DURING the past few decades, a number of possible causes of cerebral vasospasm have been proposed based mainly on the results of animal experimentation (see below). Many of these hypotheses have not been adequately tested. We have attempted to design experiments to assess the contribution of some of these factors to the cerebral arterial narrowing seen in a monkey model of chronic cerebral vasospasm. Cerebral arteries were examined 5—6 days after experimental subarachnoid hemorrhage (SAH) when cerebral arterial narrowing is maximal and invariably associated with neurologic deficit.

Some of the possible, immediate causes of narrowing following hemorrhage include 1) changes in the structural constituents of the artery wall that affect its passive properties, in particular distensibility. Such changes may be due to edema and cellular change and alterations in arterial wall integrity; 2) increased vascular smooth muscle tone possibly due to the effects of substances endogenous to the vessel wall or the brain or associated with blood or blood clot and its products; 3) changed vascular smooth muscle reactivity due, for example, to functional denervation; and 4) exaggerated or abnormal tone—changes in tone of the medial smooth muscle not dependent on external chemical stimulation. Although this latter possibility has not been formally proposed, the presence of myogenic tone in cerebral arteries, which is functionally significant, suggests this possibility.

Our examination of cerebral arteries is made only at one time, 5—6 days after the initial hemorrhagic insult. Thus, all that can be established is the state of the arteries at sacrifice. However, we do feel that our observations should be of some use in understanding the series of events that lead to the established state of chronic vasospasm. Our conclusion is that, although many factors may contribute to arterial narrowing in vivo, the most consistent of these that we can assess in vitro in the larger arteries is a decrease in wall distensibility. This may reflect the all too evident edema and the evidence of inflammation, fibrin deposit, and damage to the blood vessel wall. In addition, large, spontaneous, prolonged changes in active tone were encountered especially in the smaller arteries, which probably significantly contribute to narrowing. Changes in sensitivity to vasoconstrictors were variable. If these changes occurred in some instances, their consequences would be offset by the marked decrease in contractility that was frequently measured. The role of nerves in established vasospasm is probably minimal since these elements are damaged and are relatively ineffective. Since the calcium channel blocker, diltiazem, administered before the experimental hem-
orrhage protects against arterial narrowing in this model, the series of events that lead to the changes found on the fifth and sixth day are probably associated with calcium entry into cells.

Preliminary communication of some aspects of these experiments have already been published.16,17

Materials and Methods

The method of inducing SAH in monkeys (Macaca nemestrina) that leads to chronic cerebral vasospasm has been previously described.1 Under anesthesia a 0.4-mm needle is placed through the intracranial portion of the internal carotid artery and percutaneously removed the following day, resulting in hemorrhage. Widespread vasospasm is usually present 5 days later, primarily in the ipsilateral vessels. On the evening of the day before sacrifice—the fifth or sixth day—the animals were examined neurologically and carotid angiography was performed (see Ref. 18 for details). A total of 6 monkeys, 4 males and 2 females, weighing 6.9–13.5 kg, were used in this study.

After anesthesia with ketamine (5–20 mg/kg i.m.) followed by pentobarbitol (6–8 mg/kg i.v.), the monkey was heparinized and bled through a large venous catheter. The calvaria was removed and the brain delivered, as rapidly as consistent with exercising great care to preserve the pial arterial tree, into a bath of gassed Krebs bicarbonate solution (see below). The vertebral and internal carotid arteries were severed as they pierced the dura. The circle of Willis and its main branches were dissected from the brain and divided for in vitro study according to the plan shown in Figure 1. Once the arterial system was removed from the brain and placed in the Krebs solution, dissection could proceed without haste. Due to variation in the size and pattern of the arterial system between animals, there were small variations in the size and location of allocated segments. No attempt was made to pattern the distribution of the segments for in vitro examination to

In Vitro Contraction

Artery segments destined for contraction studies were placed in Krebs bicarbonate solution and cleaned of extraneous material under a dissecting microscope. The solution was of the following mM composition: Na+, 144.2; K+, 4.9; Ca²⁺, 1.6; Mg²⁺, 1.2; Cl⁻, 126.7; HCO₃⁻, 25; SO₄²⁻, 1.19; glucose, 11.1; and EDTA, 0.024, equilibrated with 95% O₂-5% CO₂. Segments were trimmed to 4-mm lengths and, using 2 stainless steel wires inserted into the lumen of the segment, were mounted in a tissue bath at 37.5°C as described previously by Bevan and Osher.19 A resting force based on our experience with arteries of similar size was used. The experimental conditions did not permit determination of optimum length in each instance, which would have been necessary since the monkeys differed considerably in size and since in the lesioned animals the arterial segments from the two sides differed from each other. It is our experience that the active force-length curve of arteries has a wide
plateau, and we do not consider small deviations from optimum length to be significant in relation to the measurements we made. Changes in isometric force were measured using a sensitive strain gauge and were displayed on a Soltec 220 recorder or a Grass polygraph.

Electrical field stimulation was delivered from a Grass SD-9 stimulator wired via a low-impedance voltage follower to platinum wire electrodes positioned parallel to the vessel segment approximately 0.5–1.0 mm from its outer surface. Square-wave monophasic pulse trains were delivered at frequencies of 0.5–8 Hz, with a pulse duration of 0.3 msec. The stimulation voltage was that which caused a just discernable response in the presence of tetrodotoxin (3 x 10^{-7} M). After this determination, the tetrodotoxin was washed out.

The passive wall force and vessel circumference (length between supporting wires) relation (F/L) was measured 1 hour after the segment had been set up in the tissue bath in the presence of 2 x 10^{-2} M NaNO2 or 0.1 mM MnCl2. The minimum wire separation to produce a discernable increase in recording was considered l—that approximately half the circumference. Vessels were stretched to no more than 4 x l0 in increments of 0.1 mm after equilibrium had been achieved to the preceding stretch increment.

**General Protocol for In Vitro Studies on Larger Cerebral Arteries**

Vessel segments were promptly set up in their tissue baths and arbitrarily set at 0.5 ± 0.15 g rest force (see above). After 1 hour of equilibration, F/L was determined. Resting force was adjusted, and the subsequent immediate changes in tone with time were observed. Of particular interest was an immediate myogenic response. The occurrence of spontaneous contractions, either regular or irregular throughout the experimental period, were noted. Subsequently, the effects of nor-epinephrine (NE, 10^{-6} M), transmural nerve stimulation at 4, 1, 2, 4, and 8 Hz continued to an equilibrium, and serotonin (5-HT, 10^{-7} M) were noted. Occasions were chosen to apply these stimuli, especially to segments from the lesioned side, when spontaneous changes in tone were minimal. Twenty minutes after exposure to guanethidine (5 x 10^{-4} M), segment tone was increased by tromethamine (PGF2α, 8 x 10^{-4} M and above) to a level similar to that achieved by the previous 5-HT additions. Then the effects of transmural nerve stimulation (2, 4, and 8 Hz), acetylcholine (10^{-2}–10^{-4} M), isoproterenol (10^{-6}–10^{-4} M) and papaverine (10^{-2} M) were recorded. Subsequently, in the presence of desmethylimipramine (3 x 10^{-7} M) and propranolol (10^{-5} M), a cumulative concentration-response curve to NE extending to the maximum of 10^{-4} M was recorded. These drugs were used to prevent neuronal uptake of NE (uptake) and to block β-adrenoceptors, respectively, allowing the effect of NE on the α-adrenoceptor alone to be assessed. Finally, 5-HT (10^{-3} M) followed by PGF2α (10^{-3} M) were added sequentially to establish maximum tissue contractility.

**Pial Arteries**

Small pial arteries (100–150 μm o.d.) were selected from corresponding sites of the bed of the middle cerebral artery (MCA) over the curvature of the brain and mounted in a resistance vessel myograph. The original experimental plan was to test these vascular segments using a protocol similar to that for the larger arteries. However, this proved to be almost impossible in most cases due to the unexpected, irregular, large excursions of spontaneous force development on the damaged side. In most segments, it was possible to execute the following procedures: 1) myogenic response to low levels of passive stretch, 2) effect of calcium removal from the bath fluid and of 10^{-5} M papaverine on spontaneous force change, 3) effect of electrical field stimulation in the absence of extrinsic tone, and 4) responses to graded doses of NE (10^{-2}–10^{-4} M). In the pial vessels desmethylimipramine and propranolol were not used because at the concentrations that block uptake, and β-adrenceptors, they cause changes in tone.

**Vessel Dimensions**

Measurements of wall thickness and circumference were obtained from in vitro segments cut longitudinally. Thickness of the vessel wall was obtained by focusing on the intimal and adventitial surface respectively using a microscope in which the fine focus adjustment was calibrated. The mean of 4 measurements made in different positions in the segment was used for comparison. Microscope calibration was verified by measuring known thicknesses of coverslips. The internal circumference was found using a calibrated eyepiece or a drawing tube attachment of a light microscope. Tissues were examined after they had been soaked for 2 hours in Krebs bicarbonate solution containing 2 x 10^{-3} M sodium nitrite and 10^{-3} M procaine hydrochloride.

**Tritiated Norepinephrine Neuronal Uptake**

Uptake, was estimated using tritiated NE of high specific activity ([3H]NE, 2 x 10^{-7} M [7,8-3H]norepinephrine) according to the method of Su et al. The differences between the uptake and retention of [3H]NE in the absence and presence of 10^{-4} M cocaine, an inhibitor of neuronal uptake, was taken to represent neuronal uptake.

**Choline Acetyltransferase Assay**

Blood vessels were homogenized in Potter-Elvehjem glass grinders (Kontes Glass) in a homogenization solution containing (in mM) NaCl, 300; sodium phosphate buffer, 10 (pH 7.4); EDTA, 1; and Triton X-100 (0.5% by vol; pH 7.4). The volume of the homogenization solution was adjusted to give a tissue concentration of 1.5 mg/40 μl. The procedure was carried out on ice.

The radioenzymatic assay was adapted largely from Fonnum’s method. The choline acetyltransferase (CHAT) activity was determined in 5-ml centrifuge tubes with ground glass stoppers. Ten μl of the follow-
ing substrate mixture (final concentration per 60 µl is indicated) was added to 40 µl of the homogenate: [acetyl-1-¹⁴C]coenzyme A 50–60 mCi/mmol (New England Nuclear), 0.20 mM; NaCl, 300 mM; sodium phosphate buffer, 50 mM (pH 7.4); choline chloride, 8 mM; EDTA, 10 mM; and eserine sulfate, 0.1 mM (pH 7.4). The [acetyl-1-¹⁴C]coenzyme A was diluted with unlabelled acetyl coenzyme A (Sigma Chemical) to give a final concentration of 0.2 mM. The mixture was incubated for 12 minutes at 37°C, and the reaction was stopped by the addition of 5 ml of ice-cold 10 mM sodium phosphate buffer (pH 7.4). One ml of 3-heptanone containing 15 mg of sodium tetraphenylboron (Sigma Chemical) was added to the tubes, and the contents were shaken lightly for 1 minute. After centrifugation at 3,000 rpm for 5 minutes, 0.5 ml of the organic phase containing the synthesized [¹⁴C]acetylcholine was transferred into vials containing 10 ml 3a70B scintillation cocktail (Research Products International). The radioactivity was determined in a liquid scintillation counter (Beckman LS 8000) at a counting efficiency of 89%. Blank tubes with no tissue added contained <0.1% of the added radioactivity and were subtracted from the sample results. Enzyme activity is reported as nanomoles of acetylcholine generated per gram of tissue per hour (nmol/g/h).

Data Analysis

The monkeys differed in age, sex, and weight, and their past history was completely unknown except that they had gone through the required period of quarantine. Data from segments from the side of the hemorrhage were compared with data from corresponding segments from the contralateral side using a Student's t test; p<0.05 was considered significant. Data are reported as mean ± SEM.

Results

In Vitro Studies on Large Cerebral Arteries

Relation Between Passive Wall Force and Vessel Circumference. In 7 of the 9 paired segments where F/L could be adequately executed, the curve from the vessel on the side of the lesion was displaced to the left of that from the contralateral side (Figure 2). This indicates an increased resistance to passive stretch. With regard to the other 2 segments, in 1 from the anterior cerebral artery (ACA) where the diameter on angiography was reduced to 67% of that from the contralateral side, there was no difference in the curves. In the MCA of the same monkey, F/L showed decreased resistance to stretch, and the angiographic record showed increased arterial diameter on the side of the lesion.

In Figure 2 the mean passive wall force developed for similar increases in length between the supporting wires (half the circumference) for 8 paired arterial segments are shown. The percent increase of force developed in segments from the lesioned compared with the opposite side was very similar at all lengths. The mean percent increase in passive wall force at lengths 2, 4, 6, and 8 mm beyond 10 for all pairs of segments was 449 ± 24.65% (Figure 3).

Wall Thickness. Arterial segment thickness varied in different parts of the same segment from the injured side. Mean wall thickness of the lesioned compared with the contralateral side was 114 ± 41% (n = 6 pairs; p < 0.05). Since measurements were made in the presence of NaNO₂ and procaine, thickness differences were not due to muscle contraction or spasm. There was no correlation between the resistance to stretch and change in wall thickness in the segments from the side of the hemorrhage.

Spontaneous Tone Activity. Most arterial segments showed a low level of irregularity in their baseline tension record (Figure 4). These excursions were continuous and < 5% of maximum tissue contractility and were observed in segments from both sides. Five artery preparations from the side of the lesion showed either apparently spontaneous excursions of tone or large transient increases upon changing the bath solution. These changes were invariably > 30% of maximum tissue contractility, often greater, and inconsistent in frequency of occurrence and duration of tone maintenance. These spontaneous changes in tone were recorded in segments of arteries that on angiography showed diameters 67–113% of control. Segments of arteries not exhibiting such activity were obtained.

FIGURE 2. Passive wall force in grams/length in millimeters between supporting wires (½ circumference) from anterior cerebral and middle cerebral arteries of monkeys 5–6 days after experimental subarachnoid hemorrhage. Corresponding segments were taken from the side of the arterial puncture (＃) and the contralateral side. When compared as pairs, values from the side of the lesion were significantly greater than those of the ipsilateral side (p < 0.05).
FIGURE 3. Summary of combined observations from all isolated segments of anterior cerebral and middle cerebral arteries (see Figure 1). Parameters on the side of the puncture are expressed as percent of those on the opposite side. Passive Wall Force/Length, mean of 4 length increases of 2 mm each beyond L0 (see text for details); Contractility, maximum response to norepinephrine + serotonin; NE ED50, dose of norepinephrine to cause a half-maximum NE contraction; NE (10^-7m) % Maximum, response to norepinephrine as percent of maximum contractility; SHT (10^-7m) % Maximum, response to serotonin as percent of maximum contractility; SHT (10^-7m) % NE (10^-6m), response to serotonin as percent of that to norepinephrine; Adrenergic Nerve, mean equilibrium response to stimulation of constrictor nerves at 2, 4, and 8 Hz; Dilator Nerve, mean response to stimulation of nonadrenergic nerves at 2, 4, and 8 Hz in the presence of tone induced by PGF2α; CHAT, choline acetyltransferase activity; 3HNE, neuronal uptake of tritiated norepinephrine; Pial Arteries Spont. Muscle Activity, peak response during the third hour of the experiment; NE (10^-6m), absolute response to norepinephrine. (*p<0.05 when parameter on side of puncture is compared with that on contralateral side.)

ACA = Anterior Communicating Artery
MCA = Middle Cerebral Artery

FIGURE 4. Examples of spontaneous contraction of monkey cerebral arterial segments (top) from anterior communicating and middle cerebral arteries on the side of the experimental puncture, and (bottom) from small pial arteries (= 100 μm o.d.) from the side of the hemorrhage (#) and the contralateral side (c). In 3 bottom, note the effect of Mn (10^-4 m).
from vessels that on angiography had diameters 38–83% of control. Details of angiographic diameter measurement have been presented elsewhere.18

CONTRACTILITY. The maximum capacity of the tissue to develop force measured at the termination of the in vitro experiment was markedly reduced on the side of the injury compared with the contralateral side—31.86 ± 9.95 (p < 0.05; see Figure 3). There was a positive correlation between contractility and angiographic diameter when each was expressed as a percent of its own control (r = 0.67; n = 10; p < 0.05). In the MCA, this value was taken as the mean of angiographic measurements MCA1 and MCA2.

AGONIST SENSITIVITY. Irregularity of the experimental record and the poor maintenance of tone after agonist addition made comparisons of the dose to cause half-maximal contraction (ED50) between the two sides impossible in about half the experiments. Among the remainder, 4 segments from the spasm side were more and 1 was less sensitive than their contralateral counterparts. The mean ED50 value for these 5 pairs of segments was 96.2 ± 40%—the large SEM reflecting the extreme variability (Figure 3). The greatest increase in sensitivity in any of the pairs, of interest because of the possible role of denervation hypersensitivity in the arterial narrowing, was threefold and was found in an arterial segment devoid of angiographic evidence of vasospasm.

Because of the irregularity of the experimental records, vessel wall reactivity was measured not only by ED50 agonist concentrations, but also by responses to single agonist doses. Contractions to 10−6 M NE were 39.5 ± 11.7% of tissue maximum on the lesioned and 48.50 ± 14.8% on the contralateral side (n = 8 in each case). These were determined in the presence of desmethylimipramine and propranolol (see “Materials and Methods”). For 10−7 M 5-HT, these values were 49.0 ± 16.3% and 41.67 ± 17.15% respectively. Contractions to NE on the side of the spasm measured as developed force were 73.1 ± 7.96% of control, and for 5-HT, 181 ± 90.13%. When the responses to 5-HT were expressed as percent of those to NE on the same segment, the ratios were 433 ± 225% and 157 ± 40% for the lesioned and contralateral side respectively (n = 8). These ratios for the lesioned side were significantly greater than for the opposite side, demonstrating a selective increase in reactivity to 5-HT compared with NE.

Efferent Nerve Function

ELECTRICAL FIELD STIMULATION. Transmural electrical nerve stimulation invariably produced a frequency-dependent contraction in segments from the side contralateral to the lesion (Figure 5). For convenience, since there was no trend toward a frequency-dependent change, mean ratios of contractions of ipsilateral and contralateral segments for the 3 tested frequencies (2, 4, and 8 Hz) were determined—the average of these
for ACA and MCA segments combined were 23.41 ± 6.01% (n = 8; means from 8 grouped comparisons) indicating a significant depression of neurogenic vasoconstriction. Electrical field stimulation after guanethidine and after increasing tone with PGF₂α caused a frequency-dependent relaxation (Figure 5 Bottom). When expressed in the same way, the total mean value was 54.0 ± 22.7% (n = 8). There was no correlation between the reductions in vasoconstrictor and vasodilator responses among the various segments nor between these and the corresponding angiographic alterations. The increased tone following PGF₂α was reversed in both groups of segments by 10⁻² M sodium nitrite or 10⁻⁵ M papaverine, but not by 10⁻⁶ M acetylcholine.

**Choline Acetyltransferase.** Levels of CHAT, an index of cholinergic nerve terminal function and probably a reflection of cerebral dilator nerve integrity,²⁴,²⁵ on the side of the hemorrhage were significantly reduced compared with the contralateral side to a mean of 31 ± 10.2% (n = 5).

**Neuronal Uptake of Tritiated Norepinephrine.** Cocaine-sensitive uptake of [³H]NE reflects the entry and accumulation of tritiated NE in adrenergic nerve varicosities.²⁶ This was significantly reduced on the side of the hemorrhage, and in the other the opposite side to a mean of 20-30% of the contralateral side. When accompanied by contraction, it was diminutive compared with the opposite side where modest and characteristically frequency-dependent contractions were recorded. NE (10⁻⁸–10⁻⁵ M) elicited smaller contractions on the order of 10–30% of the control side. Higher concentrations of NE (10⁻⁵–10⁻⁴ M) caused relaxation. Peak forces developed to NE on the damaged side were 20–30% of the peak spontaneous increases in tone.

### Discussion

Investigation of cerebrovascular spasm has been hampered by a number of problems, including the availability of a suitable model. An appropriate animal preparation should reflect the salient features of the clinical condition and provide an opportunity for analytical, experimental measurements and manipulation of conditions. The model of Frazee¹ has a number of excellent attributes that commend its use. After hemorrhage is produced, a widespread spasm that is long lasting, reaching a maximum after about 1 week, usually occurs. There is an associated neurologic deficit. Arterial segments from the experimental monkeys were examined at only one point in time after the hemorrhage by a variety of techniques designed to assess their passive and functional properties. Different features were assessed on anatomically different but adjacent segments, precluding an exact correlation of arterial properties.

With only one exception, the larger arterial segments on the side of the lesion showed a greater resistance to stretch than those on the opposite side. In the one instance where the opposite was the case, no angiographic narrowing was observed in the vessel from which the arterial segment had been taken. The data showed that for a given passive wall force, the internal circumference of the artery of the lesioned side would be approximately 60% of control. On the basis of Poussete’s law, assuming that there are no other changes and a proportional reduction in all arteries contributing to the regulation of blood flow, this narrowing is equivalent to an 80% reduction in blood flow. The mean angiographic diameter of arteries from the lesioned side from which segments were taken was 61 ± 5% of the contralateral side, consistent with narrowing of the larger arteries primarily due to a decrease in arterial wall distensibility.

In the only other study measuring elastic properties of the cerebral vessels, vasospasm was produced in dogs by blood injected intracisternally.³ After 2 days the artery was more distensible, and then became progressively stiffer, approaching control by the seventh day. This progressive increase in stiffness and the increased stiffness seen in our own study on the seventh
day is probably due to an increase in mural collagen. Nagasawa et al also found that the incremental elastic modulus was a positive function of the collagen:elastic ratio in their arteries. This is consistent with current understanding of the basis of the F/L curve and with our own evidence of increased collagen synthesis (unpublished results).

Vascular smooth muscle contractility indexed by the maximum force developed to NE and 5-HT was reduced by approximately 70%, and this is reflected in the size of the single responses to NE and 5-HT. There was no change in sensitivity to NE, but the response to 5-HT was disproportionately increased. Svendgaard et al injected 1–2 ml of autologous blood intracisternally into rabbits and found that cerebral arterial narrowing occurred, becoming maximal at 4 days. There was no evidence of impairment of contractility; in fact, the response to 5-HT was potentiated more than that to NE. Lobato et al produced a similar model in cats and found an increased response to NE and 5-HT, presumably indicative of increased sensitivities, and no evidence of decreased contractility. Higher concentrations of 5-HT were potentiated preferentially. Changes were maximal on the third day, after which they returned to control values, although remaining somewhat altered for up to 30 days.

Our experimental evidence strongly suggests changes in effector nerve function based on loss of structural integrity of the adrenergic neurons (see below), a reduction in nerve-induced vasoconstriction, in [H]NE uptake, and in catecholamine histofluorescence. First observations of such changes in a similar model were made by Duckles and Bevan. Responses to dilator nerve activity were also attenuated. Based on studies of cats, this neurodilation might involve both acetylcholine and vasoactive intestinal peptide (VIP) as transmitters. Reduction in CHAT levels supports this conclusion. VIP-containing neurons have been observed in cerebral arteries of control (sham-operated) monkeys (J. Brayden, personal communication), but no attempt was made to quantify VIP neuronal density in this study. Other neuropeptides (for example, substance P, calcitonin gene-related peptide, etc.) with vasoactive properties have been described in cerebral arteries, but their physiologic role is not yet established.

A reduction in neural control of cerebrovascular tone is consistent with the observations of others. Peerless and Kendall were the first to show loss of catecholamine fluorescence after experimental SAH. Subsequently, Svendgaard et al and Edvinsson et al found that fluorescence, [H]NE uptake, and NE content were reduced in rabbit basilar arteries 1 day after cisternal injection of blood—a change that persisted for more than 2 weeks. Lobato et al also showed concomitant decrease in dopamine β-hydroxylase in cats, which returned to normal over the subsequent several weeks.

Endo and Suzuki have shown that after direct exposure of cat cerebral arteries to a blood-CSF mixture incubated 5–10 days there is transformation, decrease, and gradual disappearance of small, dense vesicles in the adrenergic terminals. This activity, when occurring late in the course of SAH, may be associated with plasmin derived from clot lysis and tissue repair. The loss of nerve function in our monkeys was not due to general operative stress suggested by Rosenblum and Guilian since there were quite clear side-to-side differences.

The experimental record of force changes in the small pial arteries was dominated by spontaneous excursions in tone. Episodes often lasted up to an hour or more. These changes were not precipitated by stretch and thus most probably represent an abnormal functional state of the vascular smooth muscle cells. This activity, however, was reduced by such nonspecific vasodilators as papaverine and sodium nitrite and was inhibited when calcium was excluded from the tissue bath. Because these spontaneous excursions were so prevalent, satisfactory F/L curves could not be constructed.

Arterial diameters of small pial arteries (150 μm o.d. unstretched) cannot be estimated from angiography. Consequently, we do not know whether there is narrowing in these vessels in vivo. There were clear differences in vitro between the small pial arteries from the two sides. We infer from the magnitude of the spontaneous changes in force that if such activity occurred in vivo, it would have a very significant influence on blood flow.

These functional changes are consistent with preliminary structural studies. Small adherent blood clots were seen on the wall of the anterior cerebral artery, which was constricted at that site. These clots contained inflammatory cells, macrophages, and fibroblasts, and the adjacent artery wall was markedly abnormal with edema, separation of the internal elastic lamina from the media, and degenerative changes of varying severity in the smooth muscle cells. There were varying amounts of separation of the internal elastic lamina from the media, with obvious vacuolation in the inner media and subendothelial layer. Minor degrees of vacuolation were also seen in the arteries of the contralateral side. On the lesioned side mixed nerve bundles in the outer adventitia and nerve terminals at the adventitio-medial border showed degenerative changes in the ACA and MCA segments. Fluorescent adrenergic nerve terminals were frequently absent or diminished. In the adventitia of the terminal internal carotid artery on the side of the lesion there was patchy loss of terminal fluorescent adrenergic nerves, but large bundles containing many intensely fluorescent axons were seen. There have been suggestions that vasospasm might reflect the continuous vasoconstrictor action of substances present in the CSF. Our observation that contractility is very greatly reduced suggests that if there is a role for such a mechanism in the larger arteries, it is minimal. It is conceivable that this mechanism may play a role in small artery constriction. CSF from patients with SAH contains smooth muscle constriction activity. When it occurs late in the course of SAH, this activity may be
associated with plasmin derived from clot lysis and tissue repair. 37

The functional consequences of loss of neurogenic control is difficult to assess as little is known about this in monkeys. By inference from studies of other species, we conclude that loss of sympathetic vasomotor paralysis in humans may be due to loss of autoregulatory capacity and intrinsic control. We do not know its status in these animals; however, the arteries did not show a maintained response to stretch. The fact that vasospasm is greatest in those larger arteries with the greatest loss of contractility suggests that changes associated with active muscle contraction are not major determinants of spasm. This condition seems to be associated with loss of contractility presumably due to wall damage. Frazee et al 44 found that i.v. nitroglycerine, which was expected to remove active tone, had a clear but small effect on spasm in this model. In an autologous nonheparinized dog model, Varsos et al 47 found that aminophylline and nifedipine, which were effective dilators in the acute state, were without action in the chronic state. The literature is replete with observations that pharmacologic manipulations expected to inhibit vascular tone are relatively ineffective in chronic cerebral vasospasm. Most animal studies are in agreement. 48

It could be that the basis of vasospasm is different in different models and vessels but, at least in our monkey studies of the larger arteries, passive factors seem to dominate. Abnormal functional reactivity may be more important peripherally.

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