Experimental Subarachnoid Hemorrhage: A Study for Spasm with the Production of Aneurysms

BY VANCE C. ROY, JR., MD.,* THORALF M. SUNDT, JR., M.D.,† AND FRANCIS MURPHEY, M.D.‡

Abstract:
Experimental Subarachnoid Hemorrhage: A Study for Spasm with the Production of Aneurysms

We have shown the difficulty in producing a reliable model of vasospasm secondary to recurrent subarachnoid hemorrhage from carotid puncture in a squirrel monkey. In this animal, mild systemic hypertension had little or no effect on the clinical status after hemorrhage. The consistent production of a saccular aneurysm on the intracranial arteries was an unexpected result of the arterial puncture and the interruption of the internal elastic membrane. At this time, there is insufficient knowledge regarding the entire subject of vasospasm for correlation with human vessel reaction to injury.

ADDITIONAL KEY WORDS arterial trauma cerebral angiography experimental hypertension laboratory animal intracranial vasospasm

The urgency for an experimental model of intracranial aneurysms and prolonged cerebral vasospasm, as seen in patients with subarachnoid hemorrhage, is apparent to all. Some investigators have been successful in the production of prolonged angio- graphical cerebral vasospasm by various means of experimental subarachnoid hemorrhage; however, none has used the clinical state as the primary criterion of spasm. In an effort to produce a reliable clinical model of cerebral vasospasm, the following study was undertaken. Aneurysm formation on intracranial vessels was an unexpected but valuable product of the work.

Methods
Forty-two squirrel monkeys (Saimiri sciureus) of average weight (0.9 kg) were operated on and divided into groups on the basis of blood pressure and the number of subarachnoid hemorrhages.

RECORDING OF BLOOD PRESSURE
Mean blood pressure measurements were made, using an intra-arterial 25-gauge needle attached to an aneroid manometer (Tycos) through a bubble trap. Baseline blood pressures were obtained prior to the production of hypertension and again before intracranial surgery.

PRODUCTION OF HYPERTENSION
The technique for the production of hypertension was that suggested by Halsey. The left kidney was exposed by a transperitoneal approach and enclosed in a plastic envelope held in place by silver clips. One week later, each monkey underwent right nephrectomy by a retroperitoneal approach through the flank. After operation, each monkey received an antibiotic consisting of a penicillin-streptomycin combination (Combiotic). All surgery was done with the monkey under general anesthesia produced from intrapeurally administered pentobarbital (Nembutal) (0.25 to 0.35 mg). Some blood pressure measurements were made after local anesthesia, and when this

*Resident in Neurosurgery, University of Tennessee, Memphis. Current address: Chelsea Naval Hospital, Chelsea, Massachusetts.
†Consultant, Section of Neurological Surgery, and Section of Cerebrovascular Research, Mayo Clinic, Rochester, Minnesota.
‡Professor of Neurosurgery, University of Tennessee.

This work was performed in the Cerebrovascular Research Laboratory at the University of Tennessee. The preparation for publication was by the second author, whose present address is Mayo Clinic and Mayo Foundation, Rochester, Minnesota, 55901.

This investigation was supported in part by Research Grants NB-6822, NB-6826, NB-5651, NB-8315, and NB-6663 from the National Institutes of Health, Public Health Service.
LABORATORY SUBARACHNOID HEMORRHAGE AND ANEURYSMS

was done, 1% procaine (Novocain) (0.25 to 0.50 ml) was the agent.

CREATION OF SUBARACHNOID HEMORRHAGE
Four to five weeks after nephrectomy, each monkey was returned to the operating room. The right hemicranium was prepared in a sterile fashion, and with an endotracheal tube in place, the monkey was restrained in an atraumatic headholder. The right intracranial internal carotid artery was exposed at its bifurcation using the Hudgins modification of the retro-orbital approach previously reported by Sundt and Waltz. All intracranial surgery was performed with the operating microscope. Approaching the artery from the anterior rather than the lateral aspect eliminated the need for brain retraction. After the dura was opened, with the arachnoid left intact, a curved surgical needle (0.19 mm in diameter) was placed in the subarachnoid space, passed along an oblique path, and inserted into the artery along its posterior aspect. The dural incision (4 to 5 mm) was then sealed with a stamp of Gelfoam or muscle. The wound was closed with silk sutures, and the monkey was removed from the headholder. If the monkey was selected for one of the two subgroups undergoing a recurrent subarachnoid hemorrhage, it was returned to the operating room seven days later. The wound was reopened, and the artery was punctured a second time.

GROUPING OF ANIMALS
The monkeys were divided into two groups: a control group of 20 animals and a hypertensive group of 20 animals. Each group was further divided into subgroups of ten on the basis of having suffered either one or two subarachnoid hemorrhages. Therefore, four groups of ten monkeys each were present: ten normotensive with one subarachnoid hemorrhage, ten normotensive with two subarachnoid hemorrhages, ten hypertensive with one subarachnoid hemorrhage, and ten hypertensive with two subarachnoid hemorrhages.

ARTERIOGRAMS
On the day of sacrifice, transfemoral angiography was carried out by means of injections of contrast medium (50% Hypaque or Renografin). Single anteroposterior films were made with the x-ray machine coned to its maximal in order to provide good resolution. Each examination usually required no more than one or two injections of contrast medium (5 to 10 ml).

BLOOD PROFILE
Blood was collected just before death of the monkey by either cardiac puncture or transfemoral catheter. Blood analysis consisted of a complete blood count, platelet count, and chemistry studies by means of the SMA-20 technique.

POSTMORTEM
All monkeys were sacrificed at the end of seven days or 14 days, depending on in which subgroup they belonged. Death was accomplished by exposure of the heart through a sternal-splitting incision and insertion of a 13-gauge needle into the aortic arch, with occlusion of the distal portion of the aorta. The monkey's head was first perfused with 10% low-molecular-weight dextran (Rheomacrodex) in order to flush blood from the cerebral vessels. This blood was returned through the venous side and an aperture made by removal of the right auricle. After perfusion by Rheomacrodex, 6% glutaraldehyde was perfused into the head of the monkey. After the perfusion was completed, the intracranial contents were removed and, with the use of the dissecting microscope, the internal carotid, proximal A1 segment, and proximal and distal middle cerebral arteries were removed in toto. Representative sections were cut from the internal carotid and middle cerebral arteries bilaterally. These were fixed in formaldehyde for light microscopy and in glutaraldehyde for electron microscopy. Late in the series, after the chance observation of aneurysm formation, the operated vessels were photographed before sectioning. Monkeys with aneurysm formation had the internal carotid artery at the site of the aneurysm prepared for light microscopy with hematoxylin and eosin and Verhoeff staining techniques, after which photomicrographs were made. Hypertensive animals were randomly selected for the removal of the remaining kidney.

Results
Because of the death of two monkeys, one from renal failure and one from a perforated duodenal ulcer with peritonitis, 40 monkeys comprised the groups available for study. The two deaths occurred before any intracranial procedure.

RECORDING OF BLOOD PRESSURE
The range of normal mean baseline blood pressures was 68 to 100 mm Hg. The range of increases in blood pressure after renal surgery was 16 to 54 mm Hg. The use of either a general or a local anesthetic had no effect on the observed blood pressure levels.

PRODUCTION OF HYPERTENSION
Thirteen of the 20 monkeys subjected to renal procedures for the creation of hypertension had an elevation of the mean arterial pressure of at
least 15 mm Hg. Seven of the 20 monkeys did not have a significant elevation in the blood pressure, but remained in this group because of the possible physiological alterations accompanying renal surgery.

**Creation of Subarachnoid Hemorrhage**
In all monkeys, after arterial puncture, there was an immediate and profuse subarachnoid hemorrhage. The obliquity of the needle path and the posterior site of arterial puncture caused almost all blood to remain in the subarachnoid space. Usually there was little, if any, blood leakage into the orbit. The dura was seen to change suddenly from a slack and opalescent appearance to a tense reddish structure with absence of pulsations. In the monkeys who underwent reoperation, the dural opening was found to be occluded by the swollen brain, without evidence of spinal fluid leakage. Also at repeat operations, an opaque appearance of the arachnoid was noted at the carotid siphon. This prevented any detailed visualization of the artery and of possible aneurysm formation.

**Clinical Evidence of Spasm**
All monkeys began to awaken within three hours of operation. For one week thereafter, on a daily basis, the clinical status of each monkey was evaluated. Each monkey took food and water actively, had equal use of all extremities, and was alert and active at each observation. The only monkey that did not behave in this manner was later found to be uremic and was not included in the series. Thus, on a clinical basis, we were unable to detect any evidence of cerebral vasospasm.

**Radiographical Evidence of Spasm**
Angiograms of good quality were usually obtained. Because of the small size of the monkey and its extremely small cerebral arteries, film resolution did not permit us to differentiate normal vessel caliber from what might have been abnormal caliber. A high percentage of animals showed separation of the cranial sutures, some of which were as far apart as 5 mm, indicating increased pressure from the hemorrhages.

**Blood Profile**
Hematocrit value, leukocyte counts, and platelet counts of the monkeys generally were within the normal range for humans. Results of blood studies, including those for sodium, potassium, chloride, bicarbonate, and inorganic phosphorus, were also within the normal range for humans. The average blood urea nitrogen (BUN) in the hypertensive group was 44 mg/100 ml, and in the normotensive group 32 mg/100 ml. This BUN difference was insignificant. The remainder of the blood profile, including uric acid, cholesterol, total proteins, and assorted enzymes, did not reveal any meaningful pattern.
LABORATORY SUBARACHNOID HEMORRHAGE AND ANEURYSMS

GROSS POSTMORTEM
The most significant finding at autopsy was that of aneurysms at the site of needle punctures on the carotid arteries (fig. 1). There were no intracranial or extracranial hematomas present. The plastic-enclosed kidneys that were removed were found to be encased in a cicatrix of dense fibrous tissue.

HISTOLOGICAL FEATURES OF THE ANEURYSMS
Confirmation of the histological structure of the aneurysm and its parent vessel was obtained on the sections stained with hematoxylin and eosin. The Verhoeff stains indicate frank disruption of the internal elastic lamina at the point of aneurysm formation (fig. 2). Usually, a small amount of organizing hematoma seemed to be present in the dome of the aneurysm.

Discussion
The levels of hypertension obtained in the experimental group were not as high as expected. This might have been related to the time interval between the renal surgery and the blood pressure measurements. In any event, both the relatively small increase in blood pressure and the apparent failure of this hypertension to alter the clinical course after the subarachnoid hemorrhage were disappointing. We have not excluded some relationship between angiographical spasm and preexisting hypertensive disease in a patient with subarachnoid hemorrhage.

It was our clinical impression, along with others, that patients who had a history of hypertensive disease were more likely to have severe angiographical spasm than were normotensive patients, when patients of comparable grades were analyzed. This was our reason for creating a hypertensive group to study. Hypertension in subarachnoid hemorrhage has been documented by others and cerebral vasospasm likewise has been previously recorded in hypertensive encephalopathy. There are reports indicating a greater incidence of vasospasm in patients with subarachnoid hemorrhage and hypertension than in those without, possibly because of an increased vascular sensitivity that is secondary either to an elevated blood pressure or to abolition of normal autoregulatory mechanisms after hemorrhage.

Local vessel spasm in the region of the aneurysm may be different from generalized spasm, or perhaps the generalized form may be an extension of the former. Some investigators have reported transient local spasm in animals with hypertensive encephalopathy, and the possibility of a vasoconstrictive substance has been suspected. Bayliss has shown that the normal artery contracts with an increase in pressure and dilates with a decrease in pressure, but this is difficult to correlate with focal vascular changes.

The work of Lende concerning local vessel spasms after various stimuli is well known. Brawley and others have shown that, in a clinical setting, this focal chronic spasm is not likely due to serotonin.

The combination of a focal vessel injury and blood in the subarachnoid space seemed most likely to produce the spasm that is noted clinically. This was the technique utilized by Simeone and Landau, along with their co-workers, in producing their most severe vessel reactions. Except for the introduction of hypertension and the emphasis on clinical evidence of spasm rather than angiographical evidence, our results should not have been dissimilar from theirs. They demonstrated spasm angiographically, using the larger Rhesus or African green monkey. That the angiographical spasm in their study did not always correlate with the clinical state of the animal is not unlike actual experience, and suggests that some of our monkeys would have had angiographical spasm if not studied on a fixed schedule seven days after the hemorrhage at the time of sacrifice. The use of the Rhesus and African green monkeys could explain some differences, because, as pointed out by Echlin, there are species differences.

Notwithstanding these considerations, of the 40 squirrel monkeys subjected to one or more severe subarachnoid hemorrhages—hemorrhages severe enough to spring sutures—none suffered stupor or a focal deficit and none had electrolyte changes. In short, none had clinical or laboratory findings that we tend to associate with severe subarachnoid hemorrhage and associated vasospasm. The well-known discrepancy between the patient's clinical state and his angiographical state led us to select the clinical state as the criterion for spasm before embarking on this study, and using this as the criterion, our results were negative. In a review of the literature using this as the criterion for...
spasm, we found no consistently artifact-free positive results, regardless of the species used.

Our failure to produce an acceptable working model of spasm is typical of this area of study. The multitude of different ideas relating to spasm reveals how lacking we are in factual knowledge concerning it. Whatever may be, it seems evident that the statement of Roy and Sherrington,18 "one marked characteristic of the literature dealing with the cerebral circulation is, we think, the contradictory nature of the results which have been obtained by different investigators," is still pertinent to vasospasm.

The one noteworthy finding—a surprising and serendipitous one—in an otherwise apparently fruitless effort relating to hypertension and cerebral vasospasm is the apparent production of experimental aneurysms on intracranial arteries as a result of arterial puncture and interruption of the internal elastic membrane. The significance of this should be obvious to any investigator interested in cerebral vasospasm. A review of the literature has revealed that White and co-workers19 experimentally produced similar aneurysms in the dog in 1961.

Acknowledgments

The Baptist Memorial Hospital Department of Pathology helped in tissue preparation and microphotography. Dr. James T. Robertson also provided valuable encouragement in follow-up on aneurysm production.

References

3. Halsey J: Personal communication to the authors
17. Ehlin FA: Vasospasm and focal cerebral ischemia: An experimental study. Arch Neurol (Chicago) 47: 77-96 (Jan) 1942
Experimental Subarachnoid Hemorrhage: A Study for Spasm with the Production of Aneurysms

VANCE C. ROY, JR., THORALF M. SUNDT, JR. and FRANCIS MURPHEY

doi: 10.1161/01.STR.1.4.248

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1970 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/1/4/248

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/