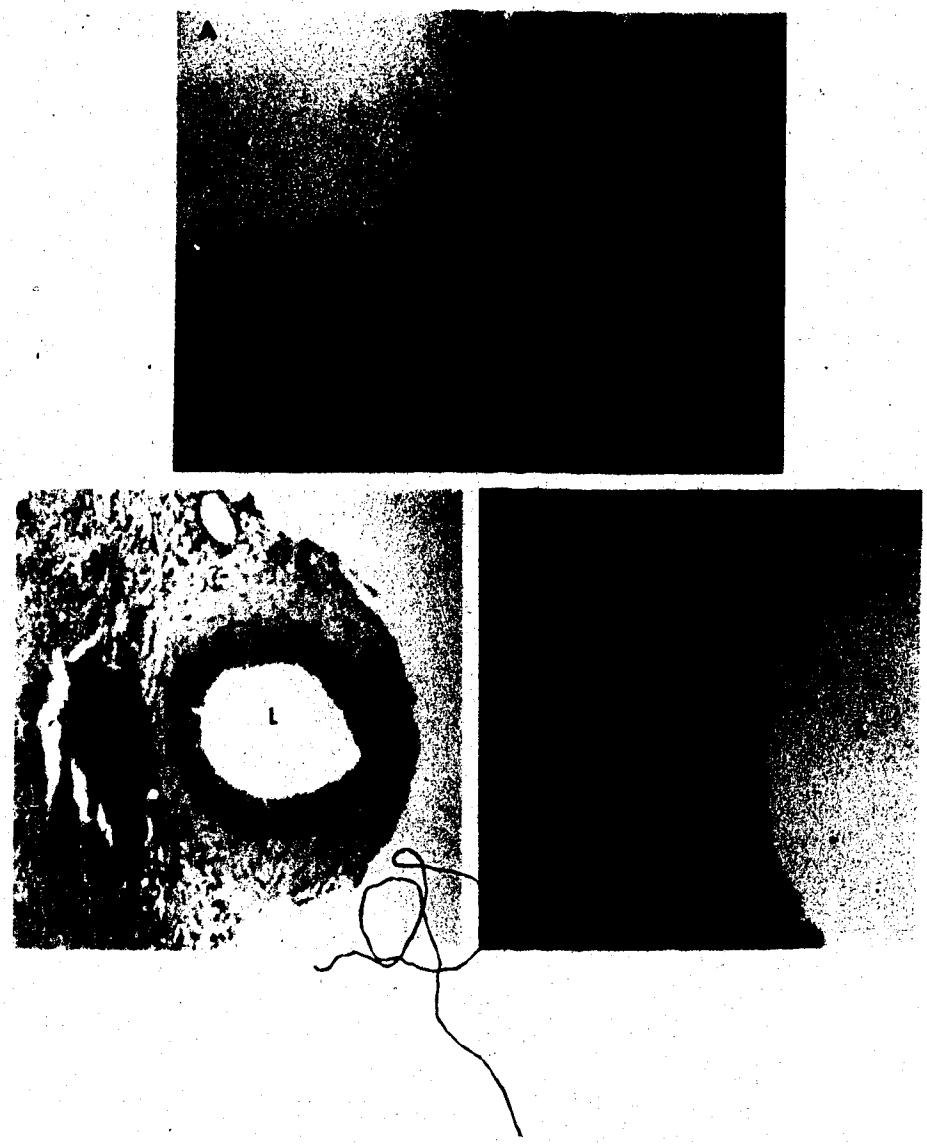


FIGURE 33

Light microscopy of cerebral vessels.

A: Basilar artery (control), showing normal endothelium, intima, smooth-muscle cells, and adventitia. X 40. B: Right middle cerebral artery partly surrounded by a hematoma (H). X 10. C: The same artery as in B but at higher magnification, showing marked undulation of the tunica intima and internal elastic lamina. X 40.

Scanning Electron Microscopy

In two of the control vessels (one MCA and one basilar artery) the endothelium was mildly convoluted. The other control specimens showed clearly defined, flat, uniform endothelium, with fusiform cells oriented longitudinally (Fig. 34).

In specimens in which angiography had demonstrated unequivocal narrowing or VSP, the endothelium was convoluted longitudinally. In some cases the endothelial cells appeared dragged in the direction of blood flow (fish-scale appearance) and the crests of the convolutions abutted one another (Fig. 35). Balloon-like protrusions, crater-like deficits, and areas of detached endothelium with platelets and leukocytes adherent were seen in some vessels in spasm (Fig. 34). The same pathologic changes were present, in various degrees of severity, in the endothelial surface of vessels from both placebo and nimodipine groups; in general there was good agreement with the angiographic appearances on the day of death (Table 6).

In most control arteries the surface of the tunica adventitia was irregular and was traversed by fibrous strands and what appeared to be nerve fibers. No vasa vasorum were seen, but numerous adventitial rounded openings, 10–35 μm in diameter, were observed. Some of these stomas appeared to be attached to or covered by strands of fibrous tissue (Figs. 36 & 37), whereas others had a clear-cut rim and were not related to other structures. The openings appeared continuous between the subarachnoid and adventitia (Fig. 38).

In specimens from the clot side in which almost all of the hematoma had been removed, the adventitia was very irregular, and it was covered with dense fibrous tissue and cellular debris, partly occluding the

TABLE 6

Degree* and Time Course of Vasospasm Apparent on Angiography, and Findings† on Scanning Electron Microscopy (SEM), in Clot-side Middle Cerebral Arteries from the 20 Monkeys Studied

Placebo Group				Nimodipine Group			
Monkey no.	VSP on Angiography Day 7	Day 14	SEM findings	Monkey no.	VSP on Angiography Day 7	Day 14	SEM findings
47	mild	mild	+	45	moderate	mild	+
50	moderate	mild	+	48	moderate	moderate	0
52	mild	mild	+, FS	49	mild	mild	+
53	severe	mild	+	57	moderate	mild	+
55	moderate	mild	++, FS	59	moderate	moderate	+
58	severe	mild	++, FS	62	severe	mild	+
61	moderate	mild	+	63	moderate	mild	+
64	moderate	moderate	++, FS	65	severe	mild	+, FS
66	moderate	mild	+	68	moderate	mild	+
67	moderate	mild	+				
69	moderate	mild	+				

* Degree of spasm (reduction in vessel caliber): mild, 11--30%; moderate, 30--50%; severe, 51-100% reduction.

† SEM findings: 0, normal endothelium; +, small endothelial convolutions; ++, medium-sized endothelial convolutions; FS, fish-scale appearance of endothelial cells.

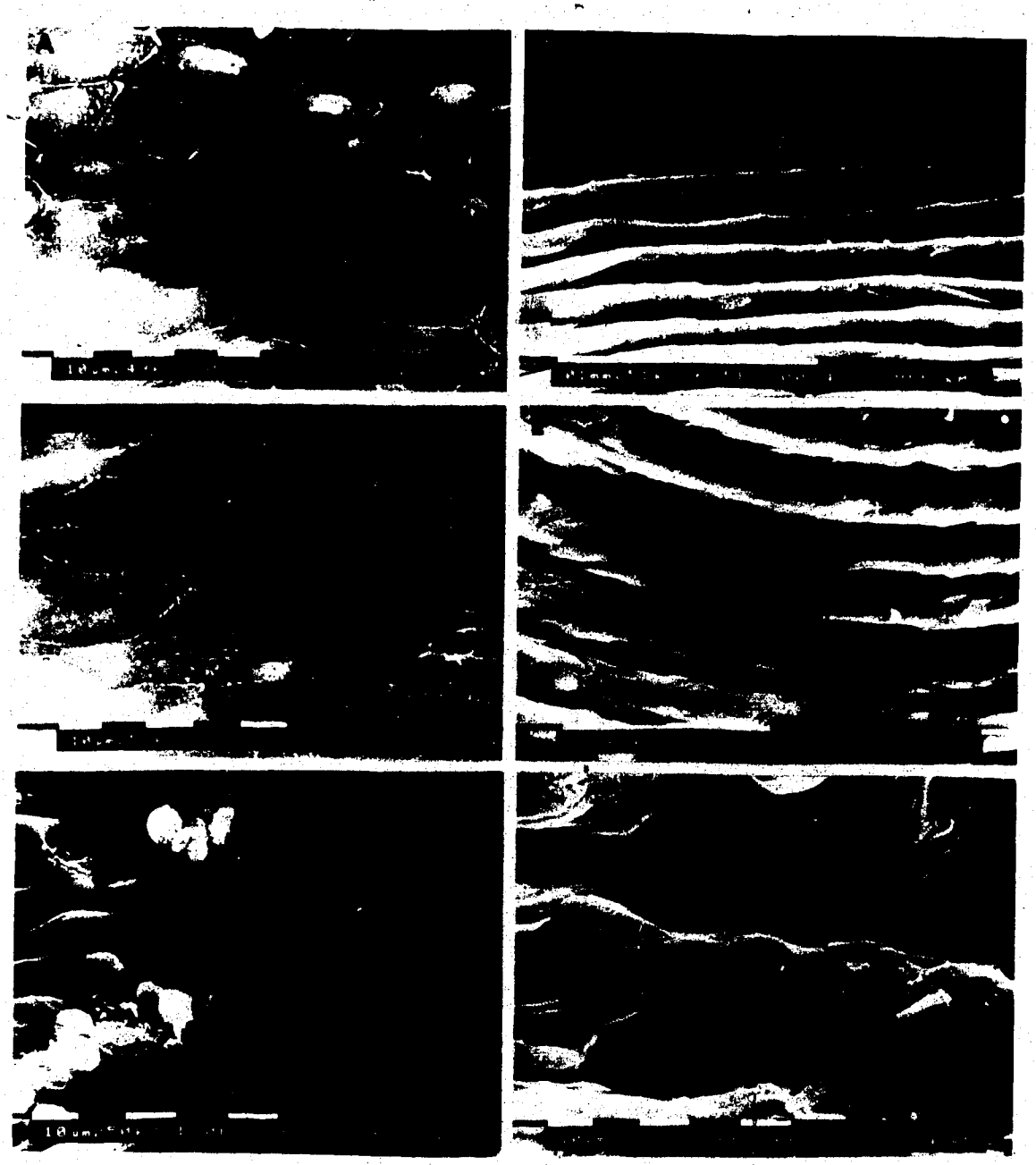
FIGURE 34

Scanning electron micrographs of the luminal surface of cerebral arteries of six monkeys fixed *in vivo* 14 days after subarachnoid hemorrhage. Magnification factors and scales are shown on each.

A & B: Control vessels. A, basilar artery; B, left middle cerebral artery (MCA) from the non-clot side. The endothelium appears normal.

C-F: Clot-side MCAs in which angiography had shown vasospasm, maximal on Day 7, abating or absent on Day 14. C: There are balloon-like protrusions and areas of detached endothelium with platelets and leukocytes adherent.

D-F: The vessel walls are folded into well-developed longitudinal convolutions. In D and F, the endothelium covering the convolutions has a fish-scale appearance.


A

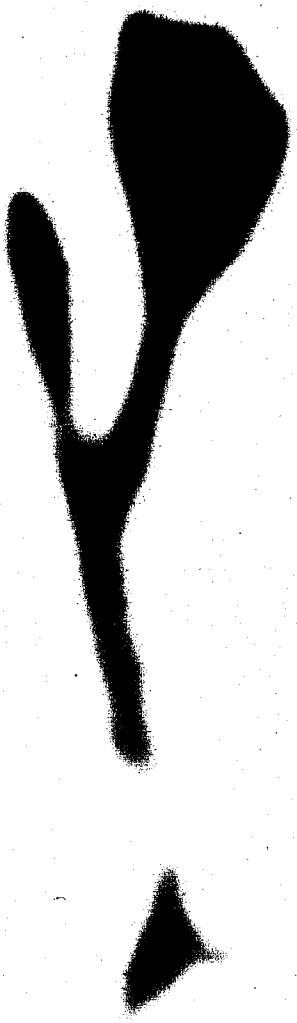


FIGURE 35

Scanning electron micrographs of middle cerebral arteries (MCA) of six monkeys, 14 days after subarachnoid hemorrhage. Magnification factors and scales are shown on each.

A-C: Control vessels (from the non-clot side). Adventitial and luminal surfaces are normal. Nerves are apparent in the adventitia.

D-F: Clot-side vessels in which angiography had shown vasospasm, maximal on Day 7, abating or absent on Day 14. The Endothelial surfaces are convoluted, and no nerves are visible in the adventitia.

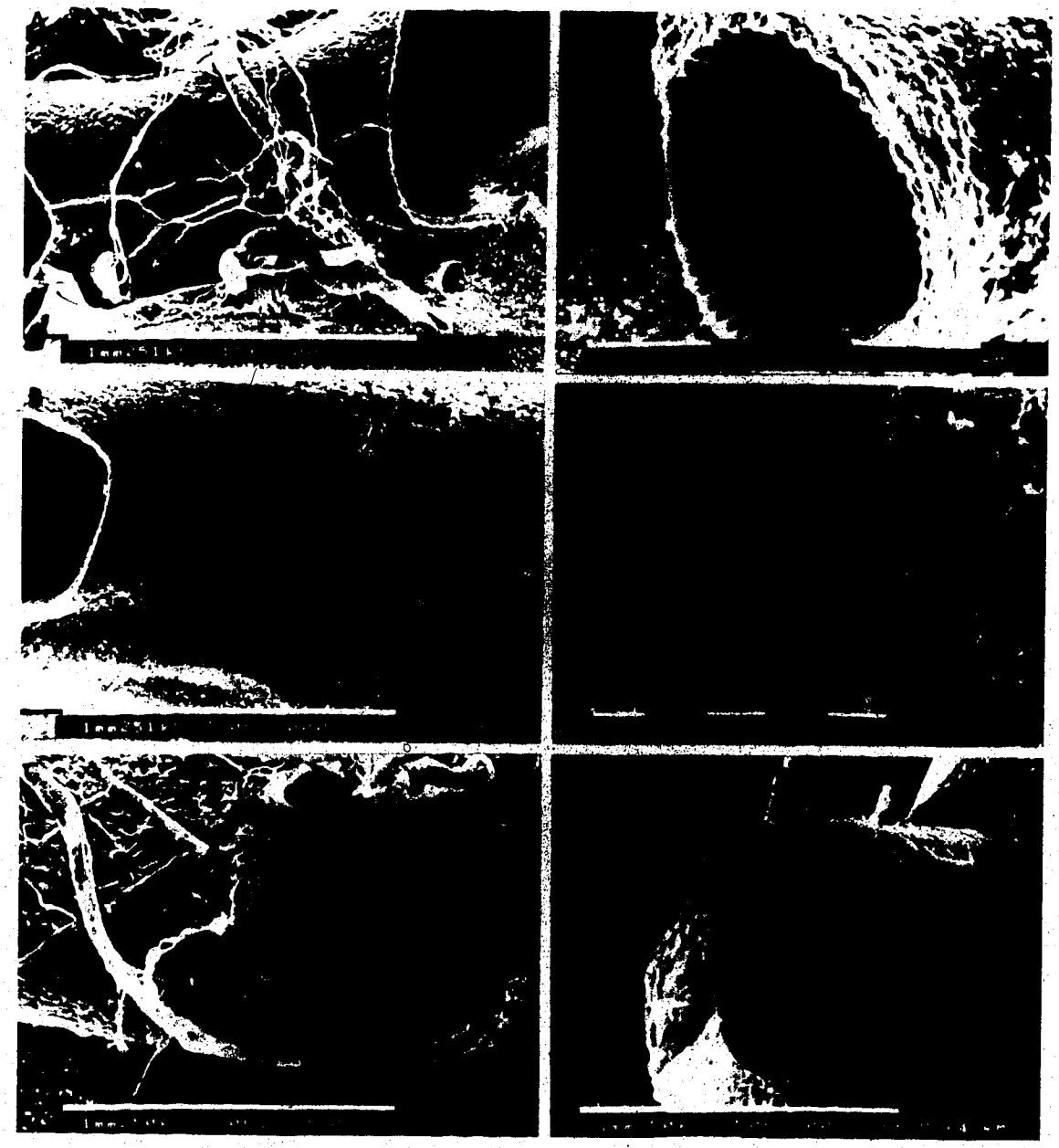
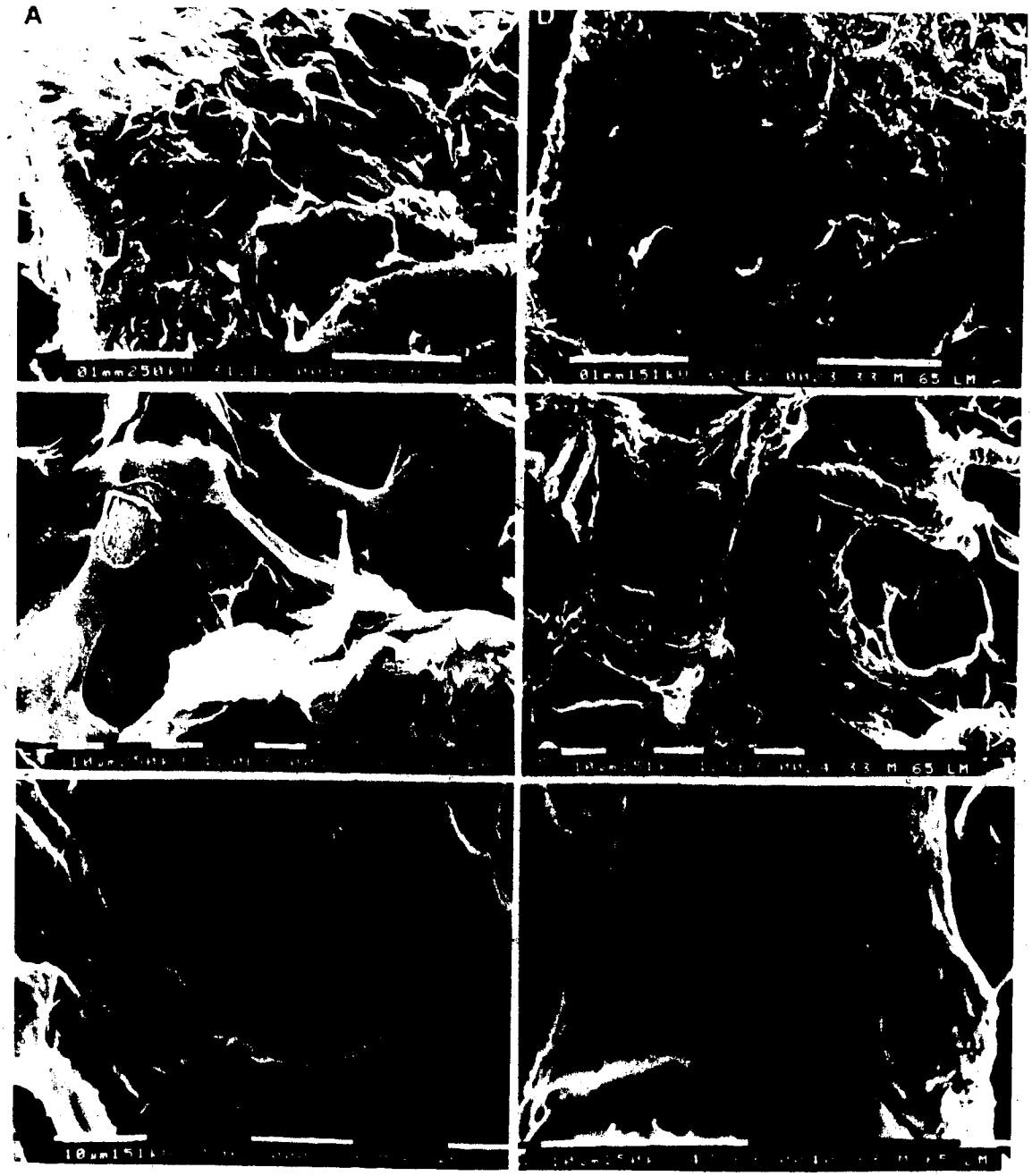


FIGURE 36

Scanning electron micrographs of two control (non-clot side) arteries. Magnification factors and scales are shown on each.. Left MCAs of monkey 67 (A-C) and monkey 65 (D-F), showing well-defined stomas on the surface of the tunica adventitia.


A

Scanning electron micrographs of the normal (vertical wide) middle cerebral arteries. Magnifications [illegible] are shown on each.

A & B: Stoma in adventitia [illegible] appears to be a fine bundle of collagen fiber.

C & D: Adventitial stoma partly covered by a [illegible] fibrous structure.

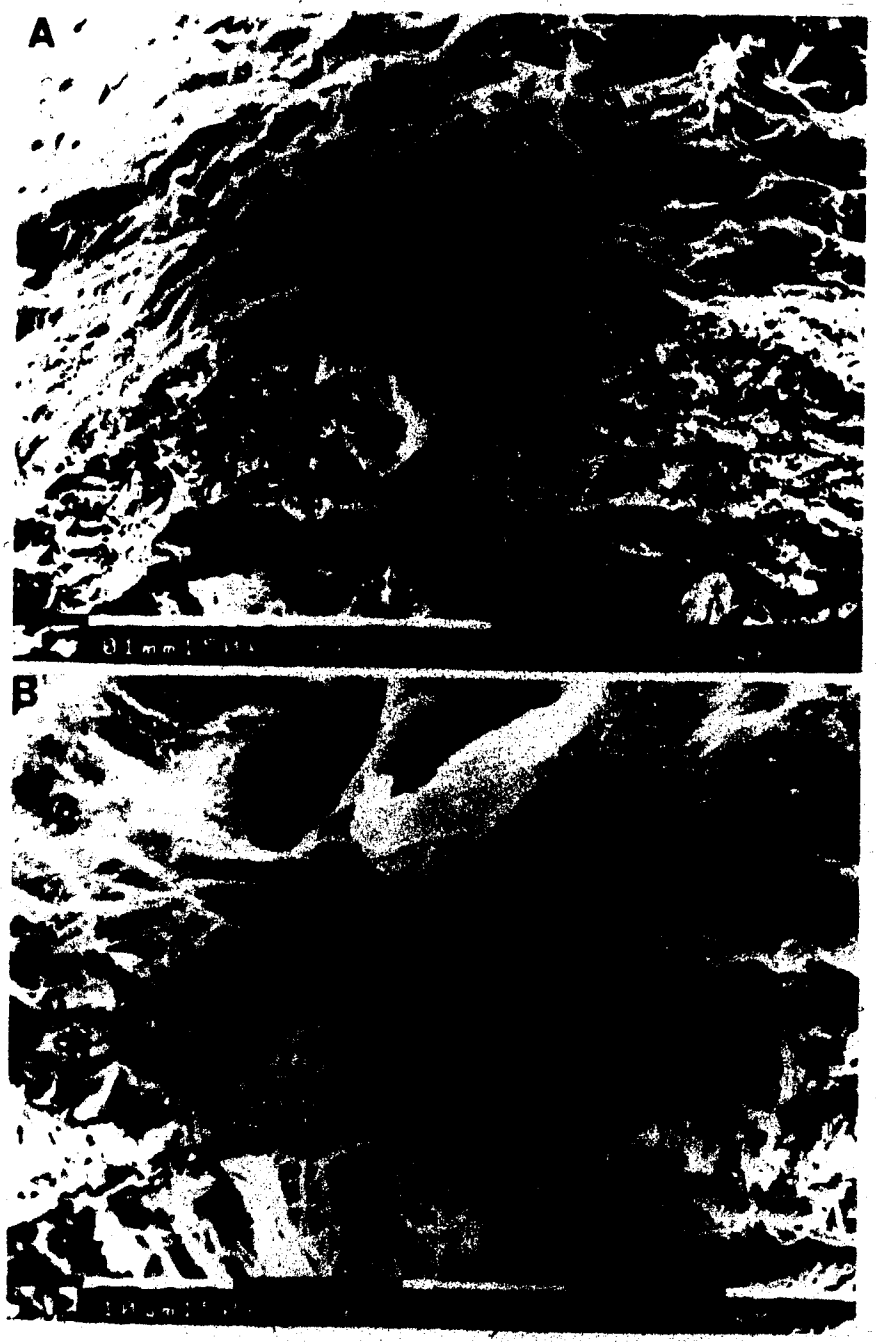


FIGURE 38

Scanning electron micrographs of the cut edge of a middle cerebral artery. Magnification factors and scales are shown on each.

A: Several stomas are visible on the surface of the tunica adventitia (arrows).

B: The stoma (arrow) appears to establish communication between the subarachnoid and subadventitial spaces (arrowheads).

stomas (Fig. 39). In arteries in which the hematoma was still attached to the wall, the adventitial surface was covered by a thick mesh of fibrous clot, with numerous deformed erythrocytes and a few leukocytes, hiding the stomas from view (Fig. 40).

The cut edge of control specimens was fairly thin and smooth, and all layers appeared normal (Figs. 41A & 42A). In arteries exposed to a hematoma, the cut edge of the wall appeared thickened and very irregular, the tunica intima was markedly corrugated, and the media and adventitia appeared fibrosed and disrupted (Figs. 41B & 42B). In some arteries from the clot side, deformed erythrocytes were seen within the wall (probably an artifact created during sectioning through the wall).

Transmission Electron Microscopy

In control vessels, normal myelinated and unmyelinated nerve-fiber bundles were observed. Despite diligent search, no nerve fibers were detected in arteries exposed to the hematoma and angiographically proved to be in spasm (Fig. 43).

No pathologic changes were seen in the control vessels except in the basilar artery of the monkey with delayed ischemia. This artery evidenced subendothelial edema, disruption of endothelial tight junctions, and fibrosis of the muscularis.

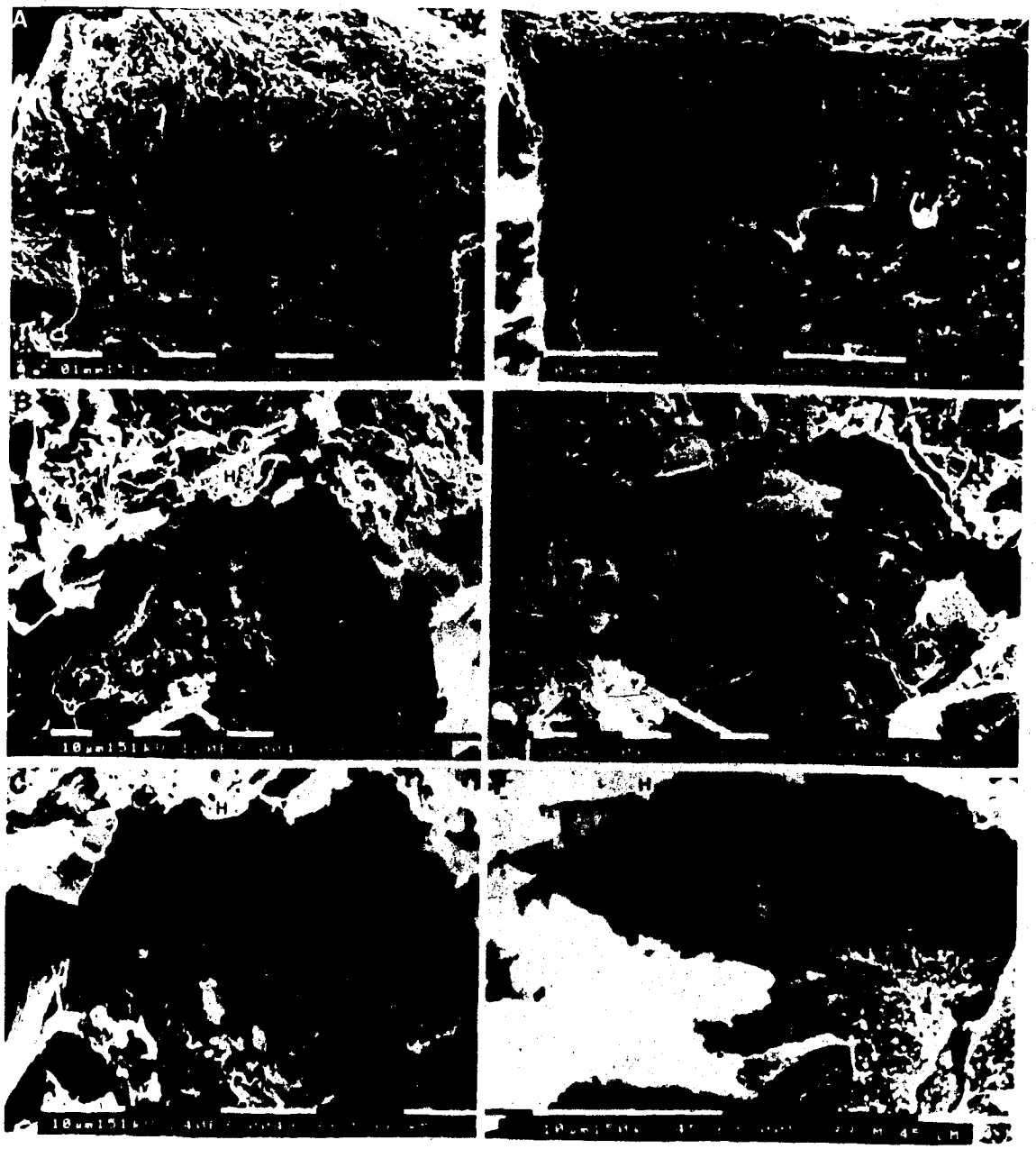
In all 10 clot-side MCAs examined with TEM, pathologic changes had occurred in all three layers; the changes were more pronounced in specimens from the placebo group. Changes in the intima included swelling and vacuolization of endothelial cells, cellular rounding or plumping, and disruption of tight junctions. In some specimens the subendothelium was swollen, and in others it had proliferated or, more commonly, its

FIGURE 39

Scanning electron micrographs of two middle cerebral arteries that had been exposed to a hematoma for 14 days. Magnification factors and scales are shown on each.

A-C: One vessel at increasing magnifications, showing a fibrous hematoma (H) partly covering the tunica adventitia and obstructing the lumen of a stoma (arrow).

D-F: Another artery at increasing magnifications, showing a dense hematoma (H) on the adventitia, partly obstructing a stoma with deformed erythrocytes (E).


A
B
C

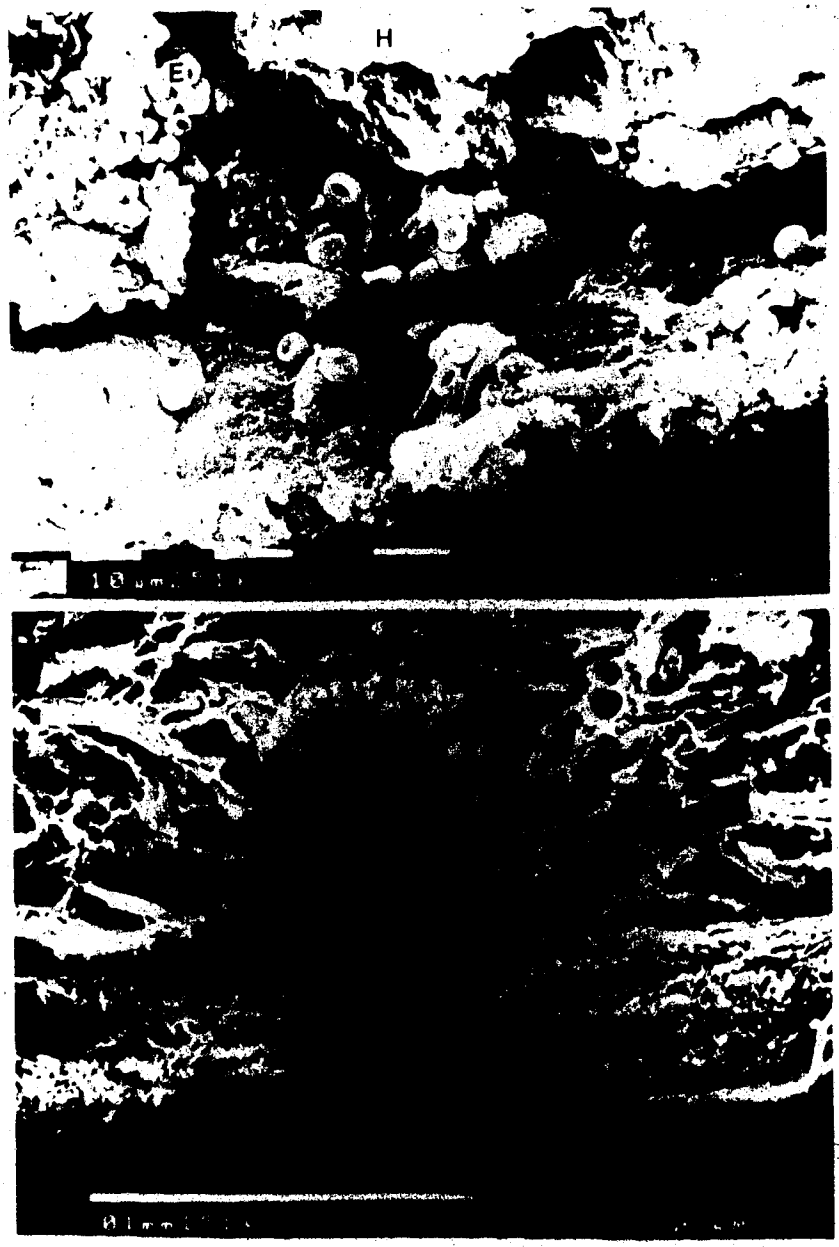


FIGURE 40

Scanning electron micrographs of two middle cerebral arteries that had been exposed to a hematoma for 14 days.

A: The tunica adventitia (TA) is covered by a dense hematoma (H) containing many deformed erythrocytes (E) and a few leukocytes (arrows). No stomas are visible. B: A thick fibrous multi-layered hematoma (H) covers the tunica adventitia. EL, elastic lamina; L, lumen.

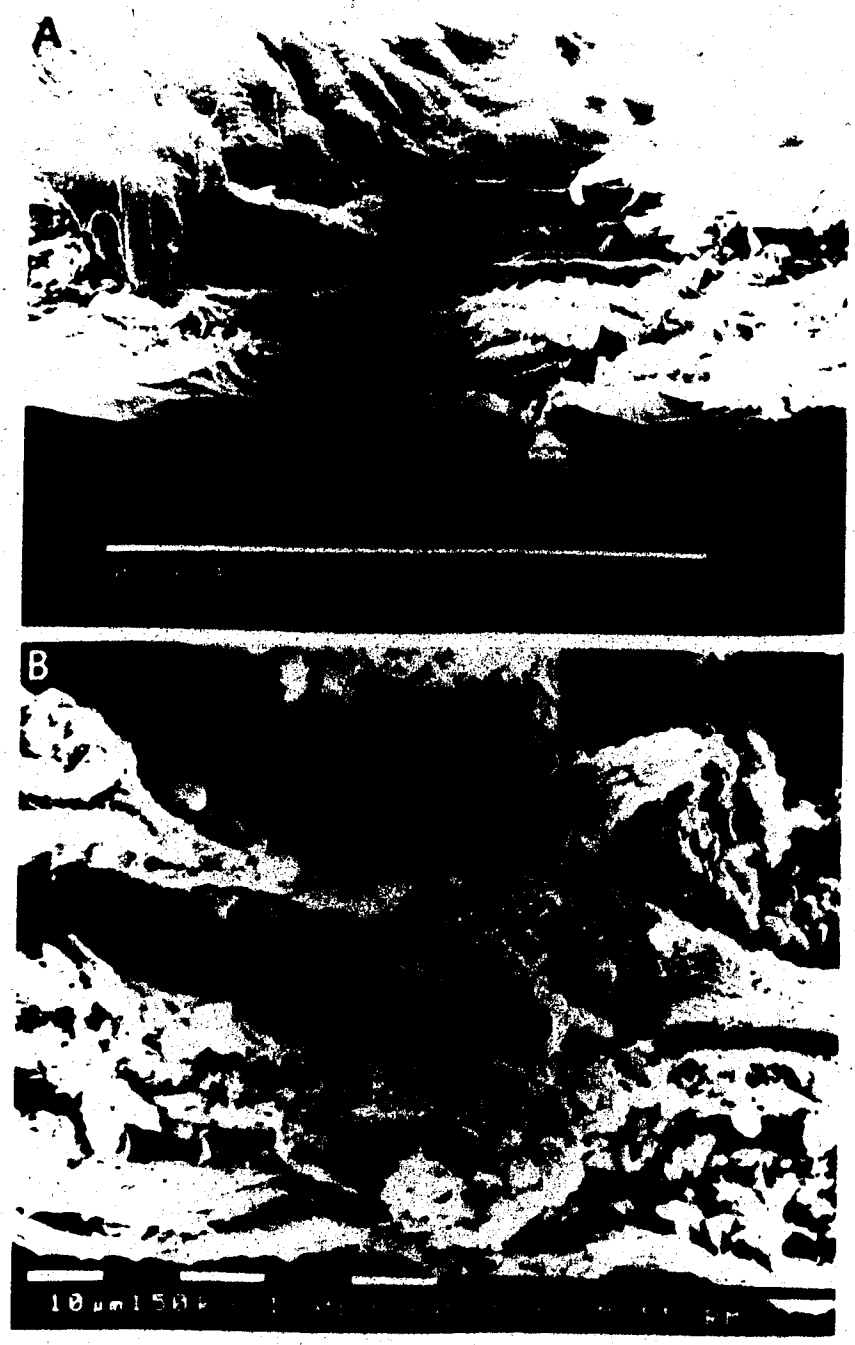


FIGURE 41

Scanning electron micrographs of the cut edge of cerebral arteries. A: Control basilar artery. B: Thick-walled middle cerebral artery that had been exposed to a hematoma for 14 days and in which angiography had shown severe VSP on Day 7. Note the severely disrupted vessel wall, numerous erythrocytes between layers are probably an artifact sectioning.

FIGURE 42

Scanning electron micrographs of the cut edge of two arteries. A: Control basilar artery, showing a thin, smooth, apparently normal vessel wall. B: Middle cerebral artery that had been exposed to a blood clot for 14 days, showing thickened vessel wall and deep corrugations of the tunica intima. NOTE: A, X 68; B, X 287.

FIGURE 43

Transmission electron micrographs of the tunica adventitia of two middle cerebral arteries.

A: Control vessels (non-clot side) showing normal myelinated (MF) and unmyelineated (UF) nerve-fiber bundles. X 12,600.

B: Clot-side specimen showing marked fibrosis and absence of nerve-fiber bundles. X 14,000.

smooth-muscle cells had migrated inward (Fig. 44). In many specimens, the internal elastic lamina bore numerous corrugations and was thinned in some areas and disrupted in others (Figs. 44 and 45). In the media, some smooth-muscle cells appeared in spasm; others had intracytoplasmic vacuoles of various sizes that appeared empty or contained fine amorphous material, and some contained numerous lysosome-like dense bodies. In general, the media evidenced swelling, abundant fibrous tissues in the intracellular space, and in some sections there were frankly pyknotic muscle cells (Fig. 46). The adventitia was generally thickened by fibrous material, and in some vessels the hematoma was still attached to it (Fig. 47).

Comment

Cerebral vasospasm is the radiological appearance of sustained arterial narrowing developing as a complication of SAH due to ruptured aneurysm (134-136). The degree of narrowing depends upon the volume of blood in the subarachnoid space (35,135), but it is not entirely clear why VSP lasts for several days to weeks.

It has been postulated that this luminal narrowing is not due solely to contraction of smooth-muscle cells, and that the major contributors are histologic changes (124,137,138) such as cellulofibrous thickening of the intima, subendothelial proliferation, and organization of luminal thrombus (134-138). In two studies (136,138) the magnitude of structural and morphologic changes in the vessel walls was found to relate to time after SAH; early changes ($\lesssim$ 3 weeks), the most prominent, were swelling of the endothelium and areas of its displacement from

FIGURE 44

Transmission electron micrographs of the tunica intima of clot-side middle cerebral arteries (L = lumen) of six monkeys, 14 days after subarachnoid hemorrhage. Angiography had shown vasospasm of these vessels, maximal on Day 7, abating or absent on Day 14.

A: Smooth-muscle cells (SM) have migrated to the subendothelium. X8250. B: The subendothelium has proliferated and is swollen. X8250. C: Smooth[illegible]ells have migrated to the subendothelium and the elas[illegible]is thin. The media (M) is fibrosed and it contains a pa[illegible]ell with an intracytoplasmic vacuole (V), indi[illegible]crosis. X6720. D: The endothelial cells appear plu[illegible]he endothelium (E) and the basement membrane (BM) are[illegible] F: The endothelium is swollen and contains va[illegible]unction has been disrupted (arrow). X22,000.

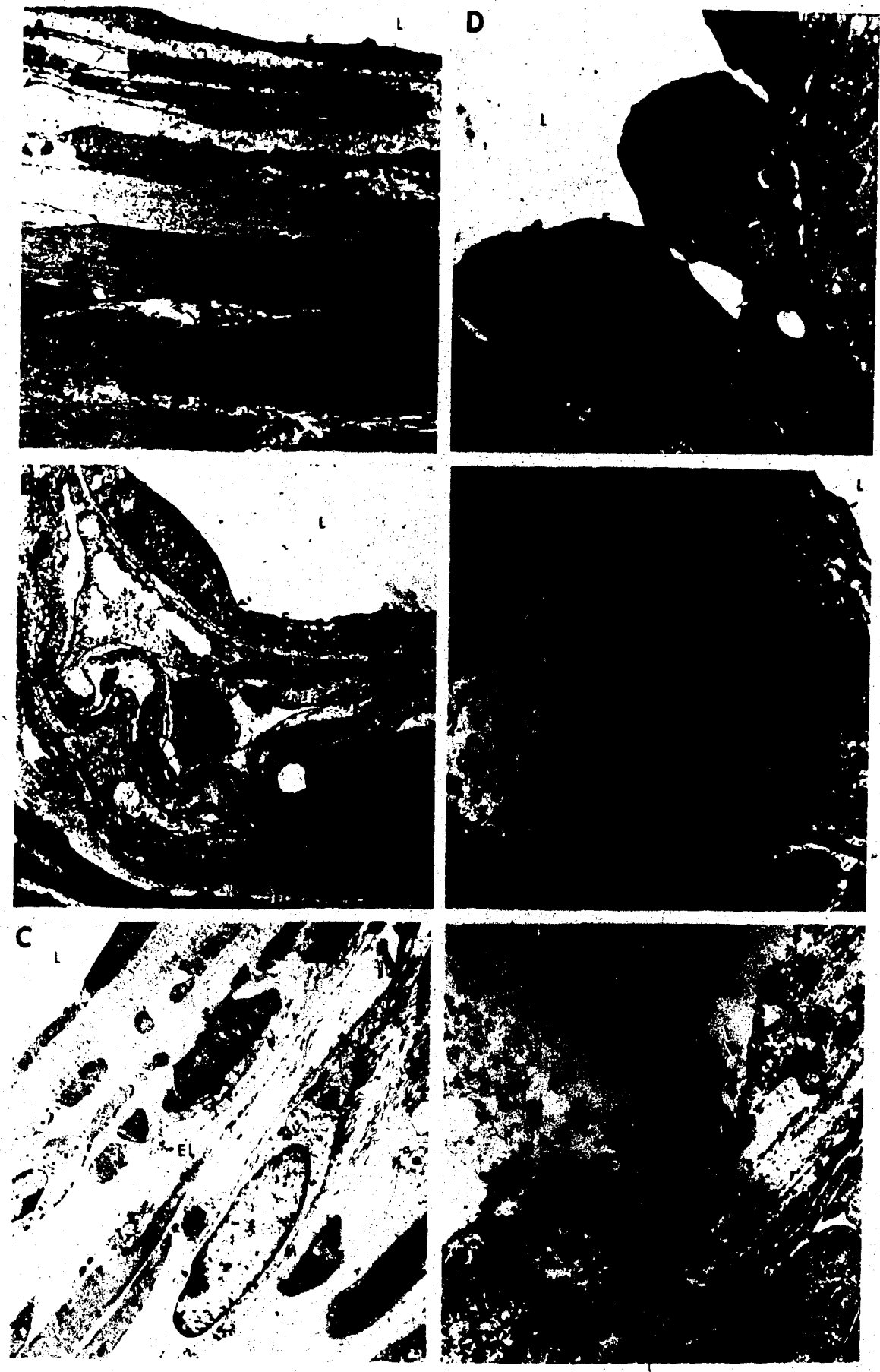

L
D
L
L
L
C
L
EL

FIGURE 45

Transmission electron micrographs of the wall of middle cerebral arteries (L = lumen) of four monkeys, 14 days after subarachnoid hemorrhage.

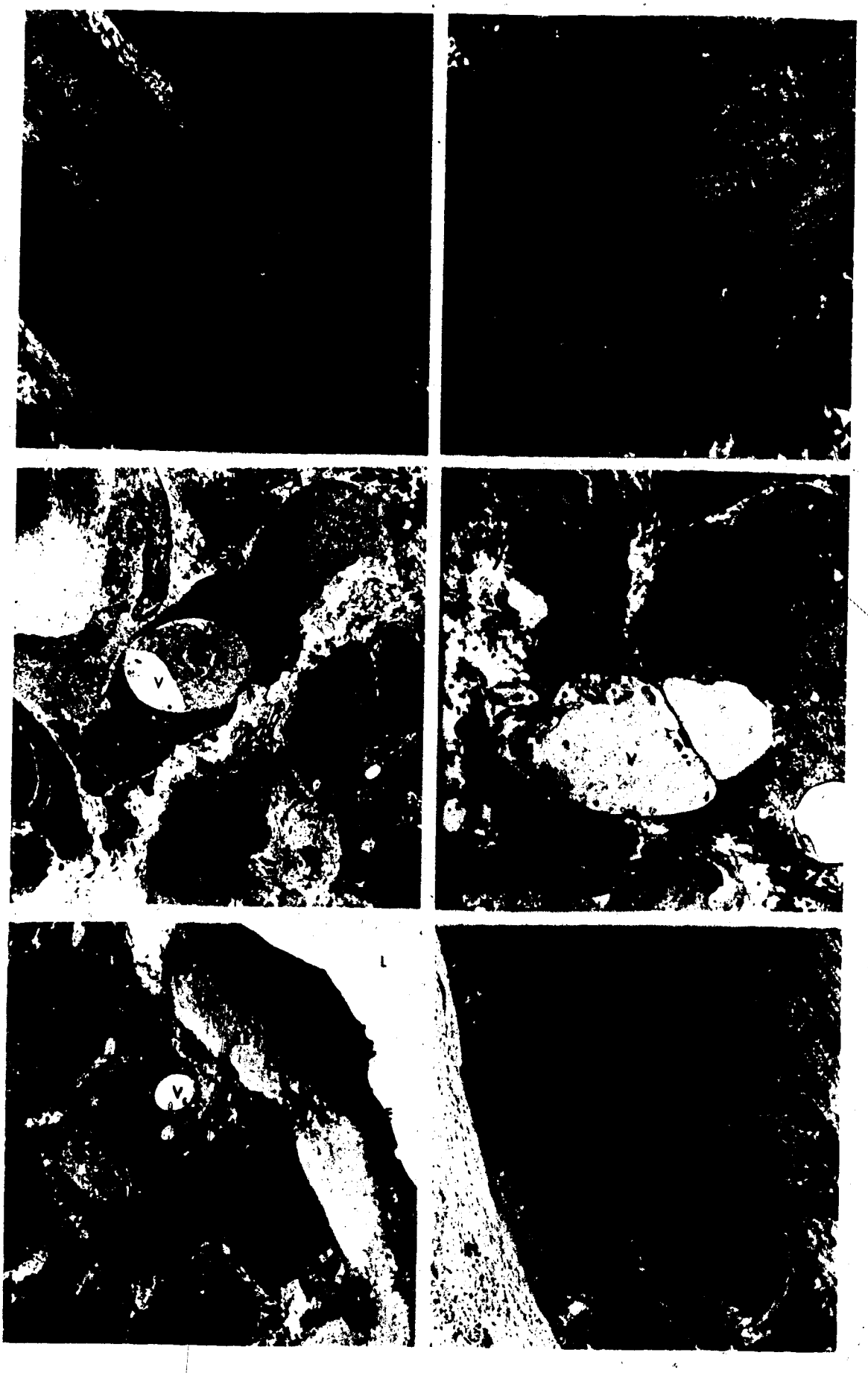
A: Control vessel (from the non-clot side). X6500. B—D: Angiography had shown vasospasm of these vessels, maximal on Day 7, abating or absent on Day 14. B: The intima on the internal elastic lamina (EL) is mildly convoluted. The endothelium (E) contains vacuoles and the muscularis (M) is swollen. X5160. C: The intima and internal elastic lamina are moderately convoluted. The endothelium is disrupted, and the endothelial and smooth-muscle cells are very swollen. X7740. D: The intima and internal elastic lamina are severely convoluted. The endothelial cells are rounded, and the media is swollen and markedly contracted. X5100.


A
L
C
L
E
B
L
E
EL
D
L
M

FIGURE 46

Transmission electron micrographs of smooth muscle of middle cerebral arteries (L = lumen), 14 days after induction of subarachnoid hemorrhage.

A: Control vessel (from the non-clot side), showing normal smooth muscle. X9900. B--F: Clot-side vessels in which angiography had shown vasospasm, maximal on Day 7, abating or absent on Day 14. B: The muscularis contains vacuoles (V) and is severely fibrosed. X5500. C: The smooth-muscle cell at left center has a pyknotic nucleus (arrow) and its cytoplasm contains vacuoles. X14,000. D: Contracted muscle cell nucleus. X22,000. E: The media contains vacuoles and is fibrosed. X10,500. F: The smooth-muscle cell contains condensed lysozomes (arrows). X17,500. EL, elastic lamina; E, endothelium.


V
V
V
L

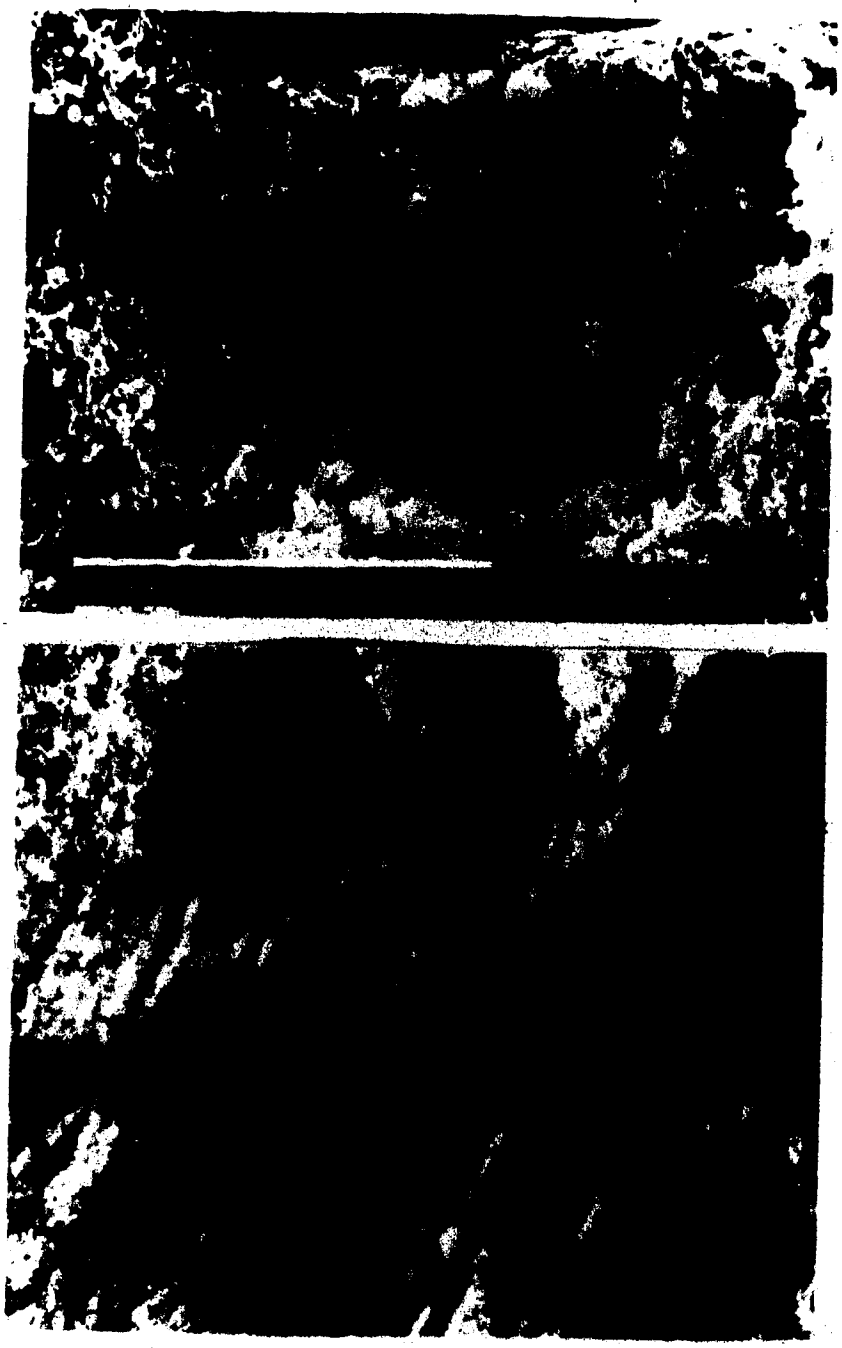


FIGURE 47

Electron micrographs of a hematoma 14 days after placement within the subarachnoid space. A: Scanning micrograph, showing deformed erythrocytes and abundant collagenous tissue. X625. B: Transmission micrograph, showing three macrophages with intracytoplasmic vacuoles and lysomes and a plasma cell (PC). X3000.

basement membrane, migration of smooth-muscle cells to the subendothelium, fragmentation and corrugation of the internal elastica, swelling and necrosis of smooth-muscle cells, and adventitial inflammatory reaction consisting of lymphocytes, plasma cells, and macrophages. Faleiro et al. (139), who found increased numbers of mast cells in the muscularis of arteries close to ruptured aneurysms, reported that patients whose arterial media contained more mast cells had less spasm and survived longer after SAH. However, the small number of their patients precluded any firm conclusions.

Eldevik et al. (140) could not confirm any morphologic changes ascribed to spasm. They studied nine patients who died from SAH and cerebral infarction from VSP, and 10 dogs weighing 11-20 kg in which an SAH was created by one or more 5-ml injections of blood into the cisterna magna. The volume of blood these workers used for inducing SAH was relatively small (in the present study an average of 7 ml of blood was used for clot placement in the 3-kg monkeys), which may explain the lack of morphologic change in the vessels in spasm: clot may not have formed in the vessels studied. It is severe chronic VSP that causes brain ischemia and is associated with morphologic changes in the arterial wall (134-136). Also, the posterior circulation is less densely innervated than its anterior counterpart (141).

Others have observed morphologic and structural changes in vessels in spasm after experimental SAH (110,142-147). After creation of SAH by intracisternal injection of 3 ml of blood or by puncture of the intradural internal carotid artery in monkeys (142), chronic VSP (> 7 days) developed only after the latter maneuver, suggesting that only this method had created a large SAH. In these cases and in another

study (110), electron microscopy showed vacuolization and fibrosis of the media and some areas of myonecrosis, denser than normal elastic lamina, and rounding of endothelial cells. Pathologic post-SAH changes in cerebral arteries of dogs (145,146) included necrosis, vacuolization and widening of the medial interstitial space, vacuoles and dense bodies in endothelial cells, detachment of the endothelium and thickening of the intima. In studies with a canine model (144,147) similar to that used by Eldevik et al. (140), changes observed in the arterial wall 30 days post-SAH consisted of subendothelial edema and accumulation of debris in the myointimal region, corrugation of the intima and elastic lamina, degeneration of smooth-muscle cells and accumulation of membrane-bound vesicles and collagen fibers within the media, and adventitial inflammatory reaction associated with VSP. The authors (144,142) suggested that adventitial more than intimal or medial changes may be concerned in the pathogenesis of VSP.

In an acute-state rat model (148) SEM of the luminal surface of spastic vessels revealed prominent convolutions not seen in control vessels and not corresponding to the changes observed on TEM. In another SEM study of the cerebral arterial endothelium, after induction of a 2-ml SAH in cats (149), mild longitudinal convolutions of the luminal surface of the basilar artery correlated with angiographically demonstrated VSP; the convolutions, which were not seen in control vessels, persisted after fixation at physiological pressure. Intimal folds can be avoided by fixation with intra-arterial perfusion at pressures above 80 mm Hg (150,151). However, luminal convolutions in normal carotid arteries of rabbits have been reported after perfusion of the animals at controlled pressure (152); probably the corrugations were

postmortem artifacts, as the rabbits were killed before the chest was opened and the thoracic aorta cannulated for infusion of the fixative.

In the present study (C), as in most previous investigations, pathologic changes in the arterial wall were associated with VSP. However, no mast cells were detected in the media, inflammatory reaction in the adventitia was minimal, there were no intraluminal thrombi in vessels in spasm and no erythrocytes or debris from them were seen in the media or intima. These findings suggest that structural and morphologic changes in vessel walls may not be a major cause of their narrowing: VSP was maximal on Day 7, and the arteries were fixed on Day 14 -- when angiography showed the lumina to be almost normal. Cerebral VSP is more likely an unphysiologic (sustained) contraction of smooth-muscle cells in response to blood in the subarachnoid space, and not the postulated narrowing resulting from histologic changes (134,137,138). These changes are probably induced by the rapid transit of blood through a narrowed arterial segment; in the present study, the endothelial cells appeared to have been dragged in the direction of blood flow. Furthermore, a high rate of blood flow might detach the damaged endothelial cells from the basement membrane, as was apparent in some of the tissues examined.

As it seems that the structural and morphologic changes in the vessel wall are the result of sustained spasm, and not the cause of the narrowing seen angiographically, therapy of VSP should be directed at preventing smooth-muscle contraction. In this study, nimodipine, in the dosage used, did not prevent morphologic change and had no significant effect on its degree.

CHAPTER THREE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

I. DISCUSSION

Cerebral aneurysms usually become manifest as SAH, and SAH from a ruptured aneurysm is a devastating disease: the prevalence of death or severe disability is about 70% (179) and the highest incidence occurs during the most productive years of life (fourth through sixth decades). Current therapy is of no benefit because of the inability to reverse severe neuronal damage. In the United Kingdom, aneurysmal SAH is responsible for 4000 deaths each year (251). In the U.S.A. (179), 36% of patients with SAH (10,000/28,000) die or remain severely disabled as a result of the initial insult; of the 18,000 patients (64%) potentially available for treatment, 9000 (32%) die from or remain severely disabled as a result of VSP, rebleeding, and/or medical and surgical complications, leaving only 9000 (32%) functioning survivors. Clearly, these figures are alarming and unacceptable; they should stimulate intensive research, directed not only to treat the SAH syndrome, but, more importantly, to prevent it.

At present, most research is directed to the study of VSP, rebleeding, and hydrocephalus, and their surgical and medical management. To devise means of preventing SAH requires emphasis on the development of noninvasive techniques with high sensitivity and specificity that would aid in the detection of asymptomatic aneurysms. With an electronic stethoscope applied on the closed eyelid, Sekhar and Wasserman (252)

recorded spikes and/or turbulent bruits in 8 of 11 patients with proven aneurysms of the carotid (cavernous or ophthalmic) and anterior communicating arteries, averaging 15 mm in diameter. The three aneurysms not detected were at the basilar bifurcation (15 mm diam) or in the carotid communicating (5 mm) or middle cerebral (7 mm) artery. Thus, the proximity of the aneurysm to the orbit and the duration of its dome anteriorly probably facilitated detection. These investigators also identified broad-band bruits (representing turbulent flow) in 4 patients with arteriovenous malformations and in 2 with carotid-cavernous fistulas, but no spikes or bruits in 7 control patients with no vascular disease or in 3 with brain tumors.

Aneurysms are said to emit sounds at a particular frequency (spikes) and, in some cases, over a range of frequencies (bruits) also, whereas arteriovenous malformations produce only bruits (259, 260). If signals from intracranial aneurysms could be quantified accurately, this would provide a noninvasive method for screening populations at high risk for SAH. Patients with positive findings could be further investigated; for example, initially with techniques such as enhanced CT, and tissue biopsy and biochemical assay for type-III collagen (251). If still positive, cerebral angiography could be performed, and if this revealed aneurysm the sac could be clipped electively. Meanwhile, the search could continue, to identify causes of aneurysm, including heritable factors.

Concurrently, we should continue attempts to treat cerebral VSP and other complications of the SAH syndrome. In general, in experimental animals and in humans, therapy for chronic VSP (reversal of the constriction) has been unsuccessful. This is due partly to our ignorance

of this condition: the great majority of investigations, including previous studies from this laboratory, have been directed to the study of acute spasm rather than chronic VSP (47,106-109,111,162,276). The early phase of vasospasm, which has not been proven in humans, may have little significance and only minor consequences; almost invariably it is chronic severe vasospasm that causes neurological deficits of delayed onset (196-198,261).

In the few studies of chronic VSP, SAH has been created in various species by methods including puncture of a vessel (116,263), avulsion of an artery of the circle of Willis (253,264), injection(s) of blood into the subarachnoid space (144,147,247,248,254,255), topical application of incubated blood or other vasogenic agents (234,246), by combining several procedures (squeeze and puncture of a vessel and SAH) (115), and by shunting blood from a systemic artery into the subarachnoid space (265). In most such studies, however, VSP has been only mild to moderate and of short duration (1 to 4 days), and any neurologic deficit that did develop occurred immediately after SAH and not a few days afterward (Days 4 to 12) as in humans (112-118,254,266).

Varsos et al. (147) studied VSP in a "double hemorrhage" canine model, injecting 4 ml of blood into the cisterna magna and repeating this on Day 2. Some of the dogs were drowsy and had a staggering gait on Day 5, but it is not clear whether this deficit developed on that day or was present immediately after the SAH. Hemiparesis did not develop in any of the dogs. Using a similar model, Chyatte et al. (254) observed the onset of neurological deterioration (hemiparesis, ataxia) immediately after the first injection of blood; this did not progress during the 8-day study and there was no deficit of delayed onset.

In the present study, the author's success in reproducing human-type chronic VSP in monkeys stems mainly from the volume of blood used to induce SAH: an average of 7 ml of blood was used for clot placement in the 3-kg monkeys, compared with 8 ml injected into the subarachnoid space of 16- to 24-kg dogs (147) and 13- to 32-kg dogs (254). Furthermore, in the present study the clot was placed in solid form against the circle of Willis, allowing direct contact of a high concentration of vasogenic/toxic substances with the arteries, especially the middle cerebral artery; this stopped CSF circulation around the spastic artery and may have blocked the passage of nimodipine into the vessel wall, preventing contact with smooth-muscle receptors.

The present experimental model appears to be the first in which a delayed ischemic deficit has been caused by VSP alone and documented both radiographically and on pathological examination. Although delayed neurological deficit occurred in only one monkey, significant vasospasm (31-100% reduction in vessel caliber) was observed in 86% of the animals. Collateral circulation was maximal when the spasm was most severe, perhaps preventing ischemia in the distribution of the affected vessel. Ischemic deficit from VSP after SAH in young patients is extremely rare (233), which may explain at least partly the very low incidence of neurological deficit in the young, healthy, female monkeys studied. Older subjects with hypertension, atherosclerosis, and reduced collateral circulation have a greater susceptibility to ischemia from VSP.

Surgical manipulation of the cerebral arteries during clot placement probably did not contribute to the development of VSP or ischemic deficit (unfortunately, sham procedures were not performed to exclude

this possibility). Studies in monkeys have shown that acute manipulation (clipping or occlusion) of the cerebral arteries may result in a severe neurological deficit acutely but not several days afterward (287-288). Embolic occlusion of the proximal segment of the MCA in conscious monkeys resulted in flaccid hemiplegia of the contralateral side within 3 min in all 25 of the animals (287). In anesthetized monkeys, persistent hemiparesis developed immediately after occlusion of the MCA with a Mayfield aneurysm clip (289). In another model, clipping of the MCA with a Scoville aneurysm clip for 4-24 hr permanently caused hemiparesis or hemiplegia and some of the animals died, whereas clipping for 1-2 hr caused at most only mild neurologic deficits and minimal histologic abnormality. In the present study, microsurgical techniques were used and great care was taken to dissect the arachnoid membrane away from the cerebral arteries; microsurgical manipulation lasted for 15-30 min and was followed by VSP only after clot placement (Fig. 6). Furthermore, in another study with the same model in this laboratory (318), angiography on Day 7 showed no VSP and neither acute nor delayed neurological deterioration occurred in two monkeys subjected to a sham operation.

Chronic VSP probably results from a cascade of events initiated by lysis of erythrocytes and release of Hb. During spontaneous oxidation of oxyHb to metHb, free radicals are produced and may damage the endothelium, compromising the synthesis of prostacyclin. Hb is a vasoconstrictor; in addition, it is a cofactor of cyclo-oxygenase and thus facilitates the production of vasoconstrictor prostaglandins (165). Thromboxane A_2 and prostaglandins-$F_{2\alpha}$ and E_2 may induce severe spasm in

the absence of prostacyclin, and lipid peroxides and other vasogenic substances (161,162,256-258) may exacerbate the spasm.

The time course of VSP matches that of clot lysis and the subsequent release of vasoactive substances (125,126,163) that may diffuse into the arterial wall through the stomas and rete pathways in the tunica adventitia (236). Zervas et al. (236) suggested that the cerebral arteries, lacking vasa vasorum (236, 295), are nourished through a labyrinthine structure or pathway in the tunica adventitia; this would allow CSF to diffuse from the subarachnoid space into the tunica media and elastic lamina and, through the latter's fenestrations (Fig. 2), to the endothelium. These authors demonstrated patency of these pathways in cats: horseradish peroxidase injected into the subarachnoid space migrated into the tunica media and subendothelium via the rete vasorum and stomas in the adventitia. Zervas et al. (236) questioned whether the stomas were artifacts of processing. In the present study, similar stomas in normal arteries (from the non-clot side) were clearly defined structures (Fig. 36-38); in clot-side arteries from which the hematoma had not been removed, the tunica adventitia was covered with remnants of the clot and no stomas were visible (Fig. 40).

It seems that not all species have adventitial stomas in the cerebral arteries. Liszczak et al. (267), studying SAH in rabbits, found none in the basilar artery, and VSP and pathological changes did not occur in any of the 27 specimens examined; probably, vasogenic and toxic substances could not enter the arterial wall. Nonaka et al. (234), studying the cerebral arteries of dogs 3 days after the injection of Hb into the subarachnoid space, stated that the Hb entered the tunicas

adventitia and media; prolonged VSP occurred, but this was released by topical applications of haptoglobin (which binds to Hb and forms a stable compound) in 14 of the 17 dogs with SAH thus treated.

A thick hematoma surrounding the cerebral artery plays three major roles in the development of chronic VSP, and may also be responsible for some of the pathological changes in the arterial wall. First, it is the source of vasogenic/toxic substances that act directly on vessel wall and its receptors to induce spasm and pathological changes; second, by blocking CSF circulation it probably disturbs normal nutrition and the efflux of waste products from the vessel wall, thus further damaging the tunicas media and adventitia; and third, by barring potential vasodilator agents from the smooth-muscle receptor (Fig. 40), the hematoma is probably an important physical factor responsible for the failure of therapy and/or prevention of VSP. This may explain the clinical efficacy of agents that can release smooth-muscle contraction when applied topically *in vivo* or tested *in vitro* - i.e., when the pharmacologic agent has free access to the specimen and is in direct contact with smooth-muscle cells. Tests *in vitro* have demonstrated the ability of numerous pharmacologic agents to reverse constriction of varied origin; these include prostacyclin (169,173,176,181-184,191), calcium antagonists (132,226,229,230,237), antiserotonin agents (238,239), inhibitors of platelet aggregation (240), papaverine and nitroglycerin (241). However, not all are effective against constrictions induced with bloody CSF from patients. Observing no significant release of spasm with the use of methysergide, mepyramine, phenoxybenzamine, propranolol or atropine, Boullin *et al.* (225) and Sasaki *et al.* (158) suggested that serotonin, histamine, acetylcholine, angiotensin II,

and/or norepinephrine are probably not involved in the genesis of chronic VSP.

It is reasonable to assume that vasodilators can act only if they reach the arterial wall, either by topical application or, if the hematoma is small and represents no significant barrier, the agent can gain access to smooth-muscle receptors via CSF pathways. In dogs and monkeys with VSP induced by puncture of the basilar artery or the application of vasoconstrictors (5% BaCl, $PGF_2\alpha$, and 5-HT), Fox and colleagues (242, 243) were able to reverse the VSP by topical application of procainamide or chloramphenicol, or methylprednisolone or cortisol, but not when these agents were given iv or into the vertebral artery. Similarly, VSP was reversed in patients and monkeys when lidocaine was applied to the spastic arteries after the blood clots had been removed to allow contact of the agent with the cerebral artery (244,245); and VSP induced by a mixture of incubated blood and CSF injected into the subarachnoid space was reversed by fusaric acid, methylprednisolone, salbutamol, o-phenanthroline or ascorbic acid (246). Others (247,248) have reversed acute spasm by altering the cAMP pathway with topical applications of isoproterenol and aminophylline, or dibutyryl-cAMP. Prostacyclin relieved VSP when applied to the spastic vessel but not when given iv or intra-arterially (184,190), and nifedipine applied to cortical arterioles in the cat reversed VSP induced with bloody human CSF (268).

Auer and colleagues (249,250) reported that nimodipine applied topically or infused intracisternally in patients undergoing surgery for aneurysmal SAH reversed VSP peroperatively and decreased the probability of symptomatic VSP postoperatively; they removed the subarachnoid hematomas before applying the nimodipine, to ensure the drug's contact with

the vessel wall. Early operation and extensive washout of blood clots (even without vasodilators) are said to help prevent or reduce the incidence of VSP (279,303-305).

For chronic VSP, however, treatment with vasodilators given orally, intravenously, or infused into the carotid, has been ineffective. Allen et al. (50) reported that nimodipine improved the neurological outcome of patients with SAH-induced VSP. However, not all of their patients underwent cerebral angiography, and in those in whom this was performed the vessel diameter was very similar in the treated and placebo groups or even favored the latter. In 14 patients with normal neurological outcome, mean vessel diameter was 50% of control (nimodipine, n=8, 49.4%; placebo, n=6, 51.0%) and in eight with poor outcome the mean diameter was 33.4% (nimodipine, n=1, 22%; placebo, n=7, 35%). Perhaps, rather than preventing VSP, nimodipine prevented neuronal death, by modifying calcium homeostasis in the brain: increased intracellular calcium may activate the endogenous phopholipases that damage the mitochondrial endoplasmic and plasma membranes (269,272). More recently, in 6 patients with SAH and proven VSP, Grotenhuis et al. (270) studied the effects of nimodipine given as a slow bolus injection into the carotid over 10 min (after which cerebral angiography was performed), then infused iv at 2 mg/hr for 5 days, followed by 60 mg q6h po for 3 days. Although this treatment was started within 5 hr of onset of the VSP, vessel caliber did not change. Similarly, failure to relieve chronic VSP in man has been reported for iv infusion of OKY/1581, which inhibits thromboxane A_2 synthetase, in patients with SAH operated on within 72 hr (271); and for reserpine and kanamycin (202,207), isoproterenol alone (273) or with aminophylline (274), nitroprusside, and phenylephrine

(275). In dogs, chronic VSP after "double hemorrhage" was not reversible by aminophylline, nifedipine, or papaverine (147).

Successful treatment of chronic VSP has been reported by a few investigators. Flamm and his colleagues (274,280,281) managed VSP in cats, and then in humans, by altering the metabolism of cAMP in the smooth-muscle cell. Aminophylline and isoproterenol caused clinical improvement in 60% (40/65) and 75% (9/12) of their patients, although reversal of VSP was not demonstrated angiographically. Furthermore, in their recent study of 65 patients with SAH (274), in which the management included monitoring CVP and BPs and using hypervolemia (with albumin or plasminate) to elevate CVP to 10 mm Hg, they assumed the improvement in neurological status was due to inhibition of spasm. More probably, however, the improvement resulted mainly from the management of volume expansion and elevation of CVP.

It is generally accepted at present that the only available therapy for neurological deficits due to VSP (but not reversal of the constriction) is volume expansion with crystalloids, albumin, and/or blood, to elevate the CVP to approximately 10 mm Hg or the pulmonary capillary wedge pressure to about 18-20 mm Hg (196,282-286), together with elevation of the systolic BP with vasopressor agents and normalization of the ICP to increase cerebral perfusion pressure. Kassell et al. (285) used a protocol of this type to treat 58 patients who had progressive neurological deterioration due to SAH-induced VSP: neurological status improved permanently in 74%, there was no change in 16%, and the deterioration progressed in 10%; complications noted, which occurred in 33%, included pulmonary edema in 17% and rebleeding from unclipped aneurysm in 19%.

In the present study, nimodipine (1 mg/kg po tid) failed to prevent VSP on the clot side but reduced ($p < 0.05$) the incidence of VSP on the non-clot side. Also, a later study (318) in the same model with larger, graded doses of nimodipine (3, 6, and 12 mg/kg po tid) showed similar severity of VSP on the clot side in all groups, including placebo, and a trend to dilation on the non-clot side; nimodipine was in therapeutic concentrations in the CSF. These findings support the concept that a hematoma acts as a physical barrier to the action of drugs on the affected vessel (Fig. 40). Furthermore, this concept of the hematoma as a physical barrier accords with the hypothesis that the cerebral vessels are nourished from the tunica adventitia, via stomas and the rete vasorum, and not from the lumen. This may explain the inability to relieve chronic VSP except when the clots have been removed and vasodilators are applied to the vessel's surface.

Some investigators (137,154) have suggested that chronic VSP is due to an inflammatory process in the vessel wall and is induced by blood in the subarachnoid space. Findings in their study of chronic VSP from "double hemorrhage" in dogs, treated for 8 days with ibuprofen or high-dose methylprednisolone, led Chyatte et al. (254) to support the theory that chronic VSP is a vasculopathy of inflammatory origin (134,137,138). However, although severe myonecrosis and degeneration of smooth-muscle cells were found in spastic arteries from untreated dogs, no inflammatory process could be identified in any of the specimens. The difference in vessel caliber in treated vs untreated dogs probably was due to variation in the size of the hematoma: a dense blood clot encased the basilar artery from untreated animals (and was said to be of similar size in the methylprednisolone group), but there was much less blood

around this artery and the brainstem in the ibuprofen group -- perhaps due to the antiplatelet effect of this agent. Methylprednisolone, also, prevented VSP; like ibuprofen, this agent has an antihemostatic effect that could interfere with clot formation (254).

Studies in humans as well as other species have demonstrated that cerebral VSP is due to sustained contraction of smooth-muscle cells and can be reversed by direct application of nimodipine, lidocaine, methylprednisolone and cortisol (243-245,249,250). Furthermore, VSP abates spontaneously 2 wk after onset (once the vasoconstrictor agents have been consumed); although possibly due to the synthesis of prostacyclin by endothelial or myointimal cells, the pathological changes in the arterial wall (but not radiographic evidence of VSP) persist for up to 4 mo after SAH (136). In the present study, in both the treated and untreated groups, on Day 14 there were marked pathological changes in (clot-side) arteries that had been in spasm on Day 7 but not in arteries from the control (non-clot) side (the cerebral vessels were fixed by intra-arterial perfusion at 110 mm Hg on Day 14, when repeat angiography showed reversal of the VSP or mild narrowing averaging 20% reduction in vessel caliber). Interestingly, the VSP had resolved completely in specimens in which myointimal cells had migrated to the subendothelium, whereas severe constriction was observed in those with single-layer endothelium and pathological changes (Figs. 44 and 45).

There is no doubt that VSP gives rise to subendothelial proliferation (136,145-147,295). However, what appears to be a marked thickening of the vessel wall (mainly by myointimal cells) is a physical artifact. If a given number of smooth-muscle cells of an artery contract, reducing the vessel's diameter, these and other cells (endothelial) and tissues

(elastic lamina) that lack or have a limited capacity for physical change (contraction/elongation) will abut one another or be forced to create crests and valleys in order to fit within the smaller area. This will give an impression of vessel-wall thickening (Fig. 41).

The smooth-muscle cells are damaged by the toxic effects of free radicals, the sustained contraction of the tunica media, and probably by impaired nutrition (due to blockage of CSF pathways). The endothelial cells are damaged similarly, initially by the free radicals and the mechanical stress of constriction (143) (Figs. 44 and 45) but also by the high velocity of blood flow through a narrowed vessel (297).

II. CONCLUSIONS AND RECOMMENDATIONS

In summary, the most important findings in all three studies in this investigation are:

1. The size of the SAH governed the occurrence of chronic severe VSP.
2. The time-course of VSP after large SAH in the monkeys was very similar to that in humans.
3. The size of the SAH seen on the initial CT scan (Day 0) correlated with the severity and time-course of vasospasm apparent radiographically for up to 14 days.
4. Large localized SAH consistently caused severe VSP on the clot side and mild or no VSP on the non-clot side; the most affected vessel was the middle cerebral artery on the clot side.
5. Hemispheric CBF was not significantly decreased, and such CBF values did not appear to correlate with the degree and/or time-course of VSP, in individual animals or in the groups overall.
6. Severe chronic VSP of the middle cerebral artery caused a severe neurological deficit of delayed onset (hemiparesis, on the opposite side) in one monkey.
7. Nimodipine (1 mg/kg po tid) significantly reduced the incidence of VSP on the non-clot side but failed to prevent VSP on the clot side.
8. On Days 7 and 14, hemispheric CBF was not increased 2-6 hr after administration of nimodipine (1 mg/kg po tid).
9. VSP was associated with severe and dramatic changes in the arterial wall, even after radiographs showed complete resolution of spasm.

10. Nimodipine (1 mg/kg po tid) did not prevent the pathological changes.
11. The VSP seen radiologically was due to long-lasting smooth-muscle contraction and not to thickening of the vessel wall.

Future investigations should include the study of methods to prevent SAH by detecting asymptomatic aneurysms; until these sacs can be treated before rupture, the morbidity and mortality rates will remain very high.

Vasospasm is due to prolonged smooth-muscle contraction caused by a thick hematoma surrounding the cerebral arteries. The clot may block the access of therapeutic agents to the vessel wall; therefore, extensive removal of blood clot during early operation is advocated, together with systemic and/or intracisternal administration of vasodilators to ensure their adequate concentration in the CSF.

As complete removal of blood clots from the subarachnoid space is not possible, we should investigate the use of agents that could dissolve hematomas in the subarachnoid space (e.g., heparin and streptokinase) after the aneurysm has been clipped; their effects on VSP, cerebral blood flow, and neurological status; and the pharmacokinetics of their administration concurrently with vasodilators.

The cerebral arteries are probably nourished by CSF diffusing into the arterial wall via the stomas in the tunica adventitia; therefore, achievement of therapeutic concentrations of vasodilators in the CSF should be emphasized.

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